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Isolement et identification de souches d'actinomycètes : Application dans un procédé de compostage contrôlé

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Résumé

Le Maroc, engagé dans la promotion d'un développement agricole durable depuis le Plan Maroc Vert, se trouve confronté à des défis majeurs relatifs à la gestion des déchets. Cette thèse se focalise sur l'analyse des Actinomycètes isolées d'un sol irrigué par les margines à différentes à doses (5L/m² et 10L/m²), planté avec trois espèces de menthe (*Mentha aquatica, Mentha piperita, and Mentha pulegium)*. Les margines sont délibérément exploitées pour exercer une pression de sélection en vue d'obtenir des souches résistantes. L'objectif central est d'évaluer le potentiel de ces Actinomycètes dans une gestion efficace des déchets, tout en utilisant les margines comme pression de sélection pour obtenir des souches compétentes et résilientes.

La méthodologie adoptée englobe l'isolement des actinomycètes, leur caractérisation, ainsi que la sélection de souches compétentes pour les exploiter pour améliorer le processus du compostage des déchets. La majorité des actinomycètes isolés (58.82%) ont produit des substances antibactériennes contre les bactéries à Gram-positives, mais ils étaient moins actifs contre les souches à Gram-négatives, et notamment contre *Escherichia coli* (20.58%). De plus, les souches d'actinomycètes isolées ont montré un large spectre d'action contre les deux levures testées, en particulier contre *Saccharomyces cerevisiae*. De plus, ces souches ont présenté une tolérance variable à différents facteurs, montrant une préférence particulière pour une température de 37°C, un pH neutre (pH=7), et une faible concentration en sel (NaCl, 2%). Le compost inoculé avec les souches compétentes sélectionnées a affiché une teneur totale en azote de 1,18 %, tandis que le témoin présentait 0,84 % d'azote. L'évaluation de l'impact de ces composts sur la culture de fèves a révélé que le compost inoculé a généré le taux de germination le plus élevé (67,4 %), améliorant significativement le rendement et la qualité des graines.

À partir d'un compost élaboré obtenu, une souche thermophile a été sélectionnée et identifiée comme *Thermoactinomycetes sacchari* OBEF23. L'isolement de cette souche souligne l'importance de poursuivre la recherche sur cet actinomycète thermophile peu étudié, qui pourrait jouer un rôle significatif dans les processus de biotechnologie et de bioremédiation. Par ailleurs, à partir des souches isolées du sol utilisé dans cette étude, une souche spécifique *Streptomyces anulatus* EFBO8, sélectionnée pour son activité inulinase, a été caractérisée comme étant la plus productive, avec une valeur de 24.024 UE/ml, ouvrant des perspectives d'application potentielle dans l'industrie agroalimentaire.

Cette thèse est une contribution significative à la compréhension des mécanismes biologiques liés à la gestion des déchets organiques au Maroc. Les résultats obtenus fournissent des orientations précieuses pour le développement de pratiques agricoles durables, soutenant ainsi les objectifs du Plan Maroc Vert en matière de durabilité et de gestion efficace des ressources agricoles.

Mots clés : Plan Maroc Vert, Géneration Green, Procédé de compostage, Actinomycètes, Agriculture durable, Enzymes dégradatives

Abstract

Morocco, committed to promoting sustainable agricultural development through the Green Morocco Plan, faces significant challenges related to waste management. This thesis focuses on the analysis of Actinomycetes isolated from soil irrigated with olive mill wastewater at different doses (5L/m² and 10L/m²), planted with three species of mint (*Mentha aquatica, Mentha piperita, and Mentha pulegium*). Olive mill wastewater is deliberately exploited to exert selection pressure to obtain resistant strains. The central objective is to assess the potential of these Actinomycetes in effective waste management, using olive mill wastewater as a means of selection to obtain competent and resilient strains.

The adopted methodology includes the isolation of Actinomycetes, their in-depth characterization, and the selection of competent strains to exploit them for improving the composting process and its outcome. The majority of isolated Actinomycetes (58.82%) produced antibacterial substances against Gram-positive bacteria, but they were less active against Gram-negative strains, especially Escherichia coli (20.58%). Additionally, the isolated Actinomycete strains showed a broad spectrum of activity against the two tested yeasts, particularly Saccharomyces cerevisiae. Moreover, these strains exhibited variable tolerance to different factors, showing a particular preference for a temperature of 37°C, neutral pH (pH=7), and low salt concentration (NaCl, 2%). The compost inoculated with selected competent strains showed a total nitrogen content of 1.18%, while the control had 0.84% nitrogen. The evaluation of the impact of these composts on fava bean cultivation revealed that the inoculated compost generated the highest germination rate (67.4%), significantly improving yield and seed quality.

From an obtained compost, a thermophilic strain was selected and identified as *Thermoactinomycetes sacchari* OBEF23. The isolation of this strain highlights the importance of further research on this understudied thermophilic Actinomycete, which could play a significant role in biotechnology and bioremediation processes. Furthermore, from the strains isolated from the soil used in this study, a specific strain, *Streptomyces anulatus* EFBO8, selected for its inulinase activity, was characterized as the most productive, with a value of 24.024 U/ml, opening potential applications in the agri-food industry.

This thesis represents a significant contribution to understanding the biological mechanisms related to organic waste management in Morocco. The obtained results provide valuable insights for the development of sustainable agricultural practices, thus supporting the goals of the Green Morocco Plan regarding sustainability and effective management of agricultural resources.

Keywords: Green Morocco Plan, Generation Green, Composting Process, Actinomycetes, Sustainable Agriculture, Degradative Enzymes

ملخص

يواجه المغرب، كدولة ملتزمة بتعزيز التنمية الزراعية المستدامة من خلال مخططات المغرب و الجيل الأخضر، تحديات كبيرة تتعلق بإدارة النفايات الصلبة الفلاحية. تركز هذه الدراسة على دراسة الأكتينومايسيتات المعزولة من تربة تم ريها بمياه مرج الزيتون (مياه الصناعة الزيتونية) بجرعات مختلفة (5 لتر/متر مربع و10 لتر/متر مربع)، والتي تم زراعتها بثلاثة أنواع من النعناع (النعناع المائي، النعناع الفلفلي، والنعناع البري). يتم استخدام مياه عصر الزيتون بشكل متعمد لفرض اختيار للحصول على سلالات مقاومة. الهدف الرئيسي هو تقييم إمكانيات هذه الأكتينومايسيتات في إدارة فعالة للنفايات، باستخدام مياه عصر الزيتون كوسيلة للحصول على سلالات كفوءة ومتأقلمة.

تتضمن المنهجية المعتمدة على عزل الأكتينومايسيتات، وتوصيفها بشكل مفصل، بالإضافة إلى اختيار سلالات كفوءة للاستفادة منها في تحسين عملية تحويل النفايات إلى سماد. أظهرت غالبية الأكتينومايسيتات المعزولة (58.82٪) إنتاج مواد مضادة للبكتيريا ضد البكتيريا موجبة الغرام ، رغم أنها كانت أقل فعالية ضد سلالات البكتيريا سالبة الغرام ، خاصةً *Escherichia coli* (20.58٪). بالإضافة إلى ذلك، أظهرت السلالات المعزولة من الأكتينومايسيتات تفوقًا واسع النطاق في مواجهة الخمائر المختبرة، خاصةً ضد الخميرة*Saccharomyces cerevisiae* . كما كانت هذه السلالات تظهر تحملًا متغيرًا تجاه مختلف العوامل، حيث تفضل درجة حرارة 37 درجة مئوية، وقيمة pH محايدة(pH = 7) ، وتركيزًا منخفضًا للملح(NaCl ، 2٪).أظهر السماد المحقون بالسلالات الكفوءة المختارة نسبة إجمالية للنيتروجين بلغت 1.18٪، بينما كانت نسبة النيتروجين للسيطرة هي 0.84٪. وأظهر تقييم تأثير هذه السماد على زراعة الفول و أن السماد الملقح أنتج أعلى معدل إنبات (67.4٪)، مما أدى إلى تحسين محصول البذور وجودتها بشكل كبير.

انطلاقا من سماد تم تحضيره، تم عزل سلالة مالة للحرارة وتم تحديدها على أنها *Thermoactinomycetes sacchari*. يسلط عزل هذه السلالة الضوء على أهمية مواصلة البحث في هذه الفطريات الشعاعية المحبة للحرارة التي لم تُدرس كثيرًا، والتي يمكن أن تلعب دورًا مهمًا في عمليات التكنولوجيا الحيوية والمعالجة الحيوية. وعلاوة على ذلك، من بين السلالات المعزولة من التربة المستخدمة في هذه الدراسة، تم تحديد سلالة Streptomyces *anulatus*، التي تم اختيارها لنشاطها في إنزيم الإينولينياز، باعتبارها الأكثر إنتاجية، ، بقيمة 24.024 وحدة إنزيم لكل ملليلتر، مما يفتح المجال أمام تطبيقات محتملة في صناعة الأغذية الزراعية.

تمثل هذه الأطروحة مساهمة كبيرة في فهمنا للآليات البيولوجية التي تنطوي عليها إدارة النفايات العضوية في المغرب. توفر النتائج التي تم الحصول عليها توجيهات قيمة لتطوير الممارسات الزراعية المستدامة، وبالتالي دعم أهداف مخطط المغرب الأخضر من حيث الاستدامة والإدارة الفعالة للموارد الزراعية.

الكلمات المفتاحية: خطة المغرب الأخضر، الجيل الأخضر، عملية التسميد، الاكتينوميسيتات، الزراعة المستدامة، الإنزيمات المحلة

بسم الله الرحمن الرحيم

« قالوا سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم »

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List of abbreviations

AFC		Factorial Analysis of Correlations
Da	**:**	Dalton
GMP	**:**	Green Morocco Plan
ISP	**:**	International Streptomyces Project
MDR	**:**	Multi Drug Resistance
MENA	**:**	Middle East and North Africa
MW	**:**	Molecular Weight
OMWW	**:**	Olive Mill Wastwaters
PCA		Principal Component Analysis
PNDM	**:**	National Household Pollution Abatement Plan
Rt	**:**	Retention Time
TOC	**:**	Total Organic Carbon
VOCs	**:**	Volatile Organic Compounds

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Introduction générale

La gestion appropriée des déchets organiques est un défi majeur dans de nombreux secteurs, y compris l'industrie agroalimentaire. L'un de ces secteurs est la production d'huile d'olive, qui génère d'énormes quantités de margines (OMWW). Ces effluents, riches en matière organique et en sels minéraux, présentent cependant un niveau élevé de pollution, caractérisé par une demande chimique en oxygène (DCO) élevée et une salinité importante. Cependant, il existe des microorganismes qui peuvent intervenir dans ce processus et offrir des solutions pratiques aux différents problèmes rencontrés. Les actinomycètes, qui sont les microorganismes les plus abondants, suscitent un intérêt particulier car ils sont connus pour leur capacité à s'adapter à différentes conditions en produisant un grand nombre de métabolites à forte valeur ajoutée.

Dans le cadre de cette thèse, nous nous intéressons à l'isolement d'actinomycètes compétentes à partir d'un sol irrigué par les margines. L'objectif est de caractériser et d'identifier ces actinomycètes, puis d'étudier leur utilisation dans l'accélération du processus de compostage. Le compostage est un processus crucial pour transformer les déchets organiques en un amendement du sol stabilisé et précieux. Au cours de ce processus, une communauté diversifiée de microorganismes, y compris des bactéries thermophiles et des actinomycètes, joue un rôle essentiel dans la décomposition de la matière organique (Mawang et al., 2021). En isolant des souches compétentes d'actinomycètes à partir de ce sol, nous visons améliorer l'efficacité du processus de compostage.

La méthodologie suivie dans ce travail multidisciplinaire converge plusieurs conceptions scientifiques vu la complexité de la thématique. Elle commence par une conception environnementale pour étudier les margines et leur effet sur la microbiologie des sols traités et leur association avec les plantes du genre Mentha testées, les micro-organismes ont été caractérisés et identifiés classiquement et par outil moléculaire. Après la selection des micro-organismes visés par cette chasse en ligne, nous avons entamé une phase biotechnologique pour une exploitation agronomique sur le terrain. Donc, dans cette optique nous avons fait appel à des techniques physico-chimiques, microbiologique, moléculaire et agronomique. Les techniques utilisées dans ces domaines ont été utilisées dans un esprit pratique et théorique.

Ce travail canalisé sur plusieurs chapitres qui traitent différents aspects dans cette l'étude. Le premier chapitre est centré sur la caractérisation physico-chimique des paramètres du sol irrigué par les margines à différentes concentrations, les paramètres physico-chimiques et minéralogiques des margine ainsi qu’une étude de la flore microbienne présente au niveau du

même sol. L'interaction entre les différentes concentrations des margines et trois espèces de menthe est également étudiée pour savoir l'effet de ces derniers sur la flore microbienne.

Le deuxième chapitre est dédié à la sélection des souches compétentes d'actinomycètes isolées à partir d'échantillons du même sol afin d'être utilisé dans le processus du compostage. Les souches isolées sont caractérisées pour leurs activités hydrolytiques et antimicrobiennes, ce qui permet de sélectionner et d'identifier les bactéries actinomycètes compétentes capables de métaboliser différentes sources de carbone et d'énergie. Les paramètres physico-chimiques tels que la température, le pH et la salinité sont étudiés pour s'enquérir de leur pression de sélection sur ces souches compétentes. Le consortium d'actinomycètes sélectionné a été testé pour son activité de compostage en mesurant la température et le pH pendant le cycle de compostage, et la qualité finale du compost a été évaluée en déterminant la conductivité, la teneur en azote total (TNA) et la teneur en carbone organique total (COT).

Le troisième chapitre est canalisé sur l'utilisation du compost dans l'amélioration de la croissance des plantes. Une étude comparative est réalisée pour évaluer les effets de deux types de compost sur la croissance et les paramètres biochimiques des plants de fèves (*Vicia faba L*). Trois traitements sont comparés : un témoin non fertilisé, un traitement fertilisé avec un compost non inoculé et un traitement fertilisé avec un compost inoculé avec le consortium d'actinomycètes *Streptomyces sp*. Les propriétés chimiques et physiques du compost sont également déterminées.

Enfin, le quatrième chapitre est dédié à l'étude des populations bactériennes thermophiles et des actinomycètes au cours du processus du compostage. Les échantillons sont collectés à différentes phases du compostage et soumis à une énumération bactérienne en utilisant les méthodes microbiologiques standard. L'isolement et l'identification d'actinomycètes compétents thermophiles sont réalisés en utilisant des milieux sélectifs.

Le cinquième chapitre de cette thèse se concentre sur la production d'inulinase, une enzyme catalysant la conversion de l'inuline en D-fructose. L'inulinase est une enzyme d'intérêt industriel, mais son extraction à partir de plantes est complexe et coûteuse en raison de la difficulté d'obtenir de grandes quantités. Ainsi, l'utilisation d'inulinase microbienne présente un potentiel prometteur pour répondre aux besoins industriels. Ce chapitre vise à sélectionner des actinomycètes capables de produire l'enzyme inulinase. Pour cela, une méthode de criblage est utilisée en utilisant un milieu de culture spécifique contenant de l'inuline en tant que source de carbone.

Cette étude met en évidence le potentiel des actinomycètes pour la production d'inulinase, offrant une alternative prometteuse à l'extraction de cette enzyme à partir de sources végétales. La purification et la caractérisation de l'inulinase permettent d'envisager son utilisation dans l'industrie, notamment dans la production d'édulcorants naturels ou la transformation de l'inuline en fructose pour diverses applications alimentaires et pharmaceutiques.

Cette thèse revêt une importance particulière dans le contexte marocain, car elle s'inscrit dans le cadre des efforts déployés par le Maroc pour développer une agriculture durable et respectueuse de l'environnement. Le Maroc a adapté le Plan Maroc Vert, une initiative nationale visant à promouvoir le développement agricole durable et à garantir la sécurité alimentaire. En mettant l'accent sur l'utilisation des ressources organiques et la réduction de l'utilisation d'engrais chimiques (Mahdavi & Vera, 2023), le Plan Maroc Vert encourage le compostage des déchets organiques pour améliorer la fertilité des sols. Les travaux de cette thèse sont donc en adéquation avec les objectifs du Plan Maroc Vert et le plan génération green. En fournissant des solutions pratiques pour la gestion des déchets organiques à travers l'utilisation des actinomycètes, cette recherche contribue à la promotion d'une agriculture durable au Maroc. De plus, en améliorant l'efficacité du processus de compostage grâce à l'utilisation des actinomycètes compétents, cette thèse permet d'atteindre les objectifs du Plan Maroc Vert en termes de valorisation des ressources organiques et de réduction de l'utilisation d'engrais chimiques.

En guise de conclusion, cette thèse met en évidence l'importance des actinomycètes compétents dans la gestion des déchets organiques et l'amélioration de la croissance des plantes. Les résultats obtenus suggèrent que l'utilisation du compost inoculé avec les actinomycètes peut constituer une approche durable pour la gestion des déchets et l'amélioration des rendements agricoles. De plus, l'isolement et la caractérisation des actinomycètes compétents ouvrent la voie à de futures recherches sur leur utilisation potentielle dans des applications industrielles telles que la biotechnologie et la bioremédiation. Cette étude contribue ainsi à la promotion de pratiques respectueuses de l'environnement dans le secteur agricole et à la valorisation des ressources organiques.

Etude bibliographique

Chapitre I

Streptomyces at the Heart of Several Sectors to Support Practical and Sustainable Applications: A Review

Streptomyces au cœur de plusieurs secteurs pour soutenir des applications pratiques et durable

Synthèse

Ce chapitre se penche sur une exploration approfondie du grand potentiel des actinomycètes en mettant particulièrement l'accent sur les bactéries hautement influentes du genre Streptomyces, dans le domaine de l'agriculture durable. Il aborde le problème pressant et de plus en plus urgent de l'utilisation répandue des engrais chimiques dans les pratiques agricoles modernes, qui a indéniablement entraîné des conséquences environnementales néfastes, la dégradation des sols et une diminution alarmante de la fertilité des sols. Pour contrer et atténuer ces défis graves, l'émergence de biofertilisants, en particulier ceux inoculés avec des actinomycètes, a ouvert la voie à une alternative prometteuse et viable.

Les actinomycètes, en particulier le genre Streptomyces, jouent un rôle irremplaçable dans la décomposition de la matière organique, la transformation des nutriments et la synthèse complexe de précieux métabolites secondaires, notamment les antibiotiques, les antifongiques et les antiviraux. Lorsqu'ils sont introduits comme inoculants dans le compost, ces remarquables bactéries filamenteuses améliorent significativement et accélèrent le processus de compostage, contribuant ainsi à la création d'un environnement du sol plus enrichi et fertile. De plus, les actinomycètes, qui englobent une grande diversité d'espèces de Streptomyces, présentent une polyvalence et une efficacité inégalées en tant qu'agents de biocontrôle, sources essentielles de progrès biotechnologiques et producteurs remarquables d'herbicides, d'insecticides et d'antibiotiques. Ainsi, exploiter le potentiel des actinomycètes sous forme de biofertilisants offre une opportunité tangible et transformative pour atténuer les impacts néfastes des engrais chimiques tout en favorisant activement et en promouvant des pratiques agricoles durables.

Ce chapitre souligne consciemment le besoin urgent de poursuivre la recherche et le développement dans le domaine des biofertilisants, dans le but de relever efficacement les défis multifacettes posés par l'utilisation indiscriminée des engrais chimiques et de favoriser finalement l'adoption généralisée de l'agriculture durable à l'échelle mondiale. En exploitant et en utilisant efficacement les capacités et les attributs innés des actinomycètes, en particulier dans les domaines du compostage et de la biotechnologie, le secteur agricole peut effectuer une transition en douceur vers des pratiques respectueuses de l'environnement et efficaces en termes de ressources. Par conséquent, l'utilisation des actinomycètes et de leur potentiel enzymatique inexploité représente un espoir prometteur, non seulement pour renforcer la qualité du compost,

mais aussi pour améliorer la fertilité des sols et réduire progressivement notre dépendance aux composés chimiques nocifs compromettant l'environnement.

En conclusion, ce chapitre met en lumière le vaste potentiel inexploité des actinomycètes, notamment les espèces du genre Streptomyces, pour révolutionner et propulser l'agriculture durable vers de nouveaux horizons. Grâce à leurs contributions inestimables au compostage, aux mécanismes de biocontrôle et à la production remarquable de composés très recherchés, les actinomycètes offrent une voie pratique et pragmatique pour atténuer efficacement les impacts néfastes associés à l'utilisation généralisée des engrais chimiques. En poursuivant activement la recherche, l'innovation et l'adoption répandue de biofertilisants enrichis des attributs extraordinaires des actinomycètes, nous pouvons collectivement œuvrer à la réalisation d'objectifs de durabilité à long terme dans le domaine de l'agriculture, assurant ainsi un avenir plus brillant et plus prospère pour l'environnement et l'humanité dans son ensemble.

Streptomyces at the Heart of Several Sectors to Support Practical and Sustainable Applications: A Review

This part of the work was published in the journal "PMMB". (Q3 - IF: 0.388).

Abstract

This article emphasizes the various contributions of actinomycetes to the well-being of human life on our planet. Agrarian virtues begin with the substitution of chemical fertilizers with bio-fertilizing microorganisms which have a versatile potential for the benefit of soil and plants by playing complementary and interconnected roles. Thus, these microorganisms improve soil health through bioremediation phenomena and elimination of several non-beneficial microorganisms. In agriculture, these microorganisms can be added to compost as inoculants to speed up the composting process and provide an additional source of beneficial microorganisms for the compost-treated soil. Streptomyces and other actinomycetes can also be used as biotechnological sources of herbicides and insecticides. In the medical and therapeutic sectors, this paper emphasizes the potential of actinomycetes, in particular Streptomyces species, in the production of antibiotics, antioxidant and anticancer agents, opening up avenues for the creation of molecules with high benefits. In biotechnology, these totipotent microorganisms produce enzymes that are widely used in a number of industries, generating considerable revenue. Sporadic data accumulated on these types of microorganisms opens up many new avenues for exploiting these natural biocatalytic resources. Papers published in the last decade have exploded the amount of information that can be put to practical use. To maximize the value of these microorganisms, it would be advisable to create common threads between the themes that bring together the areas of expertise where these microorganisms could potentially be exploited. This bibliographical synthesis is a contribution to the development of targeted database.

Keywords: Streptomyces, bio-fertilizers, antibiotics, antioxidant, anticancer, enzymes, compost, soil

1. Introduction

The widespread adoption of chemical fertilizers in modern agriculture has become common. Nonetheless, the excessive application of these fertilizers has resulted in environmental contamination, soil degradation, and soil fertility loss(Iderawumi & Kamal, 2022; Nabyonga et al., 2022; Weifeng et al., 2022). Chemical fertilizers can also be harmful and toxic to humans and animals by food, feed and water as shutter transmitters(Tyagi et al., 2022). To address these issues, biofertilizers, such as compost, have become increasingly popular in agriculture. Composting is a widely used method for recycling organic waste and producing a nutrient-rich soil amendment(Bamdad et al., 2022). Actinomycetes, a significant bacterial group commonly present in compost, have a vital role in the decomposition of organic matter and the transformation of nutrients(D. Mitra et al., 2022). In particular, Streptomyces genus is known to be implicated in compost processing and has been recognized for its potential as a biofertilizer. The Streptomyces genus, part of Streptomycetaceae family, consists of over 700 species of filamentous bacteria that are gram-positive, neutrophilic, and facultative aerobic with a G+C DNA content higher than 70%(de Lima Procópio et al., 2012a). Streptomyces bacteria life cycle is intricate and encompasses various stages, which include vegetative growth, formation of aerial hyphae, and sporulation phenomena(Pacios-Michelena et al., 2021; Xu et al., 2022). The bld genes control aerial hyphae formation, while the whi genes control cell division and spore maturation(Otani et al., 2022; Xu et al., 2022). Streptomyces is renowned for its ability to produce a huge number of secondary metabolites(Araujo, 2022; L.Lee et al., 2018), such as antibiotics, antifungals, phytotoxins, plant growth regulators, herbicides, antivirals and antitumor(Alam et al., 2022; Arakawa & Suzuki, 2022; P. Sharma et al., 2018a)(Figure1). These bacteria can colonize different ecological niches and use a wide range of carbon and nitrogen sources (Al-Enazi et al., 2022). Optimal pH growth is between 6.5 and 8, but some strains can tolerate 9 or higher levels (Pacios-Michelena et al., 2021). Streptomyces are widespread in soils and can resist drastic conditions such as long drought and nutrient scarcity, thanks to their mycelial growth and spore-forming abilities, so they are known for their ability to conquer different environmental biotopes (Worsley et al., 2020), making them valuable biocontrol tools in agriculture.

Streptomyces and other actinomycetes can be added to compost as inoculants to enhance composting process and improve final product quality(Buzón-Durán et al., 2020; Chi et al., 2020; Feng et al., 2021; Wang et al., 2022). These microorganisms can improve organic matter degradation, increase nutrient availability, and inhibit harmful microorganisms proliferation,

leading to a more stable and high value compost. Additionally, they can produce a wide range of secondary metabolites and plant hormones, which can improve their growth and health.

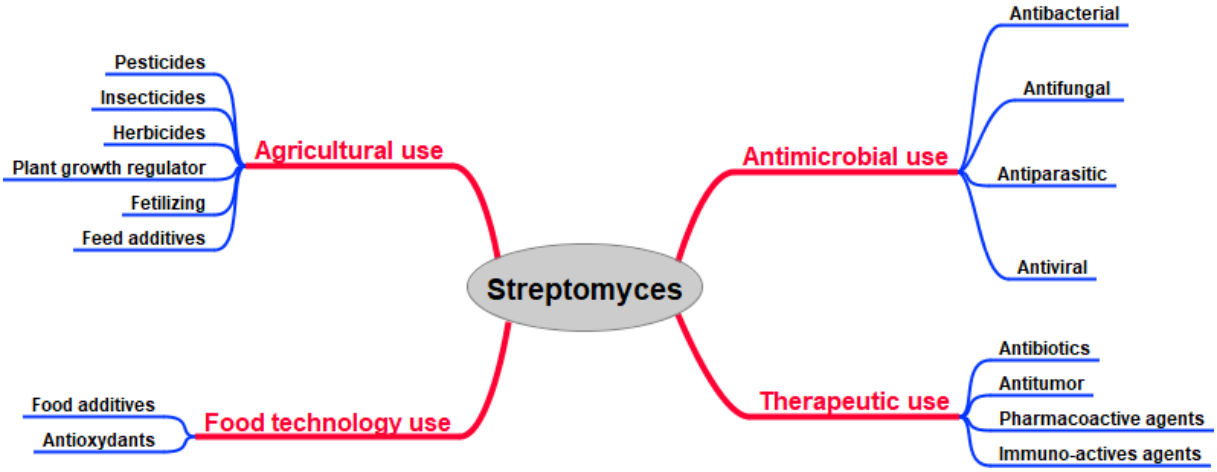

Figure 1. Mental map of Streptomyces virtues in various fields

The adverse impacts of chemical fertilizers are not limited to environmental and health concerns but also contribute to antibiotic-resistant bacteria development. The continuous application of these antimicrobial substrates in agriculture can lead to resistant strains emergence, which can give rise to serious threats to human health. Therefore, biofertilizers use, such as compost inoculated with Streptomyces, can help to reduce chemical fertilizers and antibiotics dependency in agriculture. The use of actinomycetes, especially Streptomyces, has been shown to be effective on herbicides and insecticides in soil(Arakawa & Suzuki, 2022; Kim et al., 2022; Kumar et al., 2022). However, despite their potential, very few actinomycete-based products are currently available on the market. For this reason, lots of research are being conducted to develop efficient formulations containing actinomycetes as active ingredients and improve their shelf life and stability.

One of the most notable applications of actinomycetes and their valued enzymes is the composting process. These microorganisms play a critical role in breaking down refractory plant materials, such as lignocellulose polymers, into easily metabolizable monomeric compounds(Buzón-Durán et al., 2020; Feng et al., 2021). This process not only helps to improve compost quality but also enhances soil fertility, making it an important basement for sustainable agriculture. In addition to composting, actinomycetes and their enzymes were found to have important applications in biotechnology and used in different industrial processes(Khushboo et al., 2022). Therefore, further research and development of biofertilizers

should be supported to reduce chemical fertilizers' negative impact and essentially promote sustainable agricultural practices.

The Moroccan government took proactive measures by implementing the Green Morocco Plan (GMP 2008-2020). This strategy was designed to revitalize agricultural sector and foster sustainable practices. With a specific focus on promoting organic agriculture and compost utilization as a natural soil amendment, the GMP underscores the importance of environmentally sustainable approaches. To ensure the success of this endeavor, the government enacted regulations to support the adoption of compost in agricultural practices.

In this review, we will discuss the potential of Actinomycetes, especially Streptomyces, as antibiotics and their potential applications in sustainable agriculture, with a focus on their role in compost. We will examine the mechanisms by which they enhance compost quality and composting process.

2. Overview of Streptomyces life cycle

Actinobacteria is a large phylum within the Bacteria domain widely distributed in both terrestrial and aquatic ecosystems(Barka et al., 2015). These Gram-positive bacteria are characterized by their high guanine and cytosine (G + C) content in DNA and are known for their filamentous nature, capable of forming both substrate and aerial mycelium (Mawang et al., 2021). The phylum encompasses 374 genera, with Streptomyces being the most dominant group. Non-Streptomyces actinomycetes such as Actinomyces, Kitasatospora, Micromonospora, Nocardia, Micrococcus, Arthrobacter, and Rhodococcus are also present, but are less frequently located under normal conditions and demand specialized methods for their isolation, preservation, and cultivation methods (Lee, Zainal, et al., 2014; Mawang et al., 2021).

Streptomyces species' life cycle begins with spore germination, initiated by the emergence of one or two germ tubes. Emerging shoots emerge at locations behind the tip and grow into a mesh-like structure of hyphae, known as vegetative mycelium (MV) (stage 1). Following nutrient depletion, the vegetative mycelium undergoes differentiation, transforming into aerial hyphae (stage 2). Ultimately, the aerial hyphae differentiate into elongated chains of spores (stage 3). The regulation of the polarisome, the bld gene family, and the whi gene family allow for manipulation and control of all three stages of this process (Figure 2)(Xu et al., 2022).

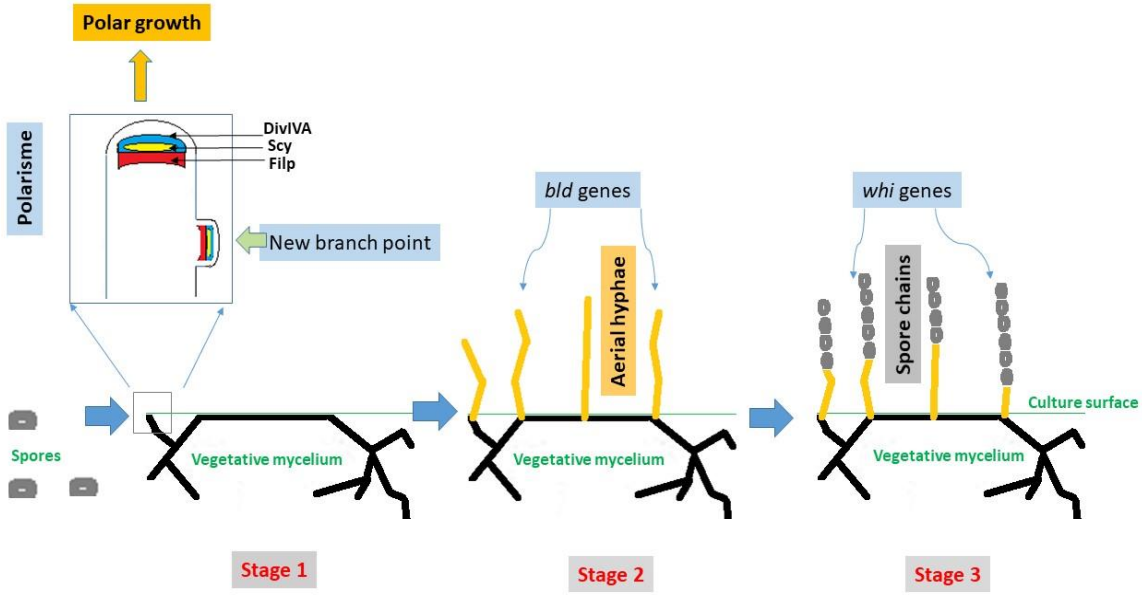

Figure 2: Streptomyces life cycle (Reproduced from (Z. Xu et al., 2022)).

Streptomyces life cycle can be divided into vegetative and reproductive phases(Lajtai-Szabó et al., 2022), with some differences in solid and liquid cultures medium(Lajtai-Szabó et al., 2022) and it is characterized by compartmentalized hyphae formation, which is a process that begins with spore germination in solid cultures or mycelial fragmentation in liquid cultures. In solid cultures, cycle life begins with spore germination and vegetative mycelium development, which grows deep into solid medium culture(Ser et al., 2017). After a period of time, programmed cell death (PCD) occurs, leading to antibiotic production and aerial mycelium growth. In contrast to solid culture where spore germination is the initial step, liquid cultures of Streptomyces begin with compartmentalized hyphae formation (MI), which is followed by programmed cell death and secondary metabolite-producing mycelia development. In some Streptomyces strains, spores formation and aerial mycelium may be hindered. Streptomyces coelicolor proteomics analysis has demonstrated that expressed proteins during mycelial growth (MII) second stage are responsible for secondary metabolites production (Figure 3). Despite these findings, the mechanisms behind Streptomyces differentiation in liquid medium are not yet fully understood, and researchers are still trying to explore the relationship between pellet formation and secondary metabolite production(Lajtai-Szabó et al., 2022).

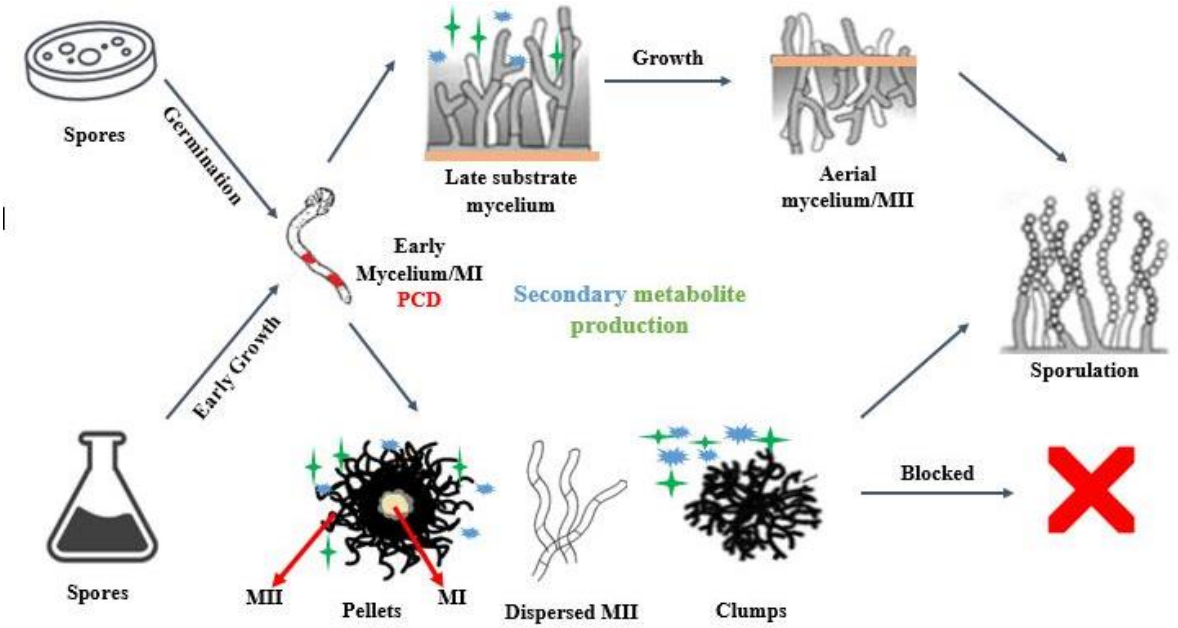

Figure 3 General life cycle of Streptomyces in solid cultures (upper panels) and liquid cultures (lower panels) (Reproduced from (Lajtai-Szabó et al., 2022))

The sporadic data accumulating on actinomycetes, and streptomycetes in particular are due to the mass discovery of a very high number of new species (J. W. F. Law et al., 2023; J. W.-F. Law, Pusparajah, et al., 2019; J. W.-F. Law et al., 2023; Ser et al., 2015) which can solve thorny problems where there are blockages in medical, cosmetic and agri-food procedures (Thye et al., 2022). These new species give hope of finding anti-cancer molecules, drugs to treat emerging diseases(Mutalib et al., 2020) or improve the living environment of mankind(J. W.-F. Law, Letchumanan, et al., 2020).

3. Industrial application: extracellular enzymes from Streptomyces spp.

Microbial-derived enzymes are widely sought after in the industrial sector because of their exceptional stability, productivity, and availability, as well as their economical nature and environmentally sustainable production methods. From an industrial standpoint, their practicality and ecological soundness further justify their use(Ferreira et al., 2021). Streptomyces sp. constitutes a group of bacteria that are well-known for their unique characteristics and capabilities to produce a wide range of extracellular enzymes(Sarmiento-Vizcaíno et al., 2022). The latter find many applications in various industrial fields (figure 4)(S. Singh & Upadhyay, 2022). Among the most commonly used enzymes produced by Streptomyces are amylase, protease, chitinase, xylanase, lipase, cellulase and laccase.

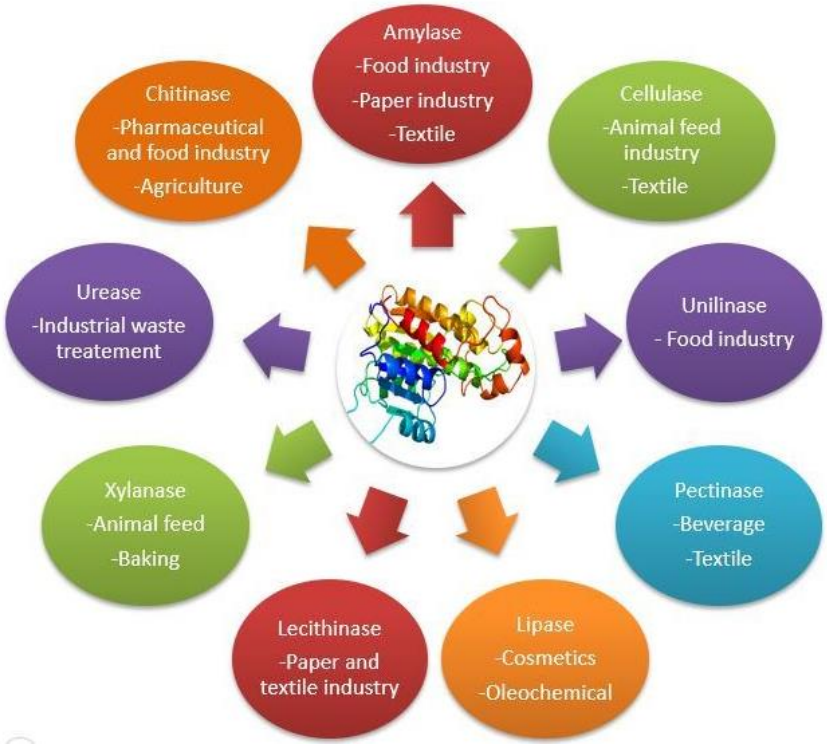

Figure 4. Diversity and functionality of extracellular enzymes produced by *Streptomyces spp.*

3.1. Amylase

Amylase (EC 3.2.1.1) is an enzyme responsible for the hydrolysis of starch into more accessible sugars like glucose and maltose. These simpler sugars serve as essential raw materials for diverse fermentative bioconversions, including ethanol, lactic acid, and citric acid fermentation. Amylases are highly regarded as one of the most significant enzymes for industrial use, due to their extensive applications(Waheeb et al., 2021), including brewing, detergent manufacturing, tanning, papermaking, textile production, biofuel production, bakery, and food processing(Mondal et al., 2022). This enzymatic activity (amylase) is still a subject of research

and development to select new species with significant physico-chemical potential(L.-H. Lee, Azman, et al., 2014). Many species of Streptomyces have been used for amylase production(Al-Dhabi et al., 2020; Boovaragamoorthy et al., 2019; Gurusamy et al., 2019; Rajkumari et al., 2019; Roopan et al., 2019; Valsalam et al., 2019). Streptomyces amylase has been used in the field of medicine(Al-Dhabi et al., 2020), and starch processing industry(Mehta & Satyanarayana, 2016), especially raw starch processing(Lakshmi et al., 2020).

Table 1 presents some of Amylase-Producing Streptomyces and their applications.

Table1: Amylase-producing Streptomyces strains and their applications

Strains	Industry	Application	References
Streptomyces spp NAA-28	Biotechnology	Food, paper, detergent, fermentation, textile, and pharmaceutical sectors	(Sobhy et al., 2023)
Streptomyces griseus	medicine	Antibiofilm agent	(Lakshmi et al., 2022)
Streptomyces lonarensis NCL 716	Food	Maltooligosaccharides production	(T. K. Sharma et al., 2012)
Streptomyces erumpens MTCC 7317	Food	Soluble starch and cassava starch hydrolysis	(Shaktimay et al., 2010)
Streptomyces enissocaesilis NRRL B-16365	Food	High fructose corn syrups production	(T. A. Singh et al., 2020)
Streptomyces spp	Food	Bakery	(Ammar et al., 2002)
Streptomyces spp SNAJSM6	Biotechnology	α-amylase production	(Anand et al., 2019)

3.2. **Protease**

Numerous research studies have documented the production of proteases from Streptomyces, making them extensively utilized in various industrial applications, notably in the field of food processing, cosmetic manufacturing, detergency, pharmaceuticals, chemical application, and waste management due to their adaptable nature(Ashraf et al., 2023). They are also used in leather processing industries in the dehairing phase, thus leading to the total elimination of chemicals application(Abdullah Al-Dhabi et al., 2020). Proteases (EC 3.4) have been shown to exhibit a high degree of tolerance to diverse environmental challenges, including extreme pH levels, temperature, and salinity conditions, with most of them displaying this characteristic(Rathnan, 2011). Additionally, proteases derived from Streptomyces spp. have been found to be effective in agro-industrial waste processes recycling such as feathers, nails, hair, and plant waste (Bentley et al., 2002).
Table 2 highlights a range of protease-producing Streptomyces strains and the various practical applications of these proteases.

Table 2: Protease-producing Streptomyces strains and their applications

Strains	Industry	Application	References
Streptomyces gulbargensis	Healthcare industry	Surgical instruments washing	(Vishalakshi et al., 2009)
Streptomyces koyangensis TN650	Detergents	Detergent formulations and non-aqueous peptide biocatalysis	(Ben Elhoul et al., 2015)
Streptomyces mutabilis TN-X30	Detergent	Serine alkaline protease formulation	(S. Mechri et al., 2022)
Streptomyces griseus K-1	Medicine, cosmetics, textiles	Pronase P preparation	(Narahashi et al., 1968)
Streptomyces coelicolor A3(2)	Biotechnology	Actinorhodin production	(Demir et al., 2019)
Streptomyces pactum	Feather industry	Chicken feathers disintegration	(Bockle & Muller, 1997)
Streptomyces flavogriseus HS1	Detergents	Laundry detergent (Detergent additive)	(Ghorbel et al., 2014)
Streptomyces nogalotor Ac 80	Leather industry	Goatskin depilation	(P. Mitra & Chakrabartty, 2005)

3.3. **Chitinase**

Chitinase (EC 3.2.1.14) is a type of enzyme that is highly valuable in industrial settings due to its ability to break down chitin. Chitinases produced by certain actinomycetes have demonstrated remarkable characteristics, such as thermostability and pH tolerance, which make them highly appropriate for various industrial applications(Kulkarni & Gadre, 2002). Streptomyces spp produces chitinases, which are used in the production of chitin derivatives, such as chitosan, a natural biopolymer with applications in the medical, agricultural, and food industries(El-araby et al., 2022b, 2022a; Khan et al., 2015). Various Streptomyces strains, including Streptomyces thermoviolaceus, have been identified as producers of chitinases. These chitinases have been used to extract chitobiose, a compound that shows potential as an antioxidant with applications in both biomedical and food industries(Bhattacharya et al., 2007; Setyahadi et al., 2019). Table 3 displays certain Streptomyces strains that produce chitinase and their industrial applications.

Table 3: Chitinase-producing Streptomyces strains and their applications

Strains	Industry	Application	References
Streptomyces alfalfa ACCC 40021	Agriculture	-Fungal Biocontrol -Chitine conversion to N-acetyl-D-glucosamine	(Lv et al., 2021)
Streptomyces sampsonii XY 2–7	Agriculture	N-acetyl chitobiose production	(W. Zhang et al., 2020)
Streptomyces spp CT02	Agriculture	Biocontrol agents against phytopathogenic fungi	(Umar et al., 2021)
Streptomyces thermocarboxydus TKU045	Medicine	Chitin oligomers	(Tran et al., 2019)
Streptomyces violaceoruber pChi	Food industry	Chitinase food enzyme	(Silano et al., 2019)
Streptomyces griseorubens E44G	Agriculture	Fusarium biocontrol	(Rashad et al., 2017)
Streptomyces cavourensis SY224	Agriculture	Anthracnose biocontrol	(S. Y. Lee, 2012)
Streptomyces hygroscopicus SRA14	Agriculture	Phytopathogenic fungi biocontrol	(Prapagdee et al., 2008)
Streptomyces rochei A-1	Food industry	Botryosphaeria dothidea post-harvest biocontrol	(Q. Zhang et al., 2016)

3.4. **Xylanases**

The principal constituent of hemicelluloses is xylan, a complex polysaccharide that is predominantly used in pulp and biobleaching industry to improve pulp quality(Priya et al., 2012). Xylanases (EC 3.2.1.8) are highly sought after in numerous industries due to their broad utility across various sectors, including pharmaceuticals, food processing, paper and cellulose manufacturing(Pinheiro et al., 2021; P. Sharma et al., 2018b; Walia et al., 2017). In addition, they are also utilized in beverages production, bakery, and probiotics(Fernandes de Souza et al., 2022). Streptomyces spp. produce xylanases which are utilized in pulp and paper industry(Kuhad & Singh, 2013; Y. Li et al., 2022) and are also able to break down various agricultural residues such as straw waste and oil cake, resulting in enhanced biogas production(Chakraborty et al., 2009; Kuhad & Singh, 2013). The industrial application of certain Streptomyces strains that produce xylanase is presented in Table 4.

Table 4: Xylanase-producing Streptomyces trains and their applications

Strains	Industry	Application	References
Streptomyces galbus NR	Pulp and paper industry	Softwood kraft pulp bleaching	(Kansoh & Nagieb, 2004)
Streptomyces sp. AOA40	Food industry	Fruit juice and bakery	(Adigüzel & Tunçer, 2016)
Streptomyces sp. Strain MS-S2	Biofuels industry	Bioethanol	(Danso et al., 2022)
Streptomyces sp. FA1	Food industry	Chinese bakery	(Y. Xu et al., 2016)
Streptomyces spp ER1	Beverage industry	Fruit juice clarification	(Rosmine et al., 2017)
Streptomyces megaspores DSM 41476	Beverage industry	Brewing	(Qiu et al., 2010, p. 414)
Streptomyces thermovulgaris TISTR1948	Food industry	Xylooligosaccharide	(Boonchuay et al., 2016)
Streptomyces spp QG-11-3	Pulp and paper industry	Eucalyptus kraft pulp bleaching	(Beg et al., 2000)

3.5. **Lipases**

Lipases (EC 3.1.1.3) have numerous applications in various industries, they are used in biodiesel production(Thatoi et al., 2021) through lipase-catalyzed transesterification and in flavor compounds synthesis by esterification in food, cosmetics, perfumery, and medicine(Vivek et al., 2022). Lipases are also used in bioremediation by lipid hydrolysis, in detergent formulation for hydrolyzing greasy and oily stains, plastic degradation, agrochemicals production, waxes, and biopolymers, along with their application in tea processing. Streptomyces derived lipases serve as biosensors in probe technologies as well(Bolina et al., 2021; Chandra, Enespa, et al., 2020; Vivek et al., 2022). Additionally, they serve as degreasing agents in leather industry, in paper industry (for pitch treatment), and in textiles (as dewaxing, desizing, and scouring agents)(Chandra, Enespa, et al., 2020). Other important applications of lipases include treating oily wastewater(K. Gao et al., 2020). Streptomyces exfoliates are known to produce lipases, enzymes that break down ester bonds in triglycerides into glycerol and fatty acids (Aly et al., 2012). These lipases have a wide range of potential applications (Parameswaran et al., 2019), including their use in fats catalyzing, cosmetics, diagnostics, and detergents (Ninawe et al., 2006). Table 5 presents industrial application of some lipase-producing Streptomyces strains

Table 5: Lipase-producing Streptomyces strains and their applications

Strains	Industry	Application	References
Streptomyces sp. DPUA1566	Detergent	Biosurfactant	(Santos et al., 2019)
Streptomyces chromofuscus	Food industry	Phospholipase D	(Wu et al., 2021)
Streptomyces violascens OC125-8	Wastewater treatement	Oily wastewater	(Boran et al., 2019)
Streptomyces spp OC119-7	Biodiesel production	Enzymatic catalyst	(Ayaz et al., 2015)
Streptomyces spp CS133	Biodiesel production	Oils transesterification	(Mander et al., 2012)
Streptomyces sp. SC734	Pharmaceutical industry	Phosphatidylserine synthesis	(Choojit et al., 2016)
Streptomyces thermocarboxydus ME168	Cosmectic and food industry	Sugar ester synthesis	(H-Kittikun et al., 2012)
Streptomyces clavuligerus CKD1119	Pharmaceutical industry	Tacrolimus	(H.-S. Kim & Park, 2007)

3.6. **Cellulases**

Cellulases, also known as glucan 1,4-β-glucosidase (EC 3.2.1.74), break down cellulose, yielding glucose, cellobiose, and cello-oligosaccharides as primary products(Zukovski et al., 2017). These enzymes work synergistically to degrade cellulose(Romsaiyud et al., 2009) and are the focus of numerous studies due to their vital role in biomass hydrolysis(Budihal et al., 2016). Due to the potential of cellulases to break down cellulosic biomass into glucose, these enzymes are in great demand in the global commercial market due to their wide-ranging applications across diverse industries (Areeshi, 2022; Ejaz et al., 2021). In order to apply cellulases in different industries such as detergents and leather, it is crucial to identify enzymes that are highly stable and capable of functioning under extreme pH and temperature conditions(Nawani et al., 2013). Certain Streptomyces spp. such as Streptomyces ruber, and Streptomyces rutgersensis, produce highly thermostable cellulases (Kar & Ray, 2008). The use of cellulases is prevalent across multiple industries such as food, brewery and wine, agriculture, textile, detergent, animal feed, pulp and paper, as well as in research and development (Ejaz et al., 2021). Table 6 presents some of cellulase-producing Streptomyces sp and their industrial applications.

Table 6: Cellulase-producing Streptomyces strains and their applications

Strains	Industry	Application	References
Streptomyces drozdowiczii	Detergent and textile	Cellulase	(Grigorevski de Lima et al., 2005)
Streptomyces spp MS-S2	Biofuels industry	Sugar bioconversion to ethanol	(Danso et al., 2022)
Streptomyces spp C48	Agriculture	Agricultural waste hydrolyzis	(Celaya-Herrera et al., 2021)
Streptomyces ruber	Textile	Cellulase	(El-Sersy et al., 2010)
Streptomyces roseochromogenes ATCC 13400	Waste treatment	Cellulase	(Restaino et al., 2023)
Streptomyces spp. NAA2	Biofuels industry	Biomass saccharification	(A. Saini & Aggarwal, 2019)
Streptomyces spp T3-1	Biofuels industry	Sugar bioconversion to ethanol	(Hsu et al., 2011)
Streptomyces clavuligerus MAC 9	Waste treatment	Biogas production	(Swarna & Gnanadoss, 2020)
Streptomyces griseoaurantiacus ZQBC691	Biotechnology	Ethanol production	(Swarna & Gnanadoss, 2020)

3.7. **Laccases**

Laccases are enzymes containing copper have a widespread presence in nature(A. N. Yadav, 2021) and have a diverse range of applications. Laccases have the potential to be used in various industrial applications due to their ability to act on a large range of substrates. These applications include treating industrial effluent, producing biofuels, bleaching, metabolizing drugs, remediating pollutants, decolorizing dyes, developing biosensor probes, and bioprinting(J. Zhang et al., 2019). These diverse applications make laccases a promising candidate for use in multiple industries. Laccase production by Streptomyces is influenced by various factors (Mishra et al., 2020) including culture conditions, substrate composition, and enzyme inducers (Gnanamani et al., 2006; Muthukumarasamy et al., 2015). Despite having a relatively low redox potential, small laccases produced by Streptomyces possess a range of exceptional traits that make them appealing for use in commercial applications. Notably, their ability to exhibit unique substrate specificity and demonstrate remarkable effectiveness over a broad pH range, particularly. Additionally, their unusual resistance to inhibitors further broadens the scope of potential applications in various industries (Kaur et al., 2022). The presented table (Table 7) provides an overview of the different types of Streptomyces strains that produce laccase enzyme and their industrial applications.

Table 7: Laccase-producing Streptomyces strains and their applications

Strains	Industry	Application	References
Streptomyces psammoticus	Wastewater treatment industry	Phenolic compounds removal	(Niladevi & Prema, 2008)
Streptomyces ipomoeae SilA	Lubricant industry	Eco-friendly oleogels production	(Domínguez et al., 2021)
Streptomyces psammoticus MTCC7334	Textile	Dye decolourization	(D.-L. Lu et al., 2023)
Streptomyces cyaneus CECT 3335	Pulp and paper industry	Kraft pulp bleaching	(Arias et al., 2003)
Streptomyces mutabilis A17	Environmental remediation	Detoxification and bioremediation of sulfonamides and synthetic dyes	(Reda et al., 2019)
Streptomyces ipomoeae CECT 3341	Textile	Dye degradation	(Blánquez et al., 2019)

In addition to previously cited enzymes, Streptomyces is renowned for its ability to produce an extensive array of other enzymes that hold significant importance in various fields. For instance, Streptomyces strains are capable of producing azoreductase, which is used in azo dyes biodegradation in textile and paper industries (Dong et al., 2019). Peroxidase is another enzyme produced by Streptomyces that has applications in environmental bioremediation, pulp and paper bleaching, and food processing (Preethi et al., 2023). Pectinase is an enzyme produced by Streptomyces strains that has applications in the fruits and vegetables processing industries (Govindaraji & Vuppu, 2020). Phenazine-1-carboxylic acid dioxygenase is another enzyme derived from Streptomyces, which plays a role in organic pollutants (Deng et al., 2023). Finally, Streptomyces is known to produce transglutaminase, an enzyme used in food processing to improve the texture and quality of meat products (Ceresino et al., 2018). The wide range of enzymes produced by Streptomyces makes it a valuable organism for industrial and biotechnological applications.

4. Streptomyces as a source of antibiotics

Since the discovery of the first antibiotic in 1942, the genus Streptomyces has gained widespread acknowledgment as an invaluable reservoir of antibiotics, with 80% of today's antibiotics originating from this group. The first antibiotic, actinomycin, was isolated from Streptomyces antibioticus, followed by streptothricin and streptomycin, obtained from S. lavendulae and S. griseus, respectively (Cuozzo et al., 2023; Waksman & Woodruff, 1940, 1942). Various antibiotics, including cephalosporins, chloramphenicol, tetracycline, nystatin, viomycin, lincomycin, vancomycin, rifamycin, kanamycin, and daptomycin, have been derived from different Streptomyces spp, as reviewed by (Cuozzo et al., 2023; de Lima Procópio et al.,

2012b; Quinn et al., 2020). Streptomyces is known to release antibiotics to hinder competitors in its telluric environment, which has led to the discovery of new compounds of commercial interest, with marketable yields (Zong et al., 2022). For Streptomyces strains, interactions with biotic or abiotic factors are essential to increase chemical diversity and compound production, as is naturally found in soil. However, recent studies have shown that antibiotic production is commonly observed when closely related strains grow together or share biosynthetic pathways for secondary metabolites, but not in defense against toxins from competitors. Moreover, nutrient limitations and competitor's presence can decrease antibiotic production output by Streptomyces (Westhoff et al., 2021). As has been reviewed by (Tyurin et al., 2018) , elicitors are also used to enhance antibiotic production by Streptomyces bacteria, and several abiotic or biotic compounds such as metals, rare earth elements, dimethyl sulfoxide, ethanol, nanoparticles, and enzymes have been identified as elicitors (Zong et al., 2022). In a recent study conducted by (Quinn et al., 2021) it was observed that Streptomyces strains isolated from soil possess the ability to combat multi-resistant Staphylococcus aureus and Pseudomonas aeruginosa infections. Genetic analysis of these strains revealed the synthesis of antibiotics resembling griseochelin, macrolactams, candicidin, and cypemycin (Cuozzo et al., 2023). Among the Streptomyces strains investigated, Streptomyces sp. 7NS3 was found to be associated with the freshwater snail Physa acuta (Cuozzo et al., 2023), produces an angucycline-like aromatic polyketide with a broad-spectrum antibiotic activity against Gram-positive bacteria (Safaei et al., 2020). The genomic analysis of the 7NS3 strain unveiled a gene cluster that may be responsible for emycin A biosynthesis. (Faddetta et al., 2022) reported the importance of extracellular vesicles produced by Streptomycetes. These vesicles were found to contain a diverse array of metabolites, including antibiotics that can be delivered (Cuozzo et al., 2023). Also, Streptomyces thermoviolaceus is capable of producing a pigmented and pH-sensitive antibiotic known as granaticin. This antibiotic is synthesized at high temperatures, specifically at 45 °C, with optimal synthesis occurring at temperatures as high as 55 °C (P. D. A. James & Edwards, 1988). More than 50% of clinically effective antibiotics are derived from Streptomyces (Jagannathan et al., 2021; Kieser et al., 2000). The exploitation of actinomycetes can be recommended to combat multidrug-resistant (MDR) pathogenic strains(Elsalam et al., 2022) and eradicate biofilms, which are the focus of much effort in the medical field to solve emergency public health problems; in particular MRSA infections associated with medical devices(Chow & Tham, 2020; Hui et al., 2021; Kemung et al., 2018; Pusparajah et al., 2021). Table 8 lists some of Streptomyces strains that have been shown to produce antibiotics.

Table 8: Selection of known antibiotic-producing Streptomyces strains

Streptomyces strains	Antibiotics	References
Streptomyces spp SM01	Picolinamycin	(Maiti et al., 2020)
Streptomyces avermitilis phiSASD1	Endolysin and Holin	(N. Lu et al., 2020)
Streptomyces spp PAL114	Mzabimycin A and B	(Tata et al., 2019)
Streptomyces spp HS-NF-780	Glutarimide	(X.-L. Zhao et al., 2019)
Streptomyces lusitanus OUCT16-27	Grincamycin L and angucycline	(L. Yang et al., 2020)
Streptomyces althioticus MSM3	Desertomycin G	(Braña et al., 2019)
Streptomyces globusus DK15	Factumycin and tetrangomycin	(Charousová et al., 2018)
Streptomyces ederensis ST13	Factumycin and tetrangomycin	(Charousová et al., 2018)
Streptomyces thermoviolaceus SRC3	Streptazolin	(Djinni et al., 2018)
Streptomyces puniceus AS13	Dinactin	(Hussain et al., 2018)
Streptomyces lydicus AZ-55	Natamycin	(Atta et al., 2015)
Streptomyces avermitilis	Avermictin	(Horii et al., 2023)
Streptomyces griseus IFO 13350	Streptomicin	(Ohnishi et al., 2008)
Streptomyces bingchenggensis CP002047	Milbemicin	(X.-J. Wang et al., 2010)
Streptomyces fradiae Tü 2717	Urdamycins	(Rohr et al., 1989)
Streptomyces lunaelactis MM109T	Lunaemycins	(Martinet et al., 2023)
Streptomyces cinnamonensis	Monensin	(Serter Kocoglu et al., 2023)
Streptomyces justiciae RA-WS2	Setomimycin	(Manhas et al., 2023)
Streptomyces cattleya NRRL 8057	Thienamycin, cephamycin C, penicillin N	(Barbe et al., 2011)
Streptomyces diastaticus TUA-NKU25	Surugamide	(Takeuchi et al., 2023)
Streptomyces spp P-56	Nonactin	(Boykova et al., 2023)
Streptomyces pluripotens MUM 16J	Glycopeptide antibiotic	(Pusparajah et al., 2023)

5. Streptomyces as sources of antioxidants and anticancer molecules

Microbial diversity offers a rich assortment of unique chemicals, serving as a valuable resource for cutting-edge biotechnology. Actinomycetes account for 42% of the more than 23,000 documented microbial secondary metabolites, while fungi produce a similar proportion (42%), and eubacteria contribute the remaining 16%(Chandra, Sharma, et al., 2020). The investigation of molecules derived from Streptomyces for their anticancer effects remains relatively understudied. Indeed, the majority of cytotoxic antibiotics in current use are sourced from Streptomyces species. Among the "classic" drugs originating from Streptomyces is the anthracyclines family, which includes well-known medications like doxorubicin and daunorubicin(Iihoshi et al., 2017; B. Pang et al., 2015). Although limited in number, studies on the potential anticancer activities of Streptomyces-derived EPS (Exopolysaccharides) have shown promising results. Ramirez-Rodriguez et al.(Ramirez-Rodriguez et al., 2018) conducted research on three Streptomyces strains (S. aburaviensis, S. gramineus, and S. psammoticus)(Cuozzo et al., 2023). These strains exhibited notable cytotoxic activity against specific cancer cell lines, including prostate cancer (PC3), breast cancer (MDA-MB-231), and lung cancer (A549)(Cuozzo et al., 2023). In another study, Streptomyces carpaticus strain, isolated from marine sediments, was exploited to produce a high-value exopolysaccharide with significant toxicity against human breast (51.7%) and colon (59.1%) tumor cells(M. S. Selim et al., 2018). Similarly, a marine Streptomyces hirsutus NRC2018-produced exopolysaccharide named EPSNC2, displayed notable and selective anticancer activity specifically against the Caco-2 cell line, without affecting other cell lines(Awady et al., 2019; Cuozzo et al., 2023). Besides this cell line (Caco-2), cancer cells models (HCT-116, HT-29, and SW480) were the subject of Streptomycetes antioxidant and anticancer activity confirmation(J. W.-F. Law, Chan, et al., 2019).

Streptomycetes are occasionally competent in the biosynthesis of compounds with anticancer activity, powered by new molecules such as streptocarbazoles A and B, streptomyceamide C and neoantimycins A and B(J. W.-F. Law, Law, et al., 2020). In a detailed study done by Ser et al.(Ser et al., 2016) , a Streptomyces strain, namely MUSC 136T, produces an extract containing cyclic peptides that have the potential to induce apoptotic cell death through the p53-associated pathway in colon cancer cells. Moreover, the researchers from the same group have discovered that in a mangrove environment, two Streptomyces strains, namely Streptomyces pluripotens MUSC 137T and Streptomyces sp. MUM 256, produced intriguing cyclic dipeptides. The presence of these cyclic dipeptides, particularly in Streptomyces pluripotens MUSC 137T, has demonstrated the regulation of genes associated with essential biological processes, including

cell cycle regulation, differentiation, apoptosis, cell adhesion, and angiogenesis(Ser et al., 2016). These compounds exhibit similarities to anthracyclines and can induce apoptosis by modifying histones and inhibiting topoisomerase I activity(Ser et al., 2017). Moreover, they have the potential to enhance the efficacy of DNA-targeted anticancer drugs. Some specific cyclic dipeptides, like pyrrolo[1,2a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)(Ser et al., 2017), have been extensively studied and demonstrated growth inhibitory effects on various cancer cell lines, including MCF-7, HT-29, and HeLa (Ser et al., 2016).

Streptomyces also exhibit efficient production of a diverse array of bioactive compounds, including antioxidants. Through a screening program focused on actinomycetes, researchers have isolated numerous compounds with antioxidant properties(Chandra, Sharma, et al., 2020; Kawahara et al., 2012). Among these compounds, Streptomyces spp. has been found to produce various antioxidant isoflavonoids, such as 4', 7, 8-trihydroxyisoflavone. Notably, these compounds have also shown promising antitumor activity(Komiyama et al., 1989). Extract derived from *Streptomyces spp* LK-3 (JF710608) has been found to possess antioxidant activity, containing key components like daidzein-8-C-glucoside (puerarin), (-) gallocatechin gallate, sesamol, cyanidin-3-O-rutinoside, and delphinidin(Karthik et al., 2013). Streptomyces, isolated from various environments, have been discovered to possess antioxidant activity. For instance, Streptomyces lydicus A2 (Lertcanawanichakul et al., 2015), Streptomyces spp. SRDP-H03 (Kn et al., 2013), and BI244 have demonstrated antioxidant activity as evaluated by the DPPH assay (Chandra, Sharma, et al., 2020; Kiruthika & Khaddaj, 2013). In a study conducted by Tan et al.(Tan et al., 2017), a strain called MUM212, isolated from mangrove soil, was found to possess remarkable antioxidant properties. The extract derived from MUM212 exhibited strong scavenging activity against various free radicals, such as superoxide anion, DPPH, and ABTS radicals. Furthermore, the extract demonstrated the ability to chelate metal ions. Another example is Streptomyces misionensis, which was isolated from soil in a mountain forest, this particular strain exhibited significant antioxidant capacity against nitric oxide, DPPH, and hydrogen peroxide free radicals(D.-R. Lee, Lee, et al., 2014, p. 10778). According to the study conducted by Tan et al.(Tan et al., 2015), Streptomyces spp. MUM256 extract was found to possess antioxidant activity. It demonstrated the ability to scavenge superoxide anion radicals, with its effectiveness being dependent on the dosage. Table 9 presents some of recent discovered Steptomyces strains with antioxidants capacities.

Table 9: recents discovered steptomyces strains with antioxidants and anticancer capacities

activity	Strain	Source	Secondary metabolites/Gene/Compounds	References
Antioxidant	*Streptomyces carpaticus K-11*	Semi-desert soil	flavonoids, alkaloids, glycosides, organic acids, alcohols, aldehydes, hydrocarbons, ethers	(Bataeva et al., 2023)
	Streptomyces tunisiensis W4MT573222	Sediments	Pigment (divaric acid)	(Ibrahim et al., 2023)
	Streptomyces flavogriseus ADEM7	Soil	sprA Gene	(El-Hadedy et al., 2023)
	Streptomyces telluris sp. AA8T	Rhizosphere soil	3,4-dihydroxybenzaldehyde	(Thayanuwadtanawong et al., 2023)
	Streptomyces sp. QZS11	Soil	Ethyl-acetate crude extract	(Saeed & Alethari, 2023)
	Streptomyces sp.MA4	Soil	Selenium nanoparticles	(Sumithra et al., 2023)
	Streptomyces sp. Sae4034	Rhizosphere soil	Alkaloid, terpenoid, flavonoid, and polyphenol	(Kurniawati et al., 2023)
	Streptomyces levis HFM-2	Human gut	2-Isopropyl-5-methyl-1-heptanol; 1-Octanol, 2-butyl-, 3-Octadecene and 3-Eicosene	(Verma et al., 2023)
	Streptomyces sp. MUSC 11, 125, 14, 273b, 292, 5	Mangrove soil	Phenolic compounds, PKS I,PKS II genes,2,4-dihydroxy-6-propylbenzoic acid, 2,4-bis(1,1-dimethylethyl), pyrazine, pyrrole, cyclic dipeptides, hydrocarbons, alcohols, triterpene	(Kemung et al., 2019; Kemung, Tan, Chan, Ser, Law, & Goh, 2020; Kemung, Tan, Chan, Ser, Law, Lee, et al., 2020; Mangzira Kemung et al., 2020; Tan et al., 2018; Tan, Mahendra, et al., 2019)
Anticancer	*Streptomyces sp. MUM265, 14, 256*	Mangrove soil	Hydrocarbons, alcohols, phenolics, cyclic dipeptides, thioholgamide A/thioholgamide B, pyrrole, pyrazine	(Ser et al., 2021; Tan, Chan, Chan, et al., 2019; Tan, Chan, Pusparajah, et al., 2019)
	Streptomyces colonosanans MUSC 93JT	Mangrove soil	Ectoine synthesis gene	(Ser et al., 2020)
	Streptomyces monashensis MUSC 1JT	Mangrove soil	phenolic compounds, pyrazines and pyrrolopyrazines, Benzoic acid, 9H-Pyrido[3,4-b]indole	(J. W.-F. Law, Ser, et al., 2019)

6. Importance of Streptomyces in agriculture

6.1. Streptomyces in agricultural biocontrol

Actinomycetes produce a number of bioactive metabolites that are beneficial to both soil and plant. They also have the capacity to act as biocontrol agents(Arora & Bouizgarne, 2022), making plants more resistant to biotic and abiotic stresses(Arora & Bouizgarne, 2022; Nagendran et al., 2021). A study done by Wang et al.(L. Wang et al., 2020) showed that certain actinomycetes (Streptomyces pactum Act12, S. globisporus Act7, and S. globisporus subsp. globisporus C28) can destroy the membrane of fungal pathogens attacking celery leaves. Streptomyces griseviridis, a light-colored actinomycete isolated from Sphagnum peat, is an example of a biocontrol agent that reduces damage caused by various soil and seed pathogens (Javed et al., 2021a; Tahvonen, 1982). Antibiotic production by actinobacteria in soil, as shown in a study done by Trejo-Estrada et al.(Trejo-Estrada et al., 1998), is also helpful against plant pathogens. For instance, Streptomyces violaceusniger YCED9 produces antibiotics such as headache, Geldanamycin, and Guanidylfingine to counteract plant pathogens. Previous studies have demonstrated actinomycetes ability of actinomycetes to enhance plant growth and effectively manage plant diseases(Javed et al., 2021a; Nagendran et al., 2021; F. Pang et al., 2022; Wang et al., 2021). Streptomyces strains are primarily recognized for its capacity to act as a biocontrol agent by producing potent volatile compounds, metabolites, and antibiotics that exhibit antipathogenic properties(Chaiharn et al., 2020). The illustration below (Figure 5) conceptually summarizes the virtues of *Streptomyces spp* consortium on good *Vicia Faba L* plant growth.

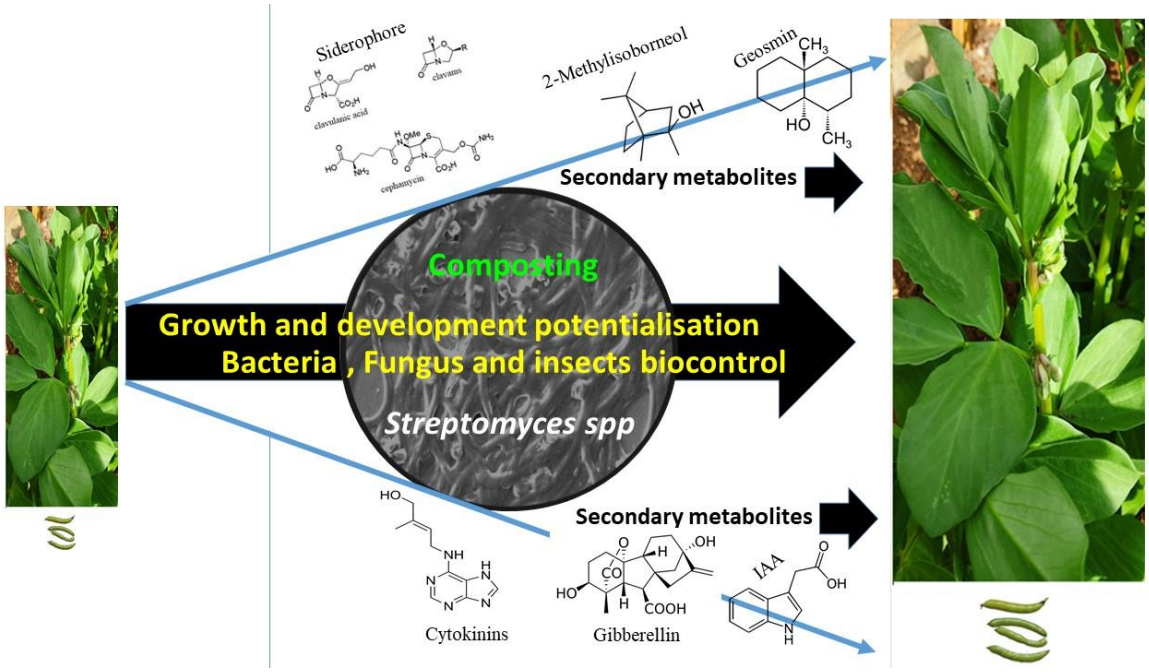

Figure 5: Different roles of secondary metabolites from *Streptomyces spp*. in plant growth stimulation

In another study done by(F. Pang et al., 2022), substances discharged from *Streptomyces coelicolor*, *Streptomyces violaceusniger*, *and Streptomyces violaceusniger* strains, including siderophore, chitinase, antifungal nigericin, and antibiotic geldanamycin (Al-Tammar & Khalifa, 2023), regulate stress metabolite and control hyphal development. While Streptomyces has been used successfully for biocontrol, one notable case is with rice plants (Myo et al., 2019). In this study, two Streptomyces strains exhibited biocontrol potential by preventing pathogenic bacteria proliferation through secondary active metabolites production. As a result, the dissemination of Burkholderia glumae, a significant bacterial menace that triggers panicle blight in rice plants and poses a serious risk to rice yields, was successfully halted (Myo et al., 2019).

The fishing and aquaculture industries can benefit from actinomycetes virtues, and Streptomyces spp. in particular, and their metabolites can be used to protect marine foodstuffs against Vibrio-contamination (Letchumanan et al., 2019; Loo et al., 2023; Tan et al., 2016, 2020; Tan, Lee, et al., 2019; Venggadasamy et al., 2021). Morocco, with its highly developed fishing and aquaculture industry, must take this thematic into consideration, as it opens up many routes of research to address the problems associated with serious foodborne infections, especially foodborne illness outbreaks.

7. Role of Streptomyces in pesticides degradation

Employing indigenous Streptomycetes for bioremediation in pesticide-contaminated environments proves to be a promising strategy, as these microorganisms are highly suited to thrive in soil and sediment habitats. Streptomyces strains have the advantage of potential metabolic diversity, mycelial growth habit, rapid growth rates, semi-selective substrates colonization, and genetic manipulability. Streptomyces can differentiate into spores that contribute to their spread and persistence, allowing their survival in soil for long periods, withstanding low nutrient concentrations and water availability (Briceño et al., 2018). Due to these advantages, different Streptomyces strains have been investigated as potential candidates for polluted environments bioremediation with various chemical pesticides families, including organochlorines, organophosphates, pyrethroids, ureas, and chloroacetanilides (Alvarez et al., 2017; Amoroso et al., 2013; Briceño et al., 2018). A study was conducted by (Bourguignon et al., 2014) have shown that Streptomyces strains isolated from sediments contaminated with pesticides are capable of thriving in the presence of methoxychlor. In another field , inoculation of biomixtures with *Streptomyces sp*. M7 increased atrazine degradation (Saez et al., 2022; Y. Zhao et al., 2023). *Streptomyces spp.* strains demonstrated enhanced propoxur degradation when starch was introduced as a co-substrate, which favored their growth in liquid medium (Rahmansyah et al., 2013). Streptomyces microorganisms have demonstrated excellent capabilities for removal or conversion of multiple pollutants simultaneously, with (Polti et al., 2014) finding, both pure and mixed cultures of Streptomyces strains displayed efficient removal of lindane and Cr(VI) from soils contaminated with both substances. Pesticides degradation by Streptomyces has been extensively explored in biotechnological processes development aimed at reducing pesticide concentrations in different environmental matrices and preventing their infiltration into the environment, ultimately reducing human exposure.

7.1. Streptomyces as biofertilizer

Streptomyces, is gaining increasing attention as commercial biofertilizers among researchers. They possess the ability to biodegrade various agro wastes and produce different enzymes in soil by contributing to plant nutrition and growth (Olanrewaju & Babalola, 2019). Moreover, actinomycetes have been found to produce plant growth hormones like indole acetic acid (IAA) (Shrivastava et al., 2021), expanding their potential applications as biofertilizers in agriculture (Anwar et al., 2016). Also, a study done by (Omar et al., 2022) have shown that *Streptomyces spp* HM8 , *Streptomyces thinghirensis* HM3, *Streptomyces spp* HM3, and *Streptomyces tricolor* HM10 produce IAA, siderophore and immobilized inorganic phosphate (Omar et al., 2022). Inoculating fields with actinomycetes has been shown to

improve plant growth and yield (Maheshwari et al., 2017), as observed in greenhouse experiments with maize (Dicko et al., 2018). However, the limited plant development and growth qualities of some actinobacteria species hinder their potential contribution to sustainable horticulture practices. Streptomyces offers a valuable benefit through its capacity to enhance phosphate availability in soil (Chouyia et al., 2022). These bacteria can transform bound phosphate into an accessible form by producing various phosphate-solubilizing acids and phytase enzymes. While the exact mechanism of acid-mediated phosphate solubilization is not fully understood (Janati et al., 2022), certain actinobacteria species like Streptomyces, Micromonospora, and Gordonia have been shown to exhibit phosphate-solubilizing activities (Sathya et al., 2017).

According to recent research (Htwe et al., 2019), biofertilizers made with strains of *Bradyrhizobium* and *Streptomyces griseoflavus* have been found to promote root and shoot growth in mung beans, soybeans, and cowpeas. This study also found that these biofertilizers increase nodulation, nitrogen fixation, phosphorus, and potassium uptake in plants, resulting in higher seed yields. Streptomyces, which can establish colonies in the rhizosphere or plant tissue, has been observed to maintain a symbiotic relationship with plant that enhances their growth (Chaiharn et al., 2020). A recent study done by Domínguez-González and his collaborators (Domínguez-González et al., 2022) suggests the use of Streptomyces spp as a biofertilizer in a biofilm form that uses perlite mineral as a carrier. This approach is recommended as an alternative to enhance crop production over an extended period and to improve plant health owing to its advantages. Notably, research done by (Omar et al., 2022) demonstrated the efficacy of Streptomyces as a biofertilizer in promoting cucumber growth and nutrient uptake under greenhouse conditions. Additionally, (Gopalakrishnan et al., 2015) found that the usage of Streptomyces strains as biofertilizers could improve chickpea crop's yield and growth.

Understanding Streptomyces's potential in agriculture is crucial for sustainable farming practices. The latest strains of Streptomyces, Table 10 highlights that the latest strains of Streptomyces from research conducted in 2022 and 2023 have shown promising results in disease control, increased plant growth, and yield enhancement.

Table 10: Summary of the latest Streptomyces strains and their agricultural significance

Strains	Crops/ plants	Results	References
Streptomyces chrestomyceticus STR-2	Rice	-Siderophore synthesis, IAA, and phosphate-solubilizing agents, -Plant growth promotion -disease reduction caused by Magnaporthe oryzae Cav	(Rahila et al., 2023)
Streptomyces spp. STRM103 and STRM104, and STRM304	Tomato Banana	-Controlling Fusarium Wilt Disease -Growth Promotion	(Kawicha et al., 2023)
Streptomyces griseus KAI-26 and MMA-32 *Streptomyces albus KAI-27* *Streptomyces albus (CAI-24 and KAI-27*	Chickepea	-Growth promotion - PGP traits enhancement -Antioxidants and grain nutrients increment	(Sambangi & Gopalakrishnan, 2023)
Streptomyces griseus MMA-32	Pearl millet	-Improving yield and nutrient content	(Srinivas et al., 2022)
Streptomyces tuirus AR26	Pepper fruit	-Development of fruit rot symptoms inhibition	(Renuka et al., 2023)
Streptomyces rochei ASH	Sorghum	-Promote plant growth -Inhibition mycelial Rhizoctonia solani and Sclerotinia sclerotioru growth	(Sudha et al., 2022)
Streptomyces sp. UTMC 313	Helianthus annuus	-Growth improvement	(Zahra et al., 2022)
Streptomyces sp. HN6	Cowpea	-Plant growth promotion	(T. Wang et al., 2022)
Streptomyces sp HM8 , Streptomyces thinghirensis HM3 , Streptomyces sp HM2, and Streptomyces tricolor HM10	Cucumber	-Production of IAA, siderophore, and immobilized inorganic phosphate -Growth improvement	(Omar et al., 2022)
Streptomyces chilikensis strain RC1830	Rice	-Preventing the F. oxysporum wilting/root rot disease -Promoting growth -Developpement of volatile organic compounds	(Behera et al., 2022)
Streptomyces sp TOR3209	Tobacco	-Growth improvement	(He et al., 2022)
Streptomyces sp. SA5	Tomato plants	-Plant growth-promoting agents production, siderophore, IAA , and ACC deaminase, phosphatase enzymes	(Alghamdi, 2022)
Streptomyces luteogriseus -8	Soybean	Yield improvement	(El-Soud et al., 2022)
Streptomyces sp SS12	Bell pepper	-Increase fruit yield	(Abbasi et al., 2022)

Streptomyces sp KS3	Rice	-Control plant disease Growth and productivity improvement -Indole-3-acetic acid (IAA) production	(Ntemafack et al., 2022)
Streptomycetes kanamyceticus CIAD-CA45	Phaseolus vulgaris	-Increase hypocotyl diameter and chlorophyll content -Increase biomass accumulation -Indole-3-acetic acid (IAA)	(Pérez-Corral et al., 2022)
Streptomycetes misionensis CIAD-CA27	Arabidopsis thaliana	-Growth improvement, -Increase biomass accumulation, ,shoot fresh weights and the root fresh weights	(Pérez-Corral et al., 2022)
Streptomyces sp UTMC 1478	Zea mays	-Plant phosphorus uptake increment increased shoot dry weight and leaf chlorophyll content	(Ghorbani Nasrabadi et al., 2022)
Streptomyces tunisiensis AI, Streptomyces enissocaesilis BYC, Streptomyces saprophyticus DE2 and Streptomyces cyaneofuscatus CYM	Sugar beet	-Growth and yield parameters improvement -High protection against root rot disease	(Aallam et al., 2021)

8. Streptomyces for boosting composting process

8.1. Inoculation techniques

Microbial inoculation methods in the composting process involve competent microorganisms addition to the compost mixture to enhance waste degradation rate and improve final quality compost. These microorganisms can either be isolated from microbial communities according to specific selection pressure or developed through culture mixtures such as soil, manure, and straw (Rastogi et al., 2020). The inoculum can be a single strain or a mixture of efficient microorganisms inoculum (Rastogi et al., 2020; Y. Zhao et al., 2016), or matured compost sample (Karnchanawong & Nissaikla, 2014; Rastogi et al., 2020). Currently, researchers are exploring the use of a combination of microorganisms that work together synergistically, known as a mixed inoculant (Jurado et al., 2014; Xi et al., 2015; J. Xu et al., 2019). The addition of microbial inoculants improves temperature profile and ammonia emissions by proliferating mesophilic and thermophilic bacterial populations. It also enhances enzymatic activity and minimizes the initial lag time of biological processes, leading to accelerated composting. Microbial inoculation techniques can efficiently decrease the release of odorous emissions, primarily volatile organic compounds (VOCs), while producing compost with elevated nutritional content (Jusoh et al., 2013; Rybarczyk, 2022). Inoculating competent microbes such as Streptomyces into compost can significantly improve the resulting compost quality; one effective way is through liquid culture inoculation. This method involves preculture elaboration, evenly distributing it onto the compost pile, and regularly turning it to ensure microorganisms mixture (Figure 6). Microbial inoculum can be introduced during various stages of the composting process, including single, two-stage, or multi-stage applications. The addition of inoculum at different stages exhibits a notable influence on the physicochemical parameters of the composting process (Zainudin et al., 2022).



Figure 6: Streptomyces inoculation method

a): Soil; b): Competent Streptomyces strains isolation ; c): Streptomyces biomass growth ; d): Single or multi stage garbage inoculation; e): Composting phases and f): Final product (good quality compost)

8.2. Streptomyces impact on composting process

Streptomyces are naturally occurring soil-dwelling bacteria. They are well known for their capacity to develop an extensive diversity of enzymes, which makes them an excellent choice for use as additives for solid waste composting (Rastogi et al., 2020). These bacteria can accelerate organic matter breakdown and promote a more effective composting process. One of the key advantages of using Streptomyces strains, in composting, is their ability to tolerate high temperatures and other drastic conditions. This means they can thrive in the high temperatures that are often present in composting environments, which is essential for rapid organic matter break. As they break down complex organic molecules, they release important nutrients into soil, improving its quality and promoting other beneficial microorganism's growth (Arora & Bouizgarne, 2022). Streptomyces are also effective in breaking down a wide range of organic materials, including plant matter, animal waste, and food residues (Sudharsan Varma & Kalamdhad, 2014). This makes them an excellent choice for use in solid waste composting, as they can reduce waste volume and promote more sustainable waste management practices. In addition to their composting benefits, Streptomyces can also help to reduce odors and prevent harmful pathogens' growth in composting environment (L. Chen et al., 2022). This can help to create a protected and more pleasant work setting for those involved in composting process. Overall, the use of Streptomyces as additives for solid waste composting can provide a number of benefits, including more efficient and effective composting, leading to improvement of soil quality, and a more sustainable waste management solution (Iutynska et al., 2017).

While multiple research projects have investigated the use of various microorganisms as additives in composting problems. The research done on Streptomyces bacteria in this application is relatively limited. Numerous studies have examined the impact of microbial inoculation on composting quality and efficiency. In general, a time reduction in the degradation process due to microbial activity is an indicator of good quality compost (Abdullah et al., 2013; Rastogi et al., 2020; Y. Zhao et al., 2016). For example, (Wei et al., 2019) studied lignocellulose degradation and found that inoculation with actinomycetes accelerated enzyme production, such as CMCase, xylanase, and lignin peroxidase, leading to increased organic matter degradation rates. (Y. Zhao et al., 2017) inoculated a cellulolytic strain of actinomycetes into dairy waste composting, resulting in cellulase activities and humic substances content improvement (Rastogi et al., 2020). (Manu et al., 2019) conducted a decentralized composting of household wet biodegradable waste. The introduction of specific inoculation in the composting process led to a significant reduction in composting time, resulting in a composting period of 30-36 days. Furthermore, the compost produced was free from pathogens. As indicated by study done by (Jusoh et al., 2013), adding microbial agents such as Streptomyces clavuligerus, Aeromonas cavia, Corynebacterium pseudotuberculosi, Shinella sp and Rhizobium, which were identified from rice straw compost, to the composting pile, resulting in an acceleration of organic matter and insoluble roughage degradation. This was due to an enhancement of key enzymes, such as CMCase and xylanase, as well as core microbial metabolisms. Numerous research studies have been conducted to investigate the impact of introducing various strains of Streptomyces bacteria into compost. For example, Shivlata and Satyanarayana(Shivlata & Satyanarayana, 2015) observed that inoculating compost with Streptomyces spp and Micromonospora spp resulted in complete degradation of yeast debris and reduced compost odor. In addition, (Mansour & Mohamedin, 2001) and (Abdulla & El-Shatoury, 2007) found that cellulolytic actinomycetes such as Streptomyces thermodiastaticus produce extracellular enzymes that can improve nutritional characteristics of compost by breaking down pathogenic fungi cells. A study conducted by (C. P. Chi et al., 2020) found that inoculating cellulolytic Streptomyces griseorubens in pig manure and rice straw compost increased temperature, extended thermophilic phase, and improved nutrient content. Additionally, the use of multiple thermotolerant cellulolytic Streptomyces spp and Actinobacteria as inocula in different composting stages enriched cellulase activities, so enhanced cellulose degradation, and increased humic substances content, thus influencing actinobacteria community structure in dairy manure-corn straw composting (Zainudin et al., 2022; Y. Zhao et al., 2017).

The potential of Streptomyces spp in pollution degradation in effluent from paper pulp has been proven through the detection of breakdown products observed after incubation period (Limaye et al., 2017). Furthermore, a recent study done by (Kocak et al., 2023) found that The introduction of Streptomyces spp. to the compost system resulted in significant changes, including the inhibition of other microbiota members. Additionally, the presence of the added strain led to noticeable alterations in organic matter degradation .In addition, (Jia et al., 2021) noted that the inclusion of a blend of Aspergillus, Penicillium, Bacillus, and Streptomyces improved cellulose degradation in mushroom residue and wood chips by enhancing the enzymatic activity of the bacterial community. These studies demonstrate the potential of Streptomyces spp. and other microorganisms to enhance organic matter degradation in different composting systems. Overall, results indicate that Streptomyces can be used as a potential bio-fertilizer that may replace conventional fertilizer in various situations (T. Wang et al., 2022). Further research is required to develop a formulation that contains these viable strains in combination with other beneficial microorganisms (co-culture) (J. Li et al., 2022) for commercial application.

8.3. Methods of Streptomyces inoculation for soil and crop enhancement

The use of beneficial microorganisms for enhancing soil and crop productivity (Arora & Bouizgarne, 2022) has gained significant attention in recent years as an alternative to conventional chemical fertilizers. Among these beneficial microorganisms, Streptomyces species have been found to play a key role in soil health and plant growth promotion. However, there is limited research on the application of Streptomyces-enriched compost on soil and crops. Currently, no studies have examined Streptomyces-enriched compost application to soil/crop. However, a study on the use of effective microorganisms-enriched manure exists, which could also be applied to compost. (Hidalgo et al., 2022) suggests various methods for effective microorganisms-enriched manure application, depending on manure type. One method is direct soil inoculation with different effective microorganisms (EM) preparations before sowing or during cultivation. Another method is fertigation, where EM formulations are added to soil through manure irrigation, with dilutions ranging from 1:1000 to 1:5000 (Khaliq et al., 2006a). Spraying EM-enriched liquid manure on plant leaves is also an option for pest control and prophylactic disease treatment. The recommended dilution for spraying is 1:1000, but 1:5000 or 1:2000 dilutions can be used depending on culture type. The required EM dose can vary based on the amount of manure to be turned into humus, ranging from 20 to 40 liters per hectare for diluted preparations and 1-3 liters per hectare for more concentrated commercial

preparations (Olle & Williams, 2013). It is advisable to increase the added EM dose if soil contains high levels of undecomposed organic matter and to dose EM in spring season whenever possible, with increased doses if no autumn treatment has been carried out, to ensure soil proper inoculation with EM. The methods proposed by (Hidalgo et al., 2022) for enriching manure with effective microorganisms may face challenges in terms of reproducibility. The lack of standardized protocols for various application methods, as well as the absence of detailed information on the specific EM formulations used and environmental factors that may affect the outcomes could make it difficult for other researchers to replicate the methods accurately and achieve consistent results. It is also important to consider the potential environmental impact of using manure-based products and to follow appropriate management practices to mitigate risks. While these methods may be effective in certain settings, further research is necessary to determine their reproducibility and reliability across different conditions. Alternative approaches such as composting and crop rotation can be used to promote soil health and reduce synthetic fertilizers reliance. Overall, a combination of different soil management techniques, including the use of EM-enriched compost, can help to promote sustainable and resilient agriculture. By carefully considering the potential benefits and limitations of different approaches, farmers and growers can make informed decisions to optimize soil health and productivity while minimizing environmental impact.

8.4. Challenges and recommendations for Streptomyces inoculation in composting

Green Morocco Plan (GMP 2008-2020) is a national strategy launched by the Moroccan government to modernize the agricultural sector and improve its sustainability. One of the key components of this plan is organic agriculture promotion and the use of compost as a natural soil amendment (Echarrafi et al., 2018). To support this initiative, Moroccan government has enacted regulations that promote the use of compost in agriculture and encourage farmers to adopt sustainable practices. Morocco passed the Organic Agriculture regulation, which regulates organic production and marketing to promote organic inputs use such as compost. The government also launched the National Compost Strategy, which aims to develop a network of composting facilities across the country and increase compost use in agriculture (Mahdavi & Vera, 2023). The strategy includes measures such as providing technical assistance and financial motivation to farmers who adopt compost-based practices, promoting research and development in composting technologies, and improving the quality and consistency of compost through standardization and certification. However, the government's commitment to promoting sustainable agriculture and reducing dependence on synthetic inputs remains strong.

Development of a robust composting industry is seen as a key component for achieving these goals. Despite these efforts, implementation of these regulations has faced several challenges, including the lack of infrastructure for compost production and distribution, limited awareness of farmers to compost, and the high cost of organic inputs. However, to overcome these challenges, the Moroccan government is working to establish a network of composting facilities and promote the application of compost as a cost-effective and environmentally friendly alternative to synthetic fertilizers by adopting compost-based agriculture practices (Epule et al., 2022). The kingdom of Morocco aims to improve soil health, reduce greenhouse gas emissions, and promote sustainable and inclusive economic growth (Perkumienė et al., 2023). In the context of the Moroccan government's efforts to promote sustainable agriculture with compost, our study focuses on improving the composting process by employing microorganisms such as Streptomyces. The use of Streptomyces bacteria in composting is an innovative approach with potential benefits for treating waste. However, several challenges need to be addressed to develop a cost-effective and environmentally sustainable technology (Adusei-Gyamfi et al., 2022). One key challenge is determining the appropriate technique and concentration of Streptomyces inoculation for optimal composting. Research is necessary to elucidate Streptomyces bacteria mechanisms during composting to identify the most suitable inoculants, taking into account their functional, physiological, adaptability, and stability. Moreover, there is a lack of large-scale studies on the use of Streptomyces bacteria in composting, and further investigations are needed to confirm the benefits observed in small-scale studies. Furthermore, developing economically viable technological procedures for Streptomyces inoculant production is crucial, including the utilization of inexpensive resources such as plant-based substrates or agro-waste for inoculant propagation. Finally, research in modelling and optimizing the composting process by engineering processing techniques is necessary to ensure composting sustainability process without compromising the quality of the final product(Policastro & Cesaro, 2023). By developing more efficient and effective composting practices, we hope to contribute to the broader goal of promoting sustainable agriculture and reducing environmental impact in Morocco and beyond.

9. Conclusion

In conclusion, this review highlights the significant contributions of Streptomyces to human well-being and their versatile potential in various sectors. Streptomycetes play a major role in agriculture by replacing chemical fertilizer, enhancing soil health through bioremediation, and acting as biological control agents for pests. Moreover, their role in the production of

antibiotics, antioxidant and anticancer agents in the medical and therapeutic sectors opens up new possibilities for high-value molecule creation. Additionally, actinomycetes contribute to biotechnology by producing valuable enzymes for various industries, leading to considerable profit margins. Finally, we can highlight new trends in nanomedicine, where streptomycetes can contribute to solving the problems of non-solubility of certain active ingredients. This is possible via the contribution of these micro-organisms in the production of active principle shuttle molecules that fail to reach targeted cites by therapeutic activities through the design of nanoparticle-based drug delivery systems (NDDS) (Ong & Tan, 2020). Overall, the numerous virtues of these microorganisms warrant further research and development to uncover their untapped potential, making them truly magical microorganisms for the betterment of our planet and human life.

Chapitre II

Isolation of competent actinomycetes strains from soil planted with three species of mint irrigated with olive mill wastewater

Isolation de souches compétentes d'actinomycètes à partir d'un sol planté de trois espèces de menthe et irriguées avec les margines

Synthèse

Ce chapitre de la thèse se concentre sur l'impact des margines (OMWW) sur la microbiote du sol, en particulier sur les actinomycètes. Pour étudier cet impact, une analyse bioclimatique de la région d'étude a été réalisée afin de comprendre l'influence du climat sur les micro-organismes du sol et leur interaction avec les plantes.

L'analyse bioclimatique a permis de déterminer des paramètres tels que les précipitations, les températures minimales et maximales en utilisant le diagramme ombrothermique d'Emberger (Debrach, 1953; Bagnouls et Gaussen, 1957). Les résultats ont démontré que les conditions climatiques semi-arides ont un impact significatif sur la croissance et le rendement de la menthe. Par conséquent, il est essentiel de prendre en compte ces facteurs climatiques lors de la plantation et de l'amélioration de cette culture dans les régions semi-arides.

De plus, le climat semi-aride peut grandement affecter la communauté microbienne du sol à Fès. Les micro-organismes du sol jouent un rôle crucial dans de nombreux processus écosystémiques, tels que le cycle des nutriments et la formation du sol. Toutefois, ces micro-organismes doivent faire face à des conditions difficiles, telles que des températures élevées, des précipitations faibles et erratiques, ainsi que des sols pauvres en nutriments. Heureusement, les actinomycètes, un groupe de bactéries, sont capables de produire des spores résistantes à des conditions environnementales extrêmes, telles que les températures élevées et les sécheresses.

Dans le cadre de cette étude, une caractérisation physico-chimique des paramètres du sol et des OMWW a été réalisée, comprenant des éléments tels que l'acidité, les solides en suspension, la matière sèche, la matière volatile, la demande chimique en oxygène (DCO), les composés phénoliques, les sucres totaux, les matières grasses, la conductivité, l'azote total, les ions ammonium, les orthophosphates, les chlorures, le calcium, le magnésium, le sodium et le potassium. De plus, une étude microbiologique des OMWW a révélé la présence de différentes flores microbiennes, notamment la flore aérobie mésophile totale, les levures et les champignons.

Par la suite, l'interaction entre différentes doses d'OMWW et trois variétés de menthe (*Mentha aquatica, Mentha piperita* et *Mentha pulegium*) a été étudiée sur une période de 120 jours. Les résultats ont montré qu'une dose de 5 L/m² d'OMWW favorise généralement la microflore du

sol, mais cet effet diminue au-delà de cette dose, à l'exception des actinomycètes qui restent résistants même à des doses de 10 L/m². De plus, les actinomycètes isolés dans les parcelles irriguées avec 5 L/m² et 10 L/m² d'OMWW, cultivée avec Mentha piperita et Mentha pulegium, ont présenté un profil enzymatique caractéristique des souches compétentes.

En conclusion, cette étude met en évidence le potentiel des actinomycètes en tant que micro-organismes capables de s'adapter aux conditions difficiles des sols irrigués avec des OMWW. Les résultats obtenus fournissent des informations importantes sur l'impact des OMWW sur la microbiote du sol, en particulier sur les actinomycètes, et ouvrent des perspectives intéressantes pour l'utilisation durable de ces effluents dans l'agriculture et la dépollution des sols.

De plus, l'étude bioclimatique a révélé que les conditions climatiques semi-arides ont un impact significatif sur la croissance et le rendement des plantes. Il est donc crucial de prendre en compte ces facteurs lors de la plantation et de l'amélioration de cette culture dans les régions semi-arides. Les actinomycètes, en raison de leur capacité à résister à des conditions environnementales extrêmes, peuvent jouer un rôle clé dans la communauté microbienne du sol, qui est également influencée par le climat semi-aride.

Ainsi, des recherches supplémentaires sont nécessaires pour optimiser la croissance de différentes variétés de menthe dans les régions semi-arides. Une gestion efficace de l'eau et des nutriments est essentielle pour atténuer les effets du climat semi-aride sur la plante et améliorer les propriétés physico-chimiques et microbiologiques du sol. En comprenant mieux les interactions entre les OMWW, la microbiote du sol et les plantes, il est possible de développer des stratégies de gestion plus efficaces pour l'utilisation des OMWW dans l'agriculture et la protection de l'environnement.

En résumé, cette étude met en lumière l'importance des actinomycètes dans les sols irrigués avec des OMWW et souligne l'impact du climat semi-aride sur la croissance des plantes et la communauté microbienne du sol. Ces résultats ouvrent la voie à de nouvelles recherches et à des approches de gestion durable pour promouvoir l'utilisation des OMWW en agriculture tout en préservant l'environnement.

Isolation of competent actinomycetes strains from soil planted with three species of mint irrigated with olive mill wastewater

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Abstract

Olives crushing produces huge quantities of olive mill wastewaters (OMWW); these effluents are rich in organic matter and mineral salts. On the other hand, OMWW have a high polluting level, resulting in high levels of chemical oxygen demand (COD) and salinity. However, there are microorganisms that interfere with this process and that could provide real and practical solutions to the various problems caused. Actinomycetes are the most abundant microorganisms that are of such particular interest, as they are known for their ability to adapt to different conditions by producing a large number of high added value metabolites. In the present work, a physico-chemical characterization of soil parameters was carried out, thus OMWW study concerned physico-chemical and mineralogical parameters to be noted: acidity (pH), suspended solids (SS), dry matter (DM), volatile matter (VM), chemical oxygen demand (COD), phenolic compounds, total sugars, fat, conductivity, total nitrogen, ammonium ions, orthophosphates, chlorides, calcium, magnesium, sodium and potassium. OMWW microbiological study showed the presence of total aerobic mesophilic flora (FMAT), yeasts and fungi whose values are respectively $7.3.10^3$, $3.8.10^2$ and $5.6.10^3$ CFU.ml^{-1}. Interaction study between different doses of OMWW and three varieties of mint (*Mentha aquatica, Mentha piperita and Mentha pulegium)* for 120 day revealed that OMWW spreading at dose of 5L/m² favors soil microflora generally, but this increase tends to fade after exceeding this dose except in the case of actinomycetes which are resistant to 10 L/m² doses. Enzymatic profile of isolated actinomycetes indicated that most competent ones are present in plots irrigated with 5L/m² and 10L/m² of OMWW, and cultivated with *Mentha piperta* and *Mentha pulegium*.

Keywords: OMWW, soil, microbiology, actinomycetes, Mentha, PCA.

1. Introduction

During olive oil extraction process, large quantities of liquid effluents called olive mill wastewaters (OMWW) are generated. They are characterized by low pH, high organic load including polysaccharides and phenolic compounds (Foti et al., 2021). Specifically, OMWWphenolic components have been reported to be responsible for phytotoxic effects and antimicrobial activity(B. Mechri et al., 2010) leading to seed germination and early plant growth inhibition (Ntougias et al., 2013), modification of soil characteristics of (Kavvadias et al., 2021) and affect soil microbial diversity (Ntougias et al., 2013).For these reasons, more and more attention has been given to finding the best spreading application methods for OMWW on agricultural land According to studies reported by (Yaakoubi et Aghenchich, 2021), a moderate supply ranging from 5 to 20 L/m² of OMWW does not seem to present any pollution risk, on the other hand, it results an improvement in soil microflora which confirms that the use of this effluent as an organic soil amendment is therefore a promising alternative for improving soil fertility (Galliou et al., 2018) and increasing soil microbial biodiversity. However, biodegradation of this liquid waste in the nature is difficult because it contains a strong antimicrobial effect exerted, by various phenolic compounds (Rinaldi et al., 2003; Sayadi et al., 2000; Yesilada et al., 1999), also the addition of such compounds may causesignificant shifts in microbial community structure and function, which in turn may influence soil viability for durable agriculture (Mekki et al., 2006). OMWW acts as an antimicrobial agent and contains compounds that are toxic, all of which result in an altered state of soil microbial diversity (Rusan et al., 2016; Mekki et al., 2013).However, some micro-organisms interfere with this process, such as actinomycetes.

Actinomycetes are very widespread microorganisms in various ecosystems. There are more than 100 genera which are considered soil saprophytic inhabitants. They possess the ability to break down organic matter, such as lignocelluloses and different types of complex carbohydrates, such as starch and chitin (Shanthi, 2021). Recently, actinomycetes have aroused much interest among microbiologists due to their various properties which make them a good source for biomolecules development. Some actinomycetes are also highly valued in various industrial applications. They are being evaluated for their use as probiotics in aquaculture, in compounds production used in plastics development, detergents and other high added value products.

Our previous work (El Hassani et al., 2022) and that carried out in our region (El Hassani et al., 2020, 2022) have raised several research questions on the fundamental and applied interest to properly valorise OMWW in Morocco. The questions raised concerned microbiology (El Hassani et al., 2008) and metabolic change as assessed by the change in metabolic pathways of

essential oil synthesis. Thus, El Hassani and his collaborators (2022) have shown that the amendment of OMWW does not modify the weight yield of the biomass and the chlorophyll contents (a and b). In contrast, these researches have reported the change in the metabolic pathways of synthesis of essential oils of the species *Mentha aquatica* (var citrata) used as a plant model for study. The authors reported that the application of OMWW affects the essential oil composition with appearance of newly synthesized compounds such as menthone and menthol and disappearance of other compounds such as linalool acetate (El Hassani et al., 2022).

Works carried out in our region (arid climate) (El Hassani et al., 2020) have cited the change in the microbiology of soils irrigated by OMWW, but no study has dealt with the potential interaction between the plants that do the phytoremediation and the microorganisms that arise after treatment of the soil with OMWW. In this light, we were interested in an experimental investigation to study the interaction between soil microorganisms and plants.

The choice of model plants was based on the availability of a well-consumed plant resource in Morocco (El Hassani, 2020) but the three plants selected are *mentha aquatica, mentha piperita* and *mentha peligium.*

Streptomyces, a group well identified among actinomycetes, show important potentialities (El Karkouri et al., 2010; K. Yadav et al., 2021) in biotechnology and bioindustry, which has focused our attention on these microorganisms.

This study target the determination of physicochemical, mineralogical and microbiological characterization of soil and OMWW used for its spreading at different doses (0 L/m², 5 L/m²and 10L/m²). The tested soil was planted with different species of mint (*mentha aquatica, mentha piperita* and *mentha peligium*). The purpose of this study is to investigate the impact of OMWW on various soil microbiota and especially Actinomycetes. Therefore, the main aims of this work is to isolate competent Actinomycetes strains from soils treated with OMWW with a high metabolic capacity.

2. Methods

Before planting the three varieties of mint (*Mentha aquatica, Mentha piperita* and *Mentha pulegium*), we carried out soil analysis before and after OMWW spreading. Soil samples contain enough core samples taken at random from all over the experimental plots, irrigated or not with OMWW.

Two pH measurements are taken on each plot. The pH with distilled water (H_2O) and pH with KCl. Measurement was carried out using a pH meter in an aqueous extract obtained according to standard (NF ISO10390). Total $CaCO_3$ (%) determination was carried out by the volumetric method using Bernard's calcimeter, by breaking down the calcium carbonates with hydrochloric acid, and measuring CO^2 released volume. The active $CaCO_3$ (%) is determined by the Drouineau-Galet method (Mehalaine & Chenchouni, 2020). Mineral nitrogen determination was determined by colorimetric determination according to the method of Bremner (1965) and organic carbon (%) is determined by Anne method (Khiari et al., 2021).

Phosphorus extraction and dosage are carried out by the Joret-Hebert method (Leclercq-Dransart et al., 2019) thus exchangeable potassium determination is carried out by Schollenberger method (Chandiona et al., 2021) by flame spectrometry. Soil salt concentration in is measured using the electrical conductivity of irrigation water (ECi). Electrical conductivity is expressed in (mmhos/cm).

2.1. OMWW sampling

OMWW samples were taken from local ponds which collect all OMWW of Fez city, and then put into 2 liter bottles for physico-chemical assays. Those intended for microbiological analyzes were collected in sterile bottles and then transported to laboratory and stored at 4°C for later use.

OMWW used in this work have physico-chemical and microbiological characteristics illustrated in the following table (Table 1), The methods used for the physico-chemical characterization are those described by (Rodier et al., 2009).

Table 1: characteristics of OMWW used in plots spreading

	Parameters	Values
Physico-chemical composition (g/l)	PH	4.5
	DCO	28
	Phenolic compounds	4,8
	SS (Suspended Solids)	3,1
	TS (Total solid)	20,5
	MV (Volatil matter)	17
	Fatty matter	10
	Total sugars	0,3
Mineral composition (g/l)	Calcium ($g.l^{-1}$)	2,1
	Magnesium ($g.l^{-1}$)	1,4
	Potassium ($g.l^{-1}$)	4,4
	Sodium ($g.l^{-1}$)	2,5
	Chlorides ($g.l^{-1}$)	3,8
	Orthophosphates ($g.l^{-1}$)	0,09
	Total nitrogen ($g.l^{-1}$)	0,14
	Ammoniums ($g.l^{-1}$)	0,01
Microbial flora	TAMF	$7.3\ 10^3$
	Fungi	$3.8\ 10^2$
	Yeasts	$5.6\ 10^3$
	Total Coliforms	0
	Fecal Coliforms	0
	Fecal Streptococci	0

2.2. Bioclimatic study of fez city

To investigate the impact of climate on soil microorganisms and interactions with plants, a climatic study utilizing the Emberger bioclimatic quotient was conducted. Bioclimatic parameters such as precipitation, minimum and maximum temperature were determined using the Ombrothermal diagram (Bagnouls & Gaussen, 1957; Debrach, 1953). The bioclimatic stage was identified by creating climatograms, which involved calculating the Emberger bioclimatic quotient (Q2) using the formulas provided in Table 2

Table 2: Emberger bioclimatic quotient (Q_2) formulas.

Formulas	References	Climatological parameters
$Q_2 = 2000*P/ (M + m + 546.4)*(M-m)$ $Q_2 = 3.43*P/ (M-m)$	(Mokhtari, 2013) (Stewart, 1968)	P: Annual precipitation in mm/m^2/year M: Maximum temperature of the warmest month in °C m: Minimum temperature of the coldest month in °C
$Q_2 = 2000*P/(M^2–m^2)$	(Emberger, 1942)	P: Annual precipitation in mm/m^2/year M: Maximum temperature of the warmest month in °K m: Minimum temperature of the coldest month in °K

2.3. . Microbiological study of the soil before spreading the vegetable waters, after spreading vegetable waters and after planting

2.3.1. Sampling

Test was carried out in an experimental field located within the Faculty of Science and Technology of Fez botanical garden. Soil sampling was carried out three times with an interval of two months at nine plots. Sampling was carried out in three periods : The first before OMWW spreading, the second after their spreading and launching of three different varieties of mint (*Mentha aquatica*, *Mentha piperita* and *Mentha pule*gium) and the third period, two months after OMWW spreading just before final harvest. Three vegetable water treatments were applied, i.e. a dose of 5 L/m², 10 L/m² against a control of 0L/m² (see figure 1). In order to guarantee a good results representativeness, sample homogeneity to be analyzed is important to evaluate OMWW spreading effect on our cultivated plots after two months. On this, three samples from the same plot were mixed and then analyzed. Soil microbiological analysis was focused on bacteria, actinomycetes, yeasts and fungi.

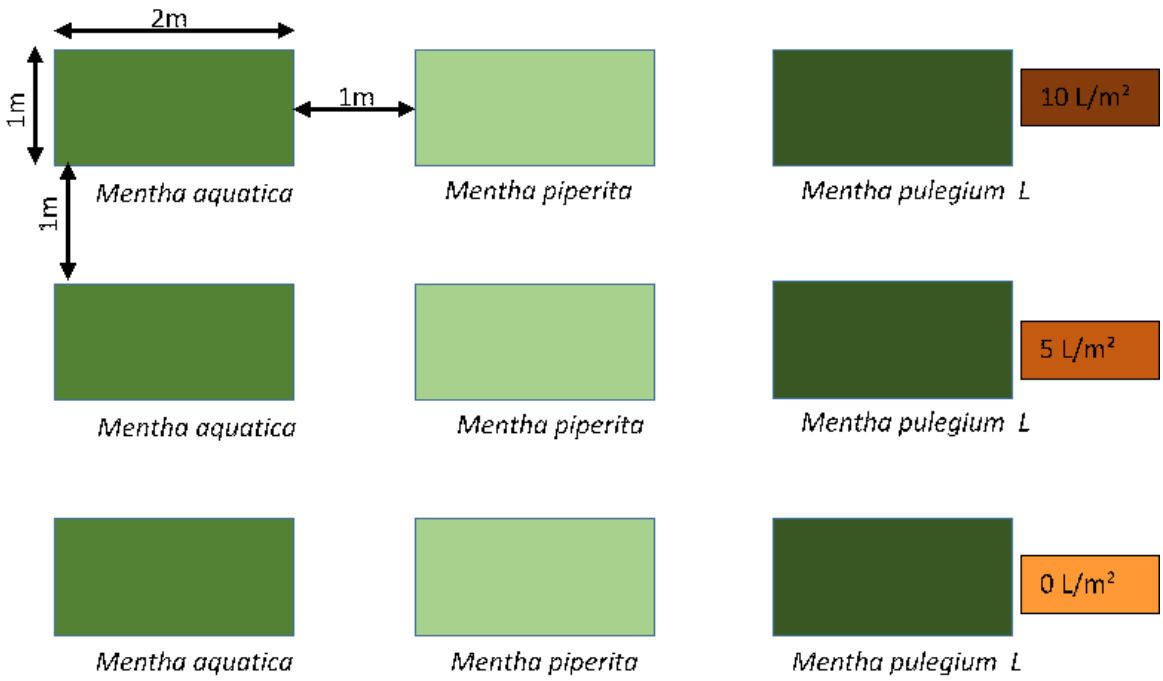

Figure 1: Experimental planting of three varieties of mint *(Mentha aquatica, Mentha piperita* and *Mentha pulegium*) applied to plots spread with two concentrations of vegetable water against a control. (0, 5 and 10 L/m²).

2.3.2. . Microbiological enumeration

Total microflora was enumerated from 0.1 ml aliquots of appropriate dilution added to solid media of LB for bacteria, ISP3 medium for actinomycetes, YPG for yeasts and malt extract medium for the mushrooms.

2.3.3. . Isolation and characterization of competent actinomycetes

In order to select the most competent actinomycete strains according to OMWW doses and mint type, a selection pressure was exerted on the latter to elect those which have the capacity to assimilate several carbon sources. For this purpose, several enzymatic activities were evaluated such as: Amylase, Cellulase, Xylanase, Gelatinase, Pectinase, Lecithinase, Caseinase, Esterase, Chitinase, and Lipase activities. The utilization of carbon and nitrogen as sole carbon sources was determinated using ISP 9 as the basal medium supplemented with a final concentration of 1 % (w/v) of the carbon sources (Shirling & Gottlieb, 1966).

3. Results and discussion

3.1. Study of the soil before and after OMWW spreading

In order to characterize the soils of the three control plots and the six treated with OMWW at different doses (0, 5 and 10 L/m²) and planted with one of the three species of Mentha, we studied the acido- base, humus content, major elements and salinity. The table below (table 3) presents soil results analysis of control and treated with OMWW.

Table 3: Soil analysis of plots irrigated with different doses of OMWW

		Doses		
Characteristics		**0L/m²**	**5L/m²**	**10L/m²**
Acid-base status	pH eau	7,11	7,32	7,08
	pH kcl	6,77	6,87	6,81
	Total limestone %	36,15	37,15	34,81
	Active limestone %	11,22	10,57	10,24
Humus rate	Nitrogen Mineral mg/100g	3,97	7,03	7,46
	Organic matter	3,4	3,88	4,69
Major elements	P assimilable P_2O_5	25,98	53,33	61,76
	K echangeable K_2O	635,4	687	747,53
Salinity mmhos/	Salinity	0,142	0,18	0,197

The acid-base status was not changed much, it seems that it was reduced by soil buffer system (Yaakoubi et al., 2010a). On the other hand, total limestone has decreased at a dose of 10L/m² and active limestone has slightly decreased at 5 and 10 L/m². Humus content has changed considerably where there is an increase in mineral nitrogen (from 3.97mg/100g to 7.46mg/100g). Regarding the major elements, there is also a remarkable increase in assimilable phosphorus and potassium. These elements are very important in soil fertilization. Salinity increase can be explained by OMWW richness in NaCl, which is widely used by farmers in olives conservation at the time of their trituration. These results made it possible to show the beneficial influence of OMWW use on soil fertility. These findings agree with those reported by certain authors (Ben Rouina et al., 1999; Yaakoubi et al., 2010b) who have underlined soil fertility improvement by OMWW spreading, particularly in terms of organic matter and potassium.

3.2. Bioclimatic study

From the ombrothermal diagram (Figure 2), we can see that F ez city has hot and dry summers and mild and wet winters.

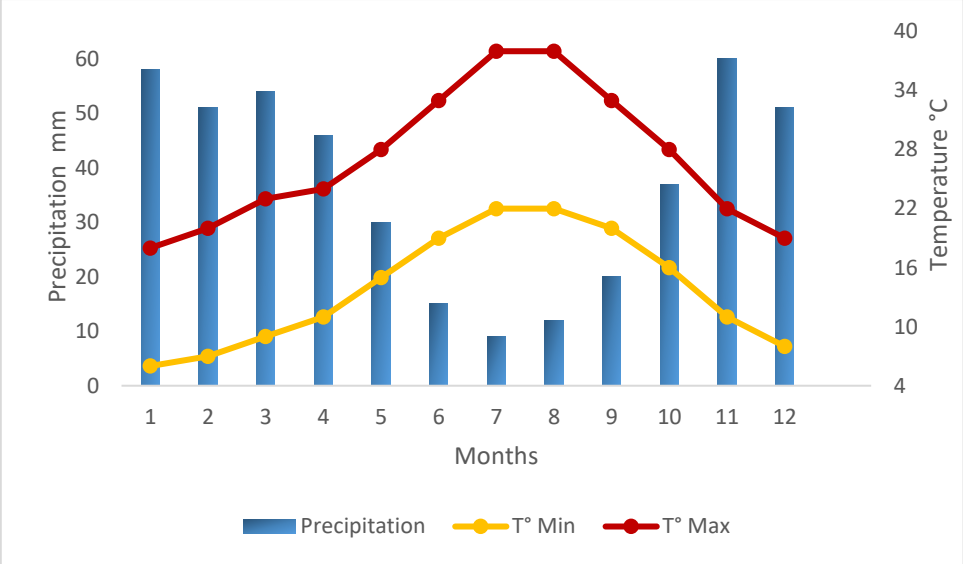

Figure 2: Ombrothermal diagram for fez city

The average maximum temperatures range from 18°C in January to 38°C in July and August, while the average minimum temperatures range from 6°C in January to 22°C in July and August. The temperature range between the average maximum and minimum temperatures is greatest in the summer months and smallest in the winter months. The precipitation values are relatively low throughout the year, with the highest average monthly precipitation occurring in November and the lowest in July. The dry season lasts from June to September, with very little rainfall during this period. The wet season starts in October and lasts until May, with the heaviest rainfall occurring in November. Overall, fez city has a relatively mild climate with low precipitation, and a clear distinction between wet winters and dry summers.

The Q2 values were obtained by applying the three formulas mentioned earlier, as presented in Table 4

Table 4: Emberger bioclimatic quotient (Q2) for the region of Fez

References	Q_2
(Mokhtari, 2013)	46,89
(Stewart, 1968)	47,48
(Emberger, 1942)	46,90
Means	47,09

Based on the climatic parameters, the studied region was positioned on the Emberger diagram (Figure 3), which indicated that Fez city is situated in a semi-arid climate zone.

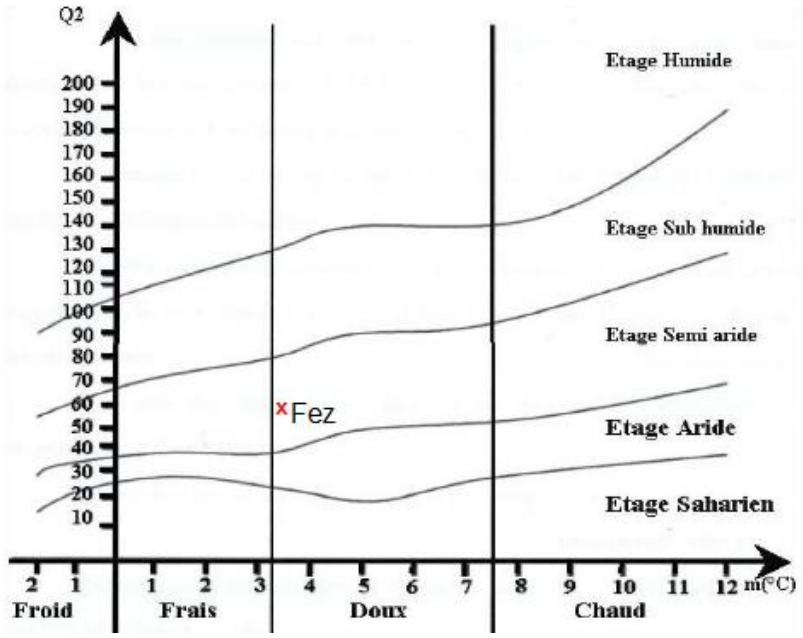

Figure 3: Emberger diagram of the bioclimatic stage of Fez city

The Emberger diagram is used in ecology to visualize the relationship between different environmental factors and the distribution of vegetation. Mint has a wide range of applications in food, pharmaceutical and cosmetic industries. However, its growth and yield can be severely affected by environmental conditions in semi-arid climates, especially water stress. (Ben Haj Yahia et al., 2019) showed that water stress leads to reductions in leaf area, plant height, and essential oil content, all of which are essential for flavor and aroma. Altitude and rainfall are also key factors affecting mint growth and yield. In a study done by (Muñoz-Bertomeu et al., 2007) and (Shams et al., 2016), results showed that populations from upper semi-arid regions had higher essential oil production than those from lower humid regions, with precipitation having a more pronounced effect than altitude. In addition, according to (Melito et al., 2016), essential oil quality varies with altitude and rainfall, and different populations show different chemotypes. Therefore, selection of populations from upper semi-arid regions for improvement programs can increase essential oil yield and quality (Rajeswara Rao, 1999). Scarcity of water resources semi-arid areas can seriously affect the growth and yield of mint (Ghamarnia et al., 2021), thus requiring effective management of water resources. However, overwatering can also negatively affect growth, and dry weight of mint plants (Nezami et al., 2016). Therefore, proper management of water and nutrients in semi-arid regions can improve the growth, yield, and quality of mint and other medicinal plants, leading to sustainable agriculture and better human nutritional health (Ebrahimi Sborezi et al., 2021). In brief, semi-arid climate conditions significantly affect the growth and yield of mint, so climate factors need to be considered when

planting and improving mint. In this context, further research is needed to optimize the growth of different mint varieties in semi-arid regions. Therefore, effective management of water and nutrients is essential to mitigate semi arid climate effects on plant and improve soil physicochemical and microbiological properties. Regarding the microbial soil community in Fez city, it can also be greatly affected by the semi-arid climate. In general, soil microbial communities play a critical role in many ecosystem processes, including nutrient cycling, and soil formation. However, the semi-arid climate can create a challenging environment for soil microorganisms, as they must cope with high temperatures, low and erratic rainfall, and nutrient-poor soils. In hot and dry conditions, soil moisture becomes limited (Geirinhas et al., 2022), which can reduce the activity and abundance of soil microorganisms. This can limit the microbial-mediated processes that occur in the soil, such as decomposition and nutrient cycling. Additionally, low rainfall can lead to a buildup of salts in the soil, which can be toxic to many microorganisms. On the other hand, during the wetter winter months, the increase in moisture can lead to an increase in microbial activity, with a corresponding increase in nutrient cycling and decomposition rates(Cruz-Paredes et al., 2021). In a study done By (Gorlach-Lira & Coutinho, 2007), in a semi-arid area, Bacteria were represented almost exclusively by Gram positive spore-forming isolates and Actinomycetes .These groups of bacteria are characterized by their physiological adaptability and ability to produce spores that are resilient to harsh environmental conditions. As a result, they are able to endure and maintain a stable population even during periods of intense stress, such as high temperatures and dry spells(Wardle, 1998). In semi-arid regions, the nutrient-poor soils can limit the growth and activity of actinomycetes. However, some species of actinomycetes have evolved mechanisms to cope with harsh conditions, such as the ability to produce drought-resistant spores or enzymes, that help them access nutrients in low-quality soils, which is confirmed by a study done by (Gorlach-Lira & Coutinho, 2007), who find that in a semi-arid area, amylase, chitinase, cellulase and protease enzymes production was more common among endospore-forming bacteria and actinomycetes. All substrates tested were 50% of actinomycetes and 8% of spore forming bacteria.

3.3. Microbiological study of the soil before spreading the vegetable waters, after spreading the vegetable waters and after planting

Figure 2 illustrates the evolution of the microbial community evaluated per gram of soil (N/g) in the different plots cultivated with the three varieties of mint *mentha aquatica* (MA), *mentha piperita* (MPp) and *Mentha pulegium* (MPl) and treated with different doses of vegetable waters (5L/m² and 10L/m²) against three designated controls for each plant.

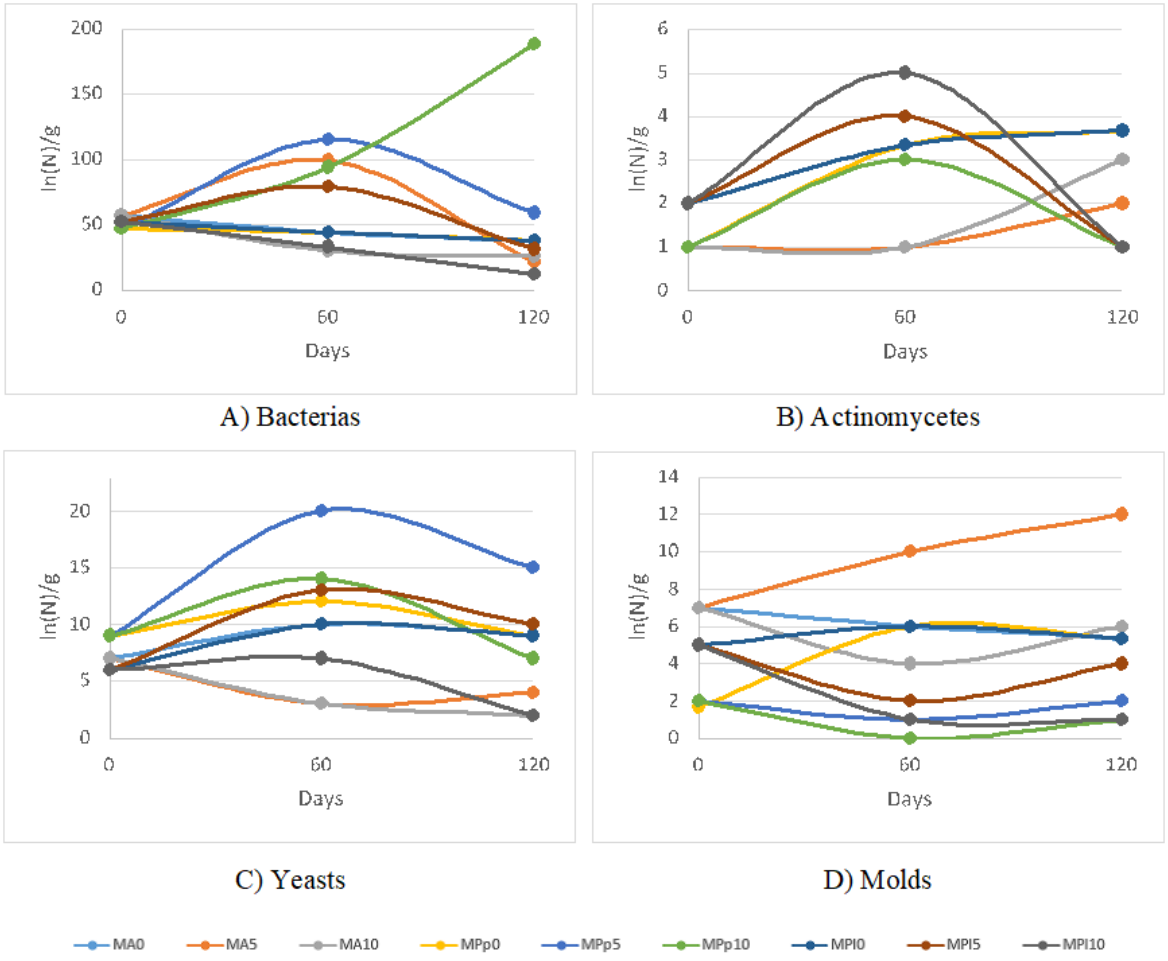

Figure 4: Microbial community evolution in plots irrigated with OMWW at different doses and planted with three genus Mentha species (a: Bacteria, b: Actinomycetes , c: yeasts, d:molds)

0 days: Before spreading the vegetable waters; 60 days: Spreading the vegetable waters and start of planting; 120 days: End of planting

MA_0 : (*Mentha aquatica* ,0L/m²); MA_5 : (*Mentha aquatica* ,5L/m²); MA_{10} : (*Mentha aquatica* ,10L/m²),
MPp_0 : (*Mentha piperita* ,0L/m²); MPp_5 : (*Mentha piperita*,5L/m²); MPp_{10} : (*Mentha piperita*,10L/m²),
MPl_0 : (*Mentha pulegium* ,0L/m²); MPl_5 : (*Mentha pulegium*,5L/m²); MPl_{10} : (*Mentha pulegium*,10L/m²)

It is noted that bacteria number per gram always increases in plots treated with OMWW only with a dose of 5L/m² (Figure 4, A). This increase can be explained by soil enrichment in mineral salts (see table 1) following the application of OMWW and then this bacterial load decreases

during treatment with a dose of 10L/m² following the pressure exerted by OMWW phenolics compounds with antimicrobial activity. Thus, we note that mint (*Mentha piperita* MPp10) at plot irrigated with 10L/m² shows an increase in bacteria number after 4 months of culture (120 day). This can be explained by the presence of released substrate by plant which presents a source of carbon and energy for bacteria and the absence of an inhibitor for the latter. This means that Mpp plant does not release toxic matter into soil, so we have a synergy between the plant (Mpp) and the bacterial load in the plot planted by this species. Our result is in agreement with (AL-Eitan et al., 2021) who reported an increase in bacteria number after the application of OMWW. According to (Di Serio et al., 2008) bacteria presence in soils treated with OMWW is explained by the fact that these microorganisms play an important role in the cycle of organic matter in nature, by their activity catalysis and their ability to decompose complex organic molecules, which is consistent with our results.

Actinomycetes monitoring in different plots shows that the most adequate dose of OMWW for their growth is 5L/m². This applies to the three mint crops (Figure 4,B) where there is an increase in of actinomycetes in the plots treated with a dose of 5L/m², and a decrease in these micro-organisms at a dose of 10L/m², except in the case of *Mentha aquatica* and *Mentha pulegium* where the load of actinomycetes increases. Actinomycetes decrease is due to the effect of high dose of OMWW rich in toxic phenolic compounds, the effect of the plant which releases toxic molecules into the soil or because of the dominance of bacteria resistant to actinomycetes antimicrobial activities on the same plot as MPp10 case.

Our results are in agreement with those found by (El Hassani et al., 2010) who also noted an abundance of actinomycetes, when applied dose was 8 L/m² on a culture of *mentha spicata* L. and also (Di Serio et al., 2008) who reported that actinomycetes increase, especially in the superficial layers treated with OMWW. Thus conducted study by (Regni et al., 2021), shows that among the most abundant genera of bacteria in soil treated with OMWW, we find actinobacteria. Similar results were also found by (Pezzolla et al., 2015) who observed that it was the only group of Gram-positive bacteria that accounted for more than 85% of total abundance in a study where OMWW digestate was used as an amendment.

Actinomycetes increase in after the agronomic application of OMWW could be explained and linked to actinomycetes ability to assimilate different carbon sources namely phenylpropenoid acids such as ferulic, vanillic and p-coumaric acids which are the commonly found phenolic acids in OMWW (Brunati et al., 2004; B. Mechri et al., 2014).

Yeasts monitoring in plots of this experimental planning shows that regardless of the dose of vegetable waters, they can develop with small variations (Figure 4, C). This can be explained by the fact that soil yeasts seem to be more adapted to the composition of OMWW and also to the presence of yeasts in OMWW microbial flora (see table 1). Thus peppermint culture of is the most suitable for these microorganisms development.

Indeed, yeasts presence during the different treatments is explained by their high capacity to degrade organic matter, in particular that which is not easily degradable such as phenolic compounds (F. El Hassani et al., 2010; Mekki et al., 2006; Oskay et al., 2004; Sassi et al., 2008).

Molds monitoring shows their presence at the level of the different OMWW (Figure 4, D) since they are already present in microflora of the latter (see table 1) thus, 10 L/m² dose of vegetable waters and the culture mint enabled them to multiply well.

Fungal populations are known for their considerable depolymerizing enzymes and resistance to recalcitrant substances. OMWW strengthened fungi, the most important organisms breaking down lignin and phenolic compounds (Mekki et al., 2013). Our result is in agreement with that found by (Di Serio et al., 2008) which underlines the presence of fungi in the areas treated with vegetable waters.

Soil microbiological study before and after OMWW spreading showed that in the case of *Mentha aquatica* and *Mentha piperita* it was observed that the microbial load (bacteria, yeasts and moulds) in the plots treated with a dose of 5L/ m² of OMWW is always higher than that of the plots treated with 10L/m² dose , except for actinomycetes. The latter are indistinguishable in the two plots treated with the different doses in the case of *mentha aquatica*, whereas in the case *of Mentha piperita* the quantity of these microorganisms is high in treated plot with 10L/m² compared to that treated with 5L/m². This can be explained by actinomycetes adaptability of different conditions, as well as their good metabolic capacity of OMWW phenolic compounds. With regard to *Mentha pulegium*, microorganisms density (bacteria, actinomycetes, yeasts and moulds) in treated plots with a dose of 5 L/m² of OMWW are higher than those of plots treated with a dose of 10 L/m².

The results of these experiments show that OMWW spreading with a dose of 5L/m² generally favors the microflora of the soil, but this increase tends to fade after exceeding this dose. Our results are similar to those cited by (Yaakoubi et al., 2010a), who report that OMWW spreading led to an increase in different microbial groups sought abundances. Indeed (Di Serio et al.,

2008) have also shown that the spreading of OMWW with doses of 8 and 16 L/m² on cultivated maize plots generally led to an increase in soil microbial flora.

Microbial community variations have been explained by several authors as possibly resulting from interactions between different factors such as micro environmental changes (decrease in oxidation conditions, strong competition for mineral nitrogen and the availability of phenolic compounds) and selective inhibition of other microbial groups by phenolic compounds and altered sources of carbone (Karpouzas et al., 2010). It has been suggested that OMWW spreading has had an impact on microbial communities structure by affecting soil nutritional status (Rousidou et al., 2010). The same authors linked microbial community changes in soil structure modification that occurs after OMWW application to the substrate. Our results also show that the amount of OMWW used can influence the microbial load in a quantitative and qualitative way.

3.3.1. Selection of competent actinomycetes strains

The table below (Table 5) presents screening results of actinomycete strains isolated from the treated plots according to their ability to assimilate the different carbon sources.

Table 5: Distribution of enzymatic activities of actinomycetes strains isolated from plots treated with OMWW and planted with different mint crops

	MA0	MA5	MA10	MPp0	MPp5	MPp10	MPl0	MPl5	Mpl10
Amylase	0	1	1	3	0	2	2	4	2
Cellulase	1	0	1	2	3	2	1	3	1
Xylanase	0	1	2	1	1	2	1	4	1
Pectinase	0	0	0	0	0	0	0	1	1
Lecithinase	1	0	0	3	2	3	0	4	2
Esterase	0	2	2	2	1	2	4	1	2
Chitinase	3	2	2	5	3	3	2	4	3
Gelatinase	3	2	2	6	4	4	4	5	3
Caseinase	0	1	0	1	1	1	2	1	0
Lipase	1	1	0	0	1	1	2	2	1

The effect of OMWW dose and the type of culture on actinomycetes population of is expressed by revelation of the number of enzymatic activities identified in each plot, or the presence of the greatest number of enzymatic activities at the level of the MP15, followed by Mpp0 and MpP10. These results corroborate results obtained in figures 2, which show that plant nature can intervene to change the microbial load of the soils treated in untreated by OMWW and this is well shown in control plots, where actinomycetes density in plots cultivated with *Mentha piperita* is higher than those of other mint crops.

The figure below (figure 3) presents the dimensional analysis (PCA) of the enzymatic profile of actinomycete strains in relation to OMWW doses and the type of menthe culture.

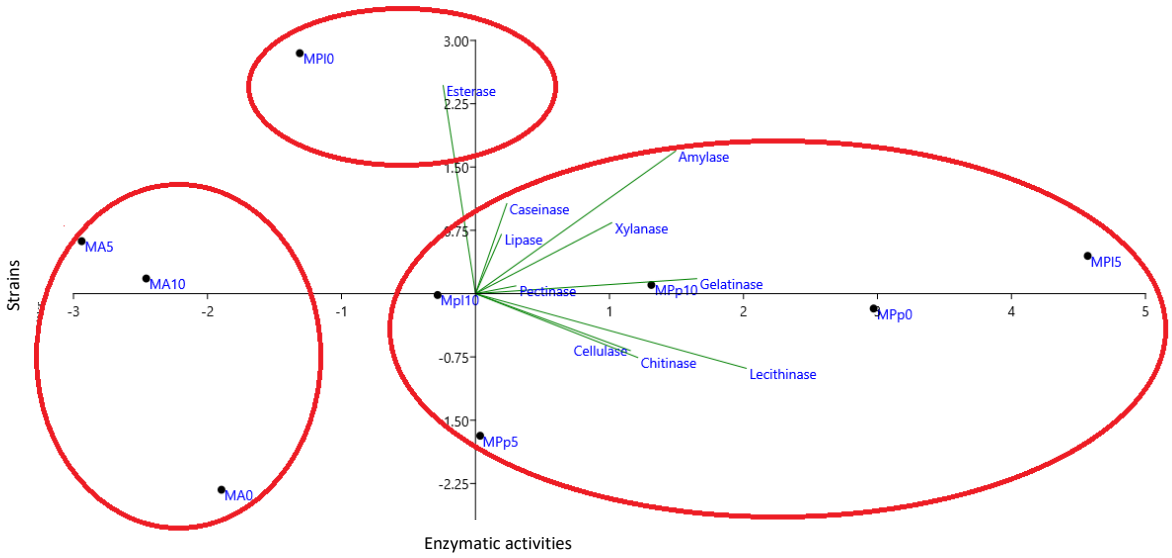

Figure 5: Correlational analysis between enzymatic activities of the strains with OMWW doses and the plantations

Correlational analysis makes it possible to interpret in a "summary" way correlations between different enzymatic activities evaluated with OMWW dose and the type of mint plantation. It is noted that the most competent actinomycete strains are positively correlated with plots irrigated with 5L/m² and 10L/m² of OMWW, and cultivated with *Mentha piperta* and *Mentha pulegium*. While for *Mentha aquatica*, the performing actinomycetes are noticed in non-irrigated OMWW plot. On the other hand, strains with a low enzymatic potential are present in plots cultivated with *Mentha aquatica* spread with different concentrations of OMWW .

This result therefore means that a culture of *Mentha piperta* and *Mentha pulegium* combined with OMWW doses ranging from 5 to 10 L/m² promotes competent actinomycetes growth having a good power of degradation of the various carbon and energy sources. Thus OMWW doses increase or decrease the metabolic pathways of actinomycetes depending on the type of plant.

All the isolated strains use most of the carbon and nitrogen sources including glucose, sucrose, fructose, galactose, mannose, citrate, glycerol, mannitol and L-proline. However, the other sources are used by only a few strains (Table 6).

Table 6: Assimilation of carbon and azote sources by the Actinomycetes isolates

carbon source	Glucose	Saccharose	Asparagine	Histidine	Fructose	Glycérol	Xylose	Maltose	Mannitol	Galactose	Sorbitol	citrate	mannose	L-proline	L-methionine	Inositol
BOEF1	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+
BOEF2	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
BOEF3	+	+	+	-	+	+	-	-	+	+	+	+	+	+	-	-
BOEF4	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-
BOEF5	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
BOEF6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
BOEF7	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
BOEF8	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	-
BOEF9	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-	+
BOEF10	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
BOEF11	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
BOEF12	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
BOEF13	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
EFBO1	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+
EFBO2	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
EFBO3	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	-
EFBO4	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-	-
EFBO5	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-	-
EFBO6	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
EFBO7	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
EFBO8	+	+	+	-	+	+	-	-	+	+	+	+	+	+	-	-
EFBO9	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	-
EFBO10	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	-
EFBO11	+	+	-	-	+	+	-	-	+	+	+	+	+	+	-	-
EFBO12	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
EFBO13	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
EFBO14	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	-
EFBO15	+	+	-	-	+	+	-	-	+	+	+	+	+	+	-	-
BOEF201	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-
BOEF204	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
BOEF205	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
BOEF202	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-
BOEF203	+	+	-	-	+	+	-	-	+	+	+	+	+	+	-	-
BOEF206	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+

The use of different carbon sources by all strains indicated a wide pattern of carbon assimilation (Valan Arazu et al., 2013). Our result is similar to those found by (Aallam et al., 2021; Do et al., 2021; Kuncharoen et al., 2022) having isolated strains that have the capacity to use various carbon and nitrogen sources as a sole source. Also, (Meliani et al., 2022) report that most actinomycetes are known to use a wide range of organic compounds as carbon source for their growth which can explain our results and justify the capacity of adaptation of our strains with the different conditions present in the plots irrigated by olive mill wastewater and planted by mint.

4. Conclusion

OMWW spreading on mint crops increased soil fertility by promoting studied microorganisms growth, but this development is attenuated when the adequate dose is exceeded, except for actinomycetes which prove to be resistant. Enzymatic profile of actinomycetes isolated from different soil samples reveals that most efficient strains are observed in plots irrigated with 5L/m² and 10L/m² of vegetable waters, and cultivated with *Mentha piperta* and *Mentha pulegium*. This work results emphasize that OMWW use of in soils treatment of planted with different varieties of mint makes it possible to select competent actinomycetes strains with a good power of degradation of different carbon and energy sources. The search for new molecules with interesting properties remains an active field of research. In this context, studies on actinomycetes would constitute a valuable complement in several fields and in particular the agronomic field.

Chapitre III

Sélection d'un consortium d'actinomycètes isolé à partir d’un sol irrigué par les margines pour améliorer le compostage

Sélection d'un consortium d'actinomycètes isolé à partir d'un sol irrigué par les margines pour améliorer le compostage

Synthèse

Le deuxième chapitre de cette thèse est focalisé sur l'isolement, la caractérisation et l'identification des souches d'actinomycètes, qui seront valorisées dans un processus de compostage. Ce dernier est un phénomène biologique qui contribue à la dépollution de l'environnement, mais il peut souvent produire des rendements insatisfaisants. Les actinomycètes, des bactéries filamenteuses à Gram-positif, présentent un cycle de développement hautement différencié et subissent une différenciation morphologique significative (Hozzein et al., 2019). Grâce à leur capacité de sporulation, ces actinomycètes peuvent résister à des conditions environnementales variables, telles que le déficit en eau ou en nutriments.

Les actinomycètes ont une grande importance biotechnologique en raison de leur production de métabolites bioactifs (Javed et al., 2021 ; Zitouni et al., 2005) et de leur capacité à interagir avec d'autres microorganismes pour restaurer les sols dégradés (Bhatti et al., 2017). De plus, ces bactéries produisent des enzymes hydrolytiques, telles que les amylases, les lipases et les cellulases, qui sont largement utilisées dans plusieurs industries, notamment l'alimentation, le textile, la pharmaceutique et la papeterie. Les actinomycètes jouent un rôle de saprophytes primaires en décomposant des polymères complexes tels que la lignocellulose des plantes et la chitine des exosquelettes des insectes.

Cependant, il y a un manque d'études sur l'effet de l'inoculation du compost avec un consortium d'actinomycètes sur le processus du compostage ainsi que sur l'enrichissement du sol. C'est pourquoi cette recherche se concentre sur l'inoculation du compost avec un consortium d'actinomycète puissant sélectionné pour améliorer la dégradation des déchets par compostage. L'étude explore également les effets de ce traitement sur la croissance des plantes de *Vicia faba*.

Pour atteindre les objectifs visés, des souches bactériennes appartenant au genre des actinomycètes ont été isolées à partir d'échantillons de sol irrigués avec des eaux usées d'huile d'olive à deux concentrations (5 L/m^2 et 10 L/m^2). Les souches isolées ont été caractérisées du point de vue phénotypique et enzymatique, ainsi que pour leur capacité antimicrobienne contre différents microorganismes pathogènes. L'objectif était d'identifier les actinomycètes compétents dans l'assimilation de composés toxiques provenant des résiduaires des huiles d'olive afin de les utiliser pour potentialiser le processus de compostage. La qualité du compost

a été évaluée en fonction de plusieurs paramètres, tels que le pH, la teneur en carbone organique total (TOC) et le rapport carbone sur azote (C:N).

Dans cette étude, une attention particulière a été accordée pour sélectionner de souches compétentes d'actinomycètes isolées à partir d'échantillons du sol afin d'améliorer le processus de compostage. Les souches isolées ont été soigneusement caractérisées pour leurs activités hydrolytiques et antimicrobiennes, afin de sélectionner et d'identifier les actinomycètes compétents capables de métaboliser différentes sources de carbone et d'énergie.

Par la suite, nous avons étudié l'effet de certains paramètres physico-chimiques tels que la température, le pH et la salinité, afin d'exercer une pression de sélection pour choisir les souches les plus compétentes. Le consortium d'actinomycètes ainsi formé a été évalué pour son activité de compostage en surveillant la température et le pH tout au long du cycle de compostage. De plus, la qualité finale du compost a été évaluée en mesurant sa conductivité, sa teneur en azote total (TNK) et le carbone organique total (TOC).

Dans le cadre de cette étude, nous avons également évalué l'effet du compost produit sur la croissance des plantes de *Vicia Faba L*. Trois paramètres ont été mesurés : le poids du système aérien (ASW), le poids du système racinaire (RSW) et la longueur de la racine principale (PRL).

Les résultats de cette étude ont révélé que la plupart des souches actinomycètes isolées présentaient une activité antimicrobienne efficace contre diverses souches bactériennes Gram-positifs et des levures. De plus, les souches ont montré une tolérance variable aux différents facteurs étudiés, avec une température de 37°C, un pH neutre (pH=7) et une faible concentration de sel (NaCl, 2%) semblant être les conditions les plus favorables pour leur croissance.

La teneur en azote total dans le compost inoculé s'est avérée significativement plus élevée que celle du témoin, indiquant une meilleure dégradation des matières organiques et une disponibilité accrue en nutriments pour les plantes. De plus, cette étude a permis d'évaluer les effets de l'inoculation du compost avec ce consortium d'actinomycètes sur la croissance des plantes de *Vicia faba*. Les résultats ont démontré une amélioration significative de la croissance des plantes traitées avec le compost enrichi en actinomycètes par rapport au témoin, ce qui confirme l'effet bénéfique de ces micro-organismes sur le développement végétal. Ces observations soulignent le potentiel du compost produit avec l'inoculation d'actinomycètes en tant qu'amendement organique pour l'agriculture durable.

En conclusion, ce deuxième chapitre de thèse met en évidence l'importance des actinomycètes dans le processus de compostage et leur potentiel pour améliorer la qualité du compost. Les actinomycètes isolés à partir d'échantillons de sol irrigués avec des eaux résiduaires des huiles d'olive présentent une activité enzymatique remarquable, une capacité antimicrobienne prometteuse et une capacité de métaboliser divers substrats carbonés. L'inoculation du compost avec un consortium d'actinomycètes a conduit à une meilleure dégradation des matières organiques, une augmentation de la teneur en azote total et une stimulation de la croissance des plantes. Ces résultats fournissent des éléments de réponse pour la poursuite des investigations sur l'utilisation des actinomycètes dans le domaine du compostage et de l'agriculture durable, en vue d'améliorer les performances du processus de décomposition des déchets organiques et de favoriser la production de compost de haute qualité.

Selection of Actinomycetes consortium from soil irrigated with olive mill wastewater to enhance composting

This part of the work was the subject of an article submitted for publication.

Abstract

This study focused on competent actinomycetes strains selection isolating from soil samples to enhace composting process. Isolated strains are characterized for their hydrolytic and antimicrobial activities to select and identify competent actinomycetes bacteria that are able to metabolize several sources of carbons and energies. After that, the effect of some physico-chemical parameters (Temperature, pH and salinity) was studied to excerce selection pressure for selecting competents stains. Actinomycetes consortium was tested for its composting activity by measuring temperature and pH during the composting cycle, and the final quality of the compost was evaluated by determining conductivity, total nitrogen content (TNK) and total organic carbon (TOC). Thus, produced compost effect was tested on *vicia faba L* plant growth is by measuring three parameters: aerial system weight (ASW), root system weight (RSW) and primary root length (PRL).

Antimicrobial activities results revealed that most isolated strains have a good inhibitory activity against several bacterial strains, mainly Gram-positive bacteria, and yeasts strains. Results showed also that isolated strains are influenced by studied factors, temperature of 37°C, a neutral pH (pH=7) and a low concentration of salt (NaCl, 2%) seem to be most tolerated by isolated actinomycetes strains. Hydrolytic enzymes confers to them ability to assimilate various polymers substrates. Total nitrogen content was 1.18% in the inoculated compost, while 0.84% nitrogen was detected in the control. A significant difference was observed in produced compost quality with bioinoculants when compared to control. Inoculation could improve the efficiency of the composting process. Therefore, Compost soil improves *vicia Faba* plants growth.

Key words: Actinomycetes, Composting, Antimicrobial activity and biopolymers hydrolysis.

1. Introduction

Composting is a biological phenomenon that contributes to environmental depollution. Unfortunately, this process is not always controllable and sometimes gives low yields. Actinomycetes, filamentous Gram-positive bacteria, present a highly differentiated development cycle that undergoes very important morphological differentiation (Hozzein et al., 2019). Through sporulation phenomenon, actinomycetes are able to withstand different conditions, such as water or nutritional deficit. Actinomycetes are not only beneficial in improving soil fertility but are also involved in biological soil buffering and suppression of plant pathogens in rhizosphere (Hozzein et al., 2019). Actinomycetes appear to be of great biotechnological interest through their production of bioactive metabolites (Javed et al., 2021b; A. Zitouni et al., 2005). Their ability to interact with other microorganisms allows them to restore degraded soils (Bhatti et al., 2017). Actinomycetes produce various hydrolytic enzymes such as amylases, lipases, and cellulases, which are widely used in many fields including food, textile, pharmaceutical and paper industries. Thus, they are primary saprophytes, they produce enzymes necessary for complex polymers decomposition, such as plants lignocellulose and insect exoskeletons chitin. The potential effects of different genera of actinomycetes on soil bioremediation and plant protection are well documented. However, up to our knowledge, the effect of soil enrichment with compost produced by a consortium of actinomycetes on plant growth is still not yet well studied. For this reason, the present work dealt with compost inoculation with potent actinomycete selected to be useful in enhacing domestic waste degradation via composting. The study also simultaneously explored the effect of our treatement on plant growth of *Vicia Faba L*. For this purpose, bacterial strains belonging to actinomycetes taxonomic genus were isolated from soil samples irrigated with olive mill wastewater (OMWW) at two concentrations (5 l/m^2) and (10 l/m^2). Phenotypic characterization, enzymatic activities profile, and study of their antimicrobial capacity against different pathogenic microorganisms are addressed. This study will allow us to identify actinomycetes competent in toxic compounds assimilation from olive mill wastewater in order to use them in composting process potentialisation. Also, the aim of the present work was to study the effects of microbial additive on composting process. Compost quality was evaluated as a function of pH, total organic carbon (TOC) and carbon to nitrogen ratio (C:N).

2. Material and methods

2.1. Sampling

Soil samples were taken from of Faculty of Science and Technology of Fez's pedagogical garden. Sampling was carried out in 9 plots, 6 of which are treated by OMWW at (5 l/m²), (10 l/m²), and 3 controls (0 l/m²). For microbial analysis, soil was sampled with a sterile spatula at a depth of 15 cm and transported to laboratory at 4°C (Tardieux & Pochon, 1962). Samples were sieved and allowed to dry at room temperature for one week before use.

2.2. Isolation of actinomycetes from soil

Isolation method

Isolation technique is used according to Boughachiche and his collaborators (2005) which consists in putting soil (10g) in 100ml of sterile physiological water (NaCl 9‰), then shaken for 5 min and underwent a decimal cascade dilutions ranging from 10^{-1} to 10^{-5}. 100µl was flooded on selectiv culture media ISP3 (Oat solution 1000ml, Trace element solution: 1ml, Agar: 20g) (Shirling & Gottlieb, 1968) and they are incubated at 30°C and observed daily for 15 days of incubation.

2.3. Strain characterization

In order to characterize isolated strains and highlight their potential, we carried out the study of their enzymatic profile as well as their tolerance to thermal, saline, and acid-base stress.

2.3.1. Metabolic characterization of Actinomycetes strains

Degradation tests of different carbon sources were carried out to investigate their degradative capacity of existing substrates in compost.

Cellulolytic activity

Cellulolytic activity was tested on Luria Bertani broth (LB) medium containing cellulose (1%) inoculated by the streak method. Clear appearance halo after 14 days around colonies indicates cellulolytic activity presence after incubation at 30°C.

Amylolytic activity

Amylolytic activity test was performed on LB medium containing 1% of soluble starch according to Gordon & Smith (1953) method. After 14 days of incubation at 28°C, agar was covered with a lugol solution. Absence of staining around colonies indicates starch hydrolysis while areas containing starch will be stained brown (Hankin & Anagnostakis, 1975).

Pectinolytic activity

The technique used to test pectinolytic activity is that of (Hankin & Anagnostakis, 1975) which consists in using LB medium with 0.5% pectin added. After 7 days of incubation, petri dishes were covered with a copper sulfate solution and left at room temperature for a few minutes. Clear halo appearance indicates pectinolytic activity presence.

Chitinolytic activity

Chitinolytic activity consists of inoculating strains to be tested on LB medium complemented with 1% of chitin. After 7 days of incubation, chitin assimilation is reflected by the clear zone appearance around colonies (Gomes et al., 2000).

Inulinase activity

This assay was performed on ″Czapex Dox″ medium supplemented containing 1% inulin, according to Das et his collaborators (2019) method. Petri dishes were treated with Lugol's iodine for 3-5 min and then washed with distilled water to remove excess iodine. The clear area around colonies indicates inulin hydrolysis.

Gelatinolytic activity

Strains were inoculated into tubes containing nutrient gelatin (0.1%) and incubated at 28°C for 21 days. Tubes were then placed in refrigerator for one hour. If gelatin becomes solid it implies that it has not been assimilated by the strains, if it remains liquid, it means that an extracellular enzyme ″gelatinase″ has hydrolyzed it.

Caseinolytic activity

To study caseinolytic activity, test is performed on an agar medium containing 5% skimmed milk. Colonies were streaked according to the method of (Williams & Cross, 1971). Appearance of any clear zone around colonies after 14 days of incubation at 28°C, shows casein hydrolysis.

Lecithinolytic activity

Lecithinase is tested on 10% egg yolk agar, strains are streaked and incubated at 30°C. The presence of the enzyme results according to (Darshit & Pandya, 2018) in the appearance of an opaque, yellowish halo with a sharp edge under colonies.

Esterolytic activity

Esterase assay was performed on Sierra medium supplemented with Tween80. After 14 days of incubation at 28°C. Opaque halo appearance around colonies reflects esterase presence (Minotto et al., 2014).

Lipolytic activity

Lipolytic activity was tested according to the method described by (Selvam et al., 2010) with a slight modification, Isolates were inoculated onto LB medium supplemented with 3% olive oil and 1% Rhodamine B (0.001%, w/v). Colonies that showed orange fluorescence under UV irradiation indicated positive lipolytic activity.

Xylanolytic activity

Xylanolytic activity test was performed on xylan-supplemented nutrient agar where strains were plated for 5-7 days. Halo Presence around colony shows a positive xylanase. (Ball & Mccarthy, 1989).

2.3.2. **Phenotypic characterization**

Growth tests at different physicochemical parameters including temperature, (37°C, 40°C, 47°C, 50°C and 57°C), pH (3-13) and salinities (2-16% NaCl) were performed on solid ISP3 medium. These tests were carried out in order to know the effect of these different physicochemical parameters on isolated strains to estimate their efficiency in composting process.

2.4. Antimicrobial activity study

Antimicrobial activity of the isolates was tested on six microorganisms, including 4 bacteria: two Gram-negatif strains: ″ *Escherichia coli* and *Pseudomonas sp* ″, two Gram-positif strains: ″ *Staphylococcus aureus* and *Bacillus sp* ″ and two yeasts: ″ *Candida tropicallis* and *saccharomyces cerevisiae* ″ according to the double layer method (Belghit et al., 2021). These strains are part of bank strain belonging to Functional Ecology and Environmental Engineering laboratory of Faculty of sciences and techniques of Fez city in Morocco.

2.5. Compost preparation

A total of 10kg of waste was introduced into designed bioreactor for composting with dimensions 85 cm × 42 cm (length × diameter). In order to ensure a homogeneity of compost, bioreactor is equipped with a motor to allow a good agitation (Figure 1). Solid waste is composed of 40% of vegetable waste, 35% of fruit waste, 5% of tea and coffee residues, 5% of sawdust and 15% of humus. 1L of competent selected actinomycetes consortium was added to the composting unit and mixed uniformly throughout the raw material (CWC). Un-inoculated control was maintained with 1L of distilled water with the raw material (CWOC). The inoculant contained 10^{12} CFU/ml of different strains.

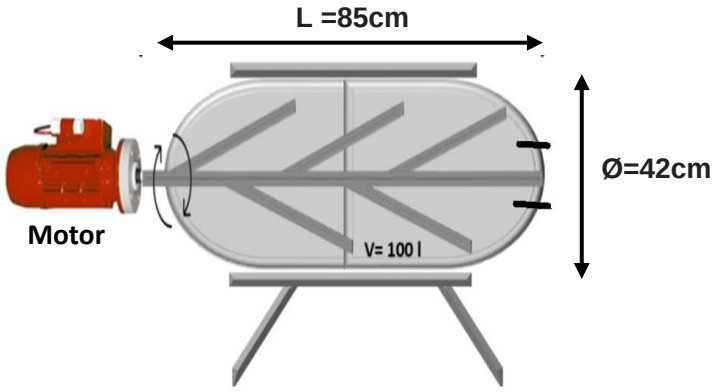

Figure 1: Designed bioreactor for lab composting

2.5.1. Kinetics of physico-chemical parameters and evaluation of final compost quality

In order to determine actinomycetes inoculation effect on composting process, temperature and pH were measured every day, as quantitative indicator with specific probes linked to composter apparatus.

The pH and electrical conductivity (EC) of a water extract were evaluated by diluting one part of the final compost by volume with 10 parts distilled water at a ratio of 1:10 (w/v). Samples were shaken and allowed to precipitate before being filtered using the procedure described by (Y. Li et al., 2019), where both EC and pH has been calibrated with standard solutions. The organic C was determined by Anne's method (1945) and Total nitrogen was determined, in the compost, by wet digestion (Kjeldahl method).

2.5.2. Compost effect on plant growth

The field experiment was conducted at the faculty of sciences and technology Fez experimental garden. Seeds of the variety aguadulce of *vicia fava L* were sown in a soil treated with compost

against a control. After plants maturity, ten whole plants of each plot were randomly uproote, then, the weight of the aerial system; root system and length of primary root were measured.

2.6. Statistical analysis

Plant data were subjected to Variance I Analysis (ANOVAI).

3. Resultats et discussion

3.1. Strains characterization

3.1.1. Metabolic characterization

Enzymatic profile of different isolates is given in table below (table1). The latter, shows that all strains present a positive gelatinase and catalase. Thus, 80% of these strains show a good capacity to degrade chitin, 50% are able to degrade starch, cellulose, lecithin, xylan, ester and unilin, while 30% of the strains hydrolyse lipids and casein. We noticed that pectin degradability is very low in most of our strains (5%).

Table 1: Enzymatic profile of isolated Actinomycetes.

Test	Cellulase	Amylase	Pectinase	Chitinase	Inulinase	Gelatinase	Caseinase	Lecithinase	Esterase	Lipase	Xylanase	Catalase
BOEF1	-	-	-	+	-	+	-	-	-	+	-	+
BOEF2	-	-	-	+	-	+	-	-	-	-	-	+
BOEF3	-	-	-	+	-	+	+	-	+	-	+	+
BOEF4	+	-	-	-	+	+	+	-	+	-	+	+
BOEF5	-	-	-	+	+	+	-	-	+	-	-	+
BOEF6	+	+	-	+	+	+	-	+	+	-	-	+
BOEF7	+	-	-	+	-	+	-	+	+	+	-	+
BOEF8	-	-	-	+	-	+	-	+	-	-	-	+
BOEF9	-	-	-	+	-	+	-	-	+	-	-	+
BOEF10	-	-	-	+	+	+	-	-	+	+	--	+
BOEF11	-	-	-	-	-	+	-	-	+	-	-	+
BOEF12	+	-	-	-	+	+	-	-	-	-	-	+
BOEF13	-	-	-	+	-	+	-	+	+	-	+	+
EFBO1	+	-	-	+	-	+	-	+	-	-	-	+
EFBO2	-	+	-	+	+	+	-	-	+	+	-	+
EFBO3	-	+	-	+	-	+	-	-	+	-	+	+
EFBO4	-	+	-	+	-	+	-	-	-	-	-	+
EFBO5	-	-	-	-	-	+	-	+	-	-	-	+
EFBO6	-	-	-	+	+	+	+	+	-	-	-	+
EFBO7	+	-	-	+	+	+	-	+	-	-	-	+
EFBO8	+	+	-	+	+	+	-	-	-	-	-	+
EFBO9	-	+	-	-	+	+	-	+	+	+	-	+
EFBO10	+	-	-	+	+	+	-	+	+	-	+	+
EFBO11	+	+	-	-	-	+	+	-	+	-	+	+
EFBO12	-	+	-	-	+	+	+	-	+	+	-	+
EFBO13	-	+	-	+	+	+	-	+	+	+	+	+
EFBO14	-	+	-	+	+	+	-	+	-	-	+	+
EFBO15	+	+	-	+	+	+	-	-	-	+	-	+
BOEF201	+	+	-	+	-	+	+	-	-	-	+	+
BOEF204	+	+	+	+	-	+	+	+	-	+	+	+
BOEF205	+	+	-	+	-	+	-	+	-	-	+	+
BOEF202	-	-	-	+	-	+	+	-	-	-	+	+
BOEF203	+	+	-	+	-	+	+	-	-	-	+	+
BOEF206	-	+	+	+	-	+	-	+	+	-	-	+

Dimensional analysis by factorial analysis of correlations (AFC) (Figure 2) reveals that enzyme profile contributes to component 1 formation and isolated strains contribute to component 2 formation. AFC results show that our strains are grouped into 3 pools , the first cluster is positively correlated with esterase, inulinase, lipase, gelatinase and catalase,where these enzymes will occupy an importance in detergent, cosmetic and food industry (Gurung et al.,

2013; Houde et al., 2004; Tamoor et al., 2021). The second pool is correlated with caseinase, amylase, cellulase, chitinase and xylanase which are widely used in pharmaceutical and food industry (Juturu & Wu, 2012; Mobini-Dehkordi & Javan, 2012). The third pool is correlated with lecithinase, amylase and chitinase which also play an important role in paper and textile industry (Kuhad et al., 2011; Mobini-Dehkordi & Javan, 2012; Rathore & Gupta, 2015) while none of the strains correlate with pectinase except EFBO204. These results confirm the importance of these strains and their interest in different biotechnological applications like composting.

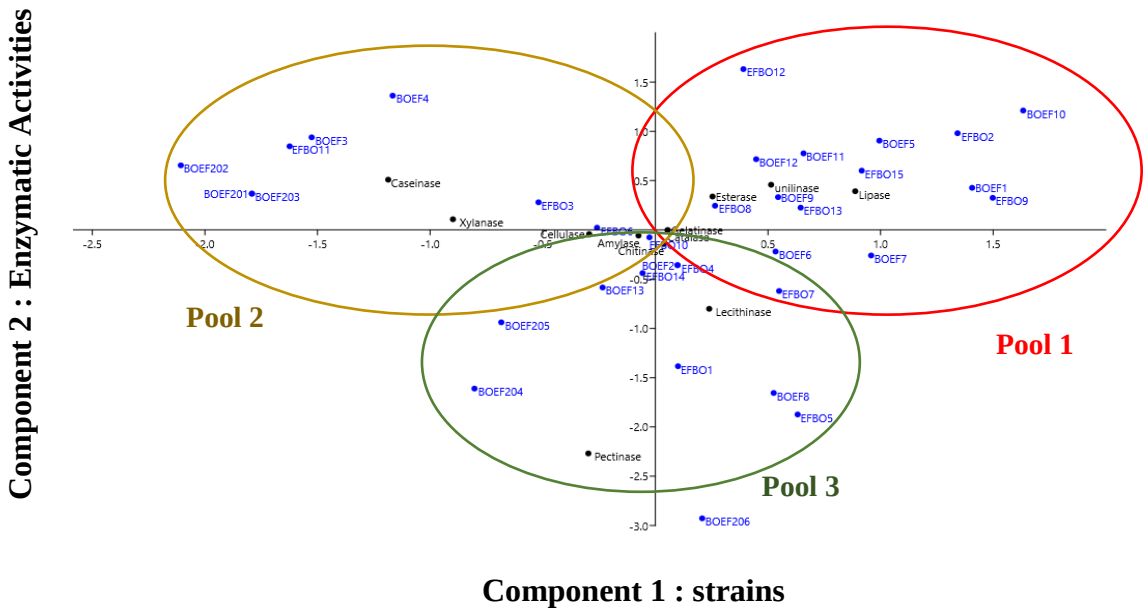

Figure 2: AFC showing strains distribution according to their enzymatic profile

In fact, many researches have reported that actinomycetes are able to produce a large number of enzymes, of high commercial value (Avramenko & Galynkin, 2010 ; Chakraborty et al., 2009). Obtained results found are in agreement with those of (Phuoc et al., 2020) who showed that among eight studied strains, only three showed strong extracellular enzyme activities. Isolated strains produce cellulase, amylase, gelatinase and chitinase. Our results are also agreeing with those found by Mobini-Dehkordi & Javan (2012) and (Hozzein et al., 2019) who report different enzymes hydrolysis including gelatinase, caseinase and cellulase by actinomycetes, which justifies researcher interest in these types of microorganisms having a wide spectrum of application of these enzymes in a wide fields range.

3.1.2. Phenotypic characterization

In order to determine strains adaptation capacity to different environmental parameters, we proceeded to a phenotypic determination. At the level of this characterization, isolates having been able to grow on different concentrations of salt, temperature and pH are given respectively on the figures below.

Actinomycetes number decreases according to salt concentration used (Figure 3) . Thus, as NaCl concentration increases, actinomycetes number decreases. We noted that all isolated strains have a good growth at concentrations of 2% (20g/l) and 4% (40g/l) NaCl, thus, among the 34 strains only 12 strains could resist to concentrations higher than 10% (100g/l) NaCl. We also noticed that from concentration 6% (60g/l), strains number decreases progressively until concentration 16% being toxic for actinomycetes survive. therefore, on all the strains, we have a survival percentage of 14.71% at a concentration of 15% of NaCl.

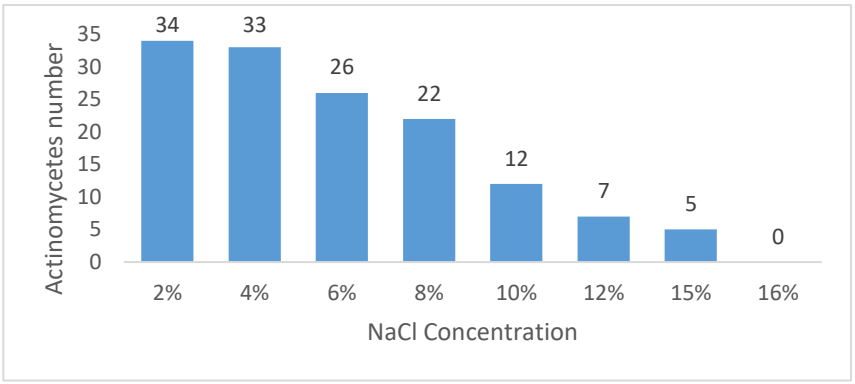

Figure 3: Actinomycetes number variation according to NaCl concentration

The actinomycetes in this study grow best at 37°C , only 8 strains growsn on 47°C and no strain is resistant to a temperature above 47°C, which means that isolated strains have a growth optimum of 37°C (Figure 4). This decrease in actinomycetes number with increasing temperature, is explained by enzymes denaturation and bacterial structures disintegration.

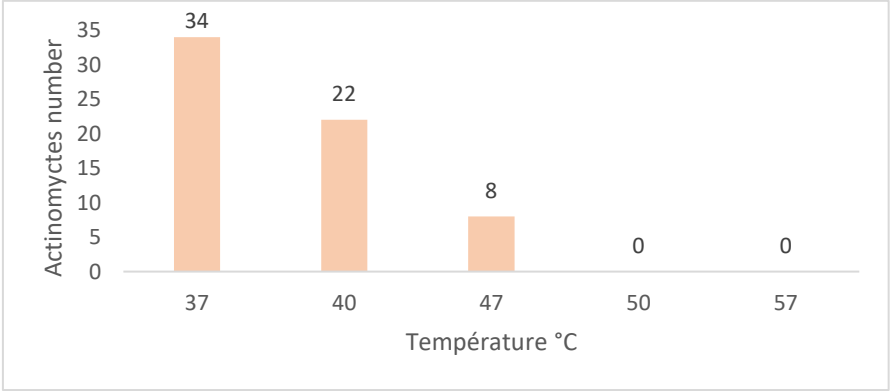

Figure 4: Actinomycetes number variation according to temperature (°C)

Growth and survival of actinomycetes are influenced by pH, thus, neutral pH medium being most favorable for these microorganisms growth (Figure 5). Acidic and basic pH seems to have a slight negative effect on our strains. This result is explained by the fact that a high or low pH values can cause weakening of bacteria by altering their membrane permeability and energy production of energy by respiratory chain. An inadequate pH attacks at the level of bacterial membrane proton pump which influences pH gradient causing the deterioration of the pedu

nculated sphere (ATP synthetize) and consequently cell death.

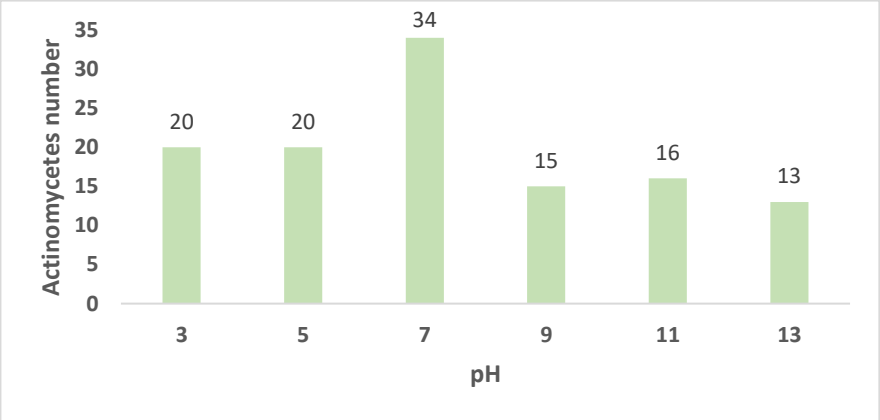

Figure 5: Actinomycetes number variation according to pH values

Overall, results show that isolates grow well at 2% NaCl, 37°C and pH=7 . Our results are in agreement with those found by (Feng et al., 2020) who reported in an isolated actinobacterium from soil a growth range up to 8% (w/v) NaCl, a temperature range of to 10-45°C, with optimal growth at 37°C. Indeed, (P. D. James et al., 1991) reported an optimal temperature of 45°C and pH between 5.5 and 6.5 for a higher growth rate of *Streptomyces thermoviolacus*, also (S. B. Kim et al., 2004) reported a new *Streptomyces* species growing abundantly in a pH range of 4.3-7.3 and at a temperature of 25-37°C. Obtained result by Maria BF and his collaborators (2018) showed that a studied actinomycete strain is able to grow on NaCl concentration ranging from 2.5 to 7%, but the 7% concentration proved to be the best, all the same, this same strain can grow on a temperature optimum of 30°C, thus, growth was observed at 45°C but not at 50°C.

3.2. **Antimicrobial activity evaluation**

Antimicrobial activity presented by the strains of our collection against different tested microorganisms are given in table below (Table2).

Table 2: Antimicrobial activity evaluation of studied strains.

	Bacteria				Yeasts	
Strains	***Pseudomonas sp***	***E.coli***	***B.subtilis***	***S.aureus***	***C.albicans***	***S.cerevisiae***
BOEF1	-	+	-	-	+	-
BOEF2	-	+	-	+	-	-
BOEF3	-	-	-	+	+	+
BOEF4	-	-	+	+	-	+
BOEF5	-	-	+	+	+	-
BOEF6	-	-	+	+	-	-
BOEF7	-	+	+	+	+	-
BOEF8	-	+	-	-	-	+
BOEF9	-	-	-	+	-	+
BOEF10	-	-	+	+	+	+
BOEF11	-	-	-	+	-	+
BOEF12	-	+	-	+	-	+
BOEF13	+	-	-	+	-	-
EFBO1	-	+	-	-	-	-
EFBO2	-	+	-	-	-	-
EFBO3	-	-	+	-	-	-
EFBO4	-	-	-	-	-	-
EFBO5	-	-	-	-	-	-
EFBO6	-	-	+	+	-	-
EFBO7	-	-	+	+	-	-
EFBO8	+	-	+	+	-	-
EFBO9	-	-	-	-	-	-
EFBO10	-	-	+	+	-	-
EFBO11	+	-	+	-	+	-
EFBO12	-	-	-	-	+	+
EFBO13	-	-	+	-	-	-
EFBO14	+	-	-	+	-	-
EFBO15	-	-	+	-	-	-
BOEF205	+	-	+	-	-	-
BOEF203	+	-	-	+	-	-
BOEF206	+	-	+	-	-	+
BOEF202	-	-	-	+	-	+
BOEF201	-	-	+	-	-	+
BOEF204	+	-	-	-	-	-

(+)-Wide inhibition, (-) -No inhibition.

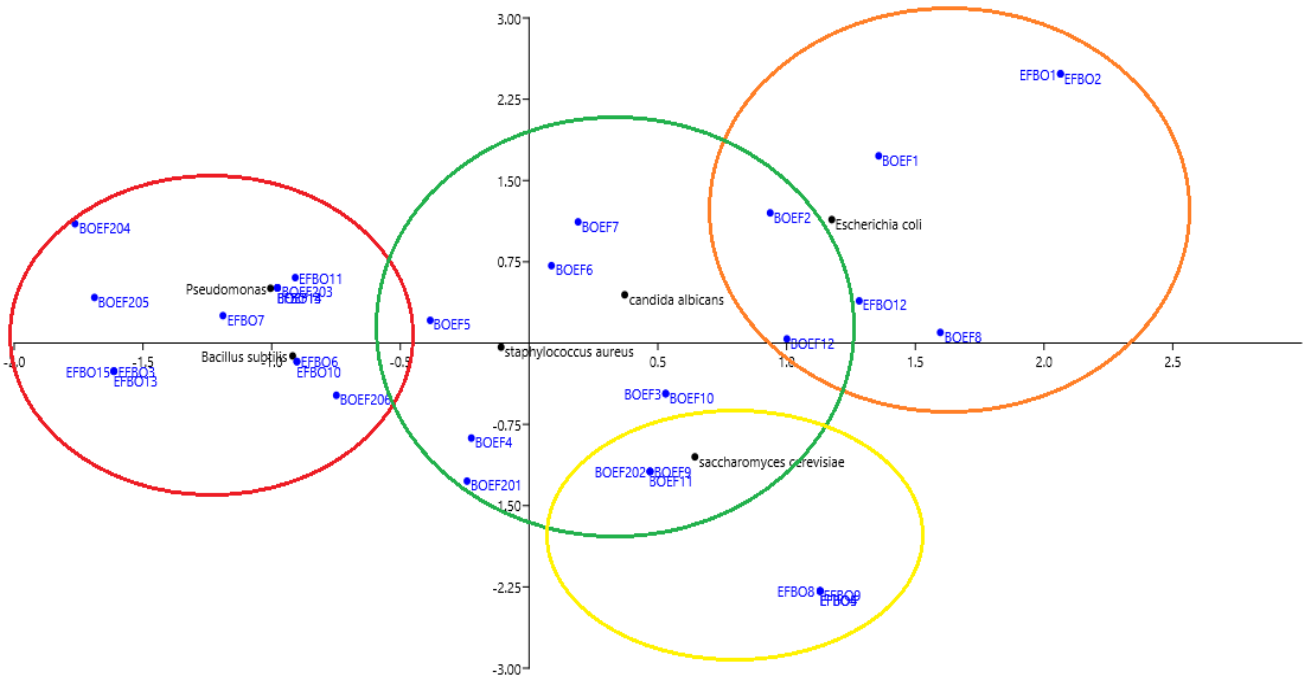

Figure 6: CFA showing Actinomycetes strains distribution according to their antimicrobial activity

Correlations factorial analysis (Figure 6) reveals that all studied actinomycetes showed antimicrobial activity against tested microorganisms. The majority of isolated actinomycetes produced antibacterial substances against Gram-positive bacteria, but they were less active against Gram-negative strains and specifically against *E.coli.* Furthermore, isolated actinomycetes strains showed a large spectrum of action against both tested yeasts, in particular against *Saccharomyces cerevisiae*.

Indeed several subsequent works have corroborated the remarkable antibacterial power of actinomycetes (McKenzie et al., 2010 ; Mythili & Ayyappa Das, 2011 ; Ng & Amsaveni, 2012; Sapkota et al., 2020). Gram-negative bacteria have an outer membrane consisting of polysaccharides, which makes cell wall impermeable to certain compounds; unlike Gram-positive bacteria, which have only an outer peptidoglycan layer that does not constitute an effective barrier and this, as had been shown by (Scherrer et al., 1971). Gram-negative bacteria have been shown to be more resistant to antibiotics than Gram-positive bacteria (Prescott, 2013). Streptomyces are widely studied due to their ability to produce a wide range of antibiotics (Hopwood & Hopwood, 2007). According to (Denizci, 1996), out of 356 isolated Streptomyces species, 36% of the isolates possess antibacterial activities against *Staphylococcus aureus* and *E. coli*. Similar result is encountered in the work of (Oskay et al., 2004) where on a total of 17 actinomycetes, strains isolated from soil, only two strains were active against *E. coli*, moreover according to (Sapkota et al., 2020), among 19 strains only 7

strains showed an activity against *E.coli* while 13 strains showed an activity against *staphylococcus aureus* which is more or less in agreement with our results.

Similarly, it was pointed out that the results are also in correlation with those of (Messaoudi et al., 2020) who report that among over 40 tested strains, 12 isolates showed antimicrobial activity against at least gram-positive bacteria, and six isolates were active against at least one gram-negative bacteria. As for the activity against yeasts, our results seem to be in agreement with those found by (Lemriss et al., 2003; Messaoudi et al., 2020) having shown that strains belonging to the genus Streptomyces show a good antifongic activity against *Candida* yeast.

3.3. Composting tests

3.3.1. . Compost physico-chemical parameters kinetics

The changes in Temperature and pH during composting process are shown in Figure 7.

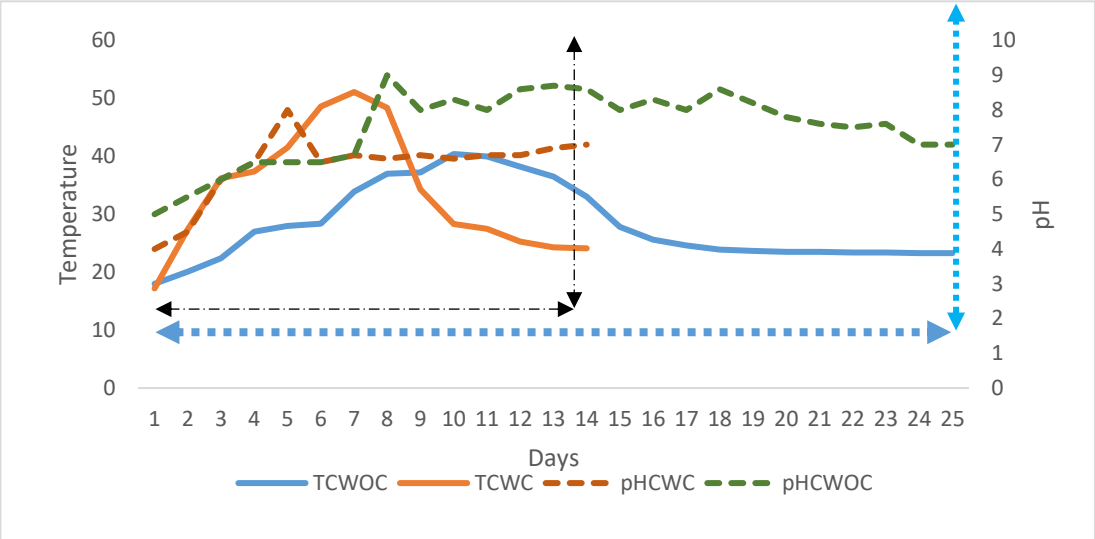

Figure 7: pH and Temperature kinetics

TCWC: Temperature of the inoculated Compost; TCWOC: Temperature of the un-inoculated compost pHCWC: pH of the inoculated compost ; pHCWOC : pH of the un-inoculated compost

Initial pH were acidic for both composts who increases to neutralization (pH=7) after 14 days for CWC compost, but reaches this value for the CWOC compost after 25 days. Microbial activity largely depends on pH conditions in composting environment. This rather rapid pH neutralization is the result of volatilization and microbial decomposition of organic acids, and ammonia release by microbial mineralization of organic nitrogen sources (Caceres et al. 2006). Thus, which confirms pH difference for the two process.

Concerning temperature, for CWOC compost, first peak appears after 8 days and maximum temperature (42°C) is reached on the 11th day. However, for the CWC compost, temperature peaks appear from 4th day and reach a maximum value of 52°C on the 7th day. This is due to actinomycetes bacteria inoculation consortium of that accelerated composting process while

causing a temperature increase. It should be noted that changes in compost temperature generally reflect microorganisms activity and overall progress of composting (Greff et al., 2022). Thermophilic phase is a crucial part of composting process because cellulose and various phytotoxic compounds are degraded by thermophilic bacteria (Greff et al., 2022).

Inoculated compost took 14 days to mature, while un-inoculated compost took 25 days, which can be explained by the fact that actinomycetes bacteria accelerate composting process because of their decomposition hydrolytic capacities. Our result is consistent with that of (Barrena et al., 2006) who reported that compost inoculation with a commercial inoculum (MicroGest 10X, Brookside Agra L.C.) allowed an optimization in terms composting time reduction (approximately 50% reduction) and economics treatment. Thus, a study by (L. Meng et al., 2017) showed that composting time was reduced by 40-43% by a mixed inoculation of Bacillus *(B. licheniformis, B. amylolitica, B. subtilis, and B. thermophilus*). Our result confirms the finding of (J. Wang et al., 2019) who reported that the addition of biological conditioners as Actinomycetes accelerate organic matter decomposition in compost, and reduce composting time.

The values of pH, electrical conductivity (CE), total nitrogen (TNK) and total organic carbon (TOC) of both composts (CWC and CWOC) are given in table 3.

Table 3: physico-chemical parameters of the final composts (mean ± SD)

	pH	CE (ms/cm)	TNK (%)	TOC (%)	C :N
Inoculated Compost (CWC)	7.8±0.08	2.69±0.10	1.18±0.01	36.15±0.08	30.63
Un-inoculated Compost (CWOC)	7.63±0.09	1.87±0.05	0.84±0.01	43.91±0.06	51.55

Measured parameters values (pH, CE, TNK) in the inoculated compost are higher than in un-inoculated compost. The inoculated compost has a EC value of 2,69 ms/cm, this increase is explained by the fact that the inoculum has favored degradation of nitrogenous organic substances and soluble salts production. Similar results were reported by (Awasthi et al., 2014), this latter worked with a thermophilic fungal consortium. Also, (Gong et al., 2017) reported high EC values in composts inoculated with two fungi (*Trametes versicolor* and *Phanerochaete chrysosporium*). The EC values of the two composts doesn't exceed 3mS/cm. (Gómez-Brandón et al., 2008) suggested that composts that are applied to soil should not have EC values higher than 3.0 mS/cm . Therefore, the produced composts were suitable for soil application. The TNK value (1.18) of the inoculated compost is higher than that of the control (0.84), which

makes the inoculum interesting. According to (Maeda et al., 2017), good nitrogen retention during composting process is essential to generate a high quality compost product as an organic fertilizer. Nitrogen loss reduction can be achieved primarily by inhibiting ammoniation, reducing nitrogen emission as NH_3, increasing nitrification, and promoting the existence of nitrogen in the form of nitrate, which is readily used by plants. Relevant research has indicated that organic waste enriched with microorganisms can effectively mitigate nitrogen losses and greenhouse gas emission through microbial assimilation (L. Meng et al., 2017). Our results are consistent with those of (Jiang et al., 2015; Milanovic et al., 2019) having confirmed that microbial inoculants containing nitrogen-fixing bacteria, in addition to bacilli and actinomycetes, were used in inoculated systems, can promote the composting process by decreasing nitrogen losses, removing phytotoxic compounds, and producing exopolysaccharides. Therefore, according to a study conducted by (Gaind et al., 2009), after the addition of a complex fungal inoculant consisting of *Aspergillus awamori, Aspergillus nidulans, Trichoderma viride, and Phanerochaete chrysosporium* to a mixture of poultry droppings and wheat straw, a rapid decrease in the C:N ratio was observed due to the higher TKN content. However, TOC value of inoculated compost is lower than control. Apparently, inoculation affected carbon and nitrogen availability, while the changed resources would balance biological metabolisms and energy efficiency in the composting system (Xie et al., 2021). (Jiang et al., 2015) reported that an initial inoculation with 1% of a bacterial culture facilitated composting process by reducing N loss and improving TOC degradation rate. In addition, Microbial additives can improve C degradation and N retention, resulting in a product with a reduced C/N ratio.

3.3.2. Compost effect on plant growth

The data in Table 4 show the effect of the two compost (CWC and CWOC) application on Vicia Faba L growth.

Table 4: Response of faba bean to the different composts (mean ± SD)

	C	CWC	CWOC
Root system (RS) Weight (g)	3,88±1,17a	4,88±6,81a	3,5±3,80a
Aerial System (AS) Weight (g)	47,78±11,61a	61,6±7,26a	37,56±0,62a
RS/AS (g)	12,15±a	12,62±a	10,73±a
Primary Root Length (cm)	10,56±1,85a	16,3±0,62b	13,14±0,84a

C: unfertilized soil; CWC: inoculated compost ; CWOC: un-inoculated compost
Different letters in column indicate significant dfferences according to the SNK test at $P \leq 0.05$.

Soil fertilization with inoculated compost significantly influenced *vicia faba L* growth, where it is noted that aerial and root system weight values as well as primary root length are significant

compared to control soil and soil fertilized with non-inoculated compost. This may be explained by Actinobacteria can increase shoots and roots length of various plants and such an increase may have benefits for the plants in terms of growth (Greff et al., 2022).

ANOVA I analysis show that differences between parameters are statistically significant (F=41,17>F'=6,94 and P=0,00). These results agree with those of (Lamsal, 2013) who reported influential growth in tomato using rhizobacterial isolates, where root and plant length were 26% and 18% longer, respectively. According to (Dicko et al., 2018), actinomycete inoculums increased maize growth in greenhouse. As a result, actinomycetes can improve plant growth when inoculated in the field by compost.

4. Conclusion

Nowadays, it is evident that actinomycetes are one of the most attractive sources of new enzymes and bioactive metabolites. This work proves to be of great interest in acquiring and understanding knowledge about actinomycetes isolated from an irrigated soil with olive mill wastewater (OMWW) that is a very specific ecosystem with respect to occurrence of new microflore and holds promising sources of extracellular enzymes and antimicrobial compounds. Enzymatic profile showed that the strains are capable of hydrolyzing most of studied substrates. They have optimal growth on media containing different ranges of NaCl concentrations (2%-8%), temperature (37°C-40°C) and pH (3-7), thus they have good antimicrobial power. Inoculation of a microbial additive into the compost can improve efficiency in terms of lower total organic carbon content and lower carbon to nitrogen ratio. The results of the study showed that inoculated compost stimulates and improves the growth of *Vicia faba*. Isolated actinomycetes present a high diversity, representing a vast unexplored resource; their metabolic reserves offer an important field of research. In this sense, obtained results during this study, underline biotechnological potential of some of the isolated strains, and can contribute to the development of new industrial processes such as pharmacy, agriculture and especially composting.

Chapitre IV

Promotion de l'agriculture durable : Évaluation de l'impact du compost inoculé par Streptomyces sur paramètres physiologiques et biochimiques de *Vicia Faba L*

Promotion de l'agriculture durable : Évaluation de l'impact du compost inoculé par Streptomyces sur la croissance, le rendement et les paramètres biochimiques de *Vicia Faba L*

Synthèse

Le chapitre 3 vise à synthétiser une étude comparative portant sur les effets de deux types de compost sur la croissance et les paramètres biochimiques du Fève (*Vicia Faba L*). L'objectif de cette recherche est d'évaluer l'efficacité de deux traitements de compost, à savoir un compost non inoculé (CS) et un compost inoculé avec *Streptomyces sp* (CA), sur les caractéristiques agromorphologiques, la qualité des graines et leur composition.

La fève (*Vicia Faba*) a été choisie comme plante model en raison de son importance agronomique et nutritionnelle. Malgré la présence de composés antinutritionnels tels que les tanins, la fève est riche en protéines, en glucides, en fibres, en vitamines et en minéraux, ce qui lui confère une valeur nutritionnelle élevée. Elle joue également un rôle important en tant que culture de rotation dans les systèmes céréaliers intensifs, contribuant à la prévention de l'érosion des sols.

Dans le cadre de cette étude, deux types de compost ont été évalués pour leur impact sur la croissance du haricot et la qualité des graines. Le compost inoculé avec Streptomyces (CA) a été comparé au compost non inoculé (CS) et aux plantes non fertilisées (T). Différents paramètres ont été mesurés, notamment la teneur en chlorophylle, la teneur en protéines, la teneur en matières grasses, la teneur en sucres, les éléments minéraux, le pourcentage de germination, le schéma de floraison, le poids, l'épaisseur, la longueur, la largeur des gousses et des graines.

Les résultats ont montré que le compost inoculé avec *Streptomyces* (CA) avait un effet significatif sur la germination, le rendement et la qualité des graines par rapport au compost non inoculé (CS) et aux plantes non fertilisées (T). Le compost CA a présenté une amélioration notable de la teneur en éléments minéraux des plantes, ainsi qu'une diminution de la teneur en tanins et en matières grasses des graines. Cela suggère que l'inoculation du compost avec *Streptomyces* favorise une meilleure assimilation des nutriments par les plantes, ce qui se traduit par une croissance plus vigoureuse et une meilleure qualité des graines.

L'utilisation du compost inoculé avec *Streptomyces* a permis d'améliorer significativement la germination des graines, le rendement des plantes et la qualité des graines en termes de teneur en éléments minéraux, de sucre total, de teneur en protéines et de contenu en flavonoïdes. De

plus, le compost inoculé a réduit la teneur en tanins et en matières grasses des graines, ce qui améliore leur qualité nutritionnelle et leur valeur commerciale. Ces résultats suggèrent que l'inoculation du compost avec des micro-organismes bénéfiques tels que *Streptomyces* peut être considérée comme une stratégie pour améliorer l'efficacité des engrais organiques et réduire la dépendance aux engrais chimiques. Cette approche favorise une meilleure assimilation des nutriments par les plantes, ce qui se traduit par une croissance plus importante et une meilleure qualité des graines.

En conclusion, cette étude démontre l'importance de l'utilisation de compost inoculé avec *Streptomyces* pour améliorer la croissance et la qualité des plantes. Les résultats obtenus soutiennent l'utilisation de méthodes durables en agriculture, telles que le compostage, afin d'optimiser la productivité des cultures tout en préservant l'environnement. Les avantages du compost inoculé avec *Streptomyces* sont multiples. En plus d'améliorer la germination, le rendement et la qualité des graines, il entraîne une augmentation de la teneur en éléments minéraux et en sucres, ainsi qu'une réduction de la teneur en tanins et en matières grasses des graines. L'utilisation du compost inoculé favorise également une meilleure assimilation des nutriments par les plantes, ce qui permet d'optimiser l'efficacité des engrais organiques. Cela contribue à réduire la dépendance aux engrais chimiques et à diminuer les impacts néfastes sur les écosystèmes.

Les résultats de cette étude soutiennent l'utilisation du compost inoculé avec *Streptomyces* comme une stratégie viable pour améliorer la croissance et la qualité des plantes. Cette approche offre des perspectives intéressantes pour des pratiques agricoles durables, en favorisant la fertilité des sols et en réduisant l'utilisation d'engrais chimiques. Cependant, des recherches supplémentaires sont nécessaires pour approfondir notre compréhension des interactions entre le compost, les micro-organismes bénéfiques et les plantes, ainsi que pour optimiser les techniques de compostage et d'inoculation afin de maximiser les bénéfices pour les cultures et l'environnement.

En conclusion, cette étude contribue à la recherche de solutions agricoles respectueuses à l'environnement et la durabilité. L'utilisation de compost inoculé avec *Streptomyces* peut constituer une alternative efficace pour améliorer la productivité agricole tout en réduisant l'utilisation d'engrais chimiques et les impacts néfastes sur les écosystèmes. Ces résultats encouragent la mise en œuvre de pratiques agricoles plus durables, préservant ainsi les ressources naturelles et soutenant une production alimentaire de qualité.

Promotion of Sustainable Agriculture: Assessment of the Impact of Streptomyces-Inoculated Compost on the Growth, Yield, and Biochemical Parameters of *Vicia Faba L*

This part of the work was the subject of an article submitted for publication.

Abstract

The development of green approaches to enhance plant growth and crop productivity is important for sustainable agriculture. Therefore, a comparative study of the effects of two types of compost on the growth and biochemical parameters of the bean (*Vicia Faba L*) was carried out . An essay was conducted at the experimental station belonging to the Faculty of Science and Technology in Fes, three treatments were compared: Unfertilized control (T), a trial fertilized with a non-inoculated compost (CS) and a trial fertilized with a compost inoculated with *Streptomyces sp* (CA). In this study, some chemical and physical properties have been determined. Chemical properties such as chlorophyll content, protein content, fat content, sugar content, mineral elements and physical properties such as germination percentage, flowering pattern, weight, thickness, length, width of pods and seeds involved in the study. Application of compost inoculated with streptomyces (CA) gave the highest germination rate (67.4%), while the CA compost result was almost the same as the control. Compared to the two fertilized experiments CA and CS, the unfertilized one (T) had a lower chlorophyll index. The CA compost increased yield and seed quality (Total sugar, protein content, flavonoides content) and decreased seeds tanins content and lipid content. The results indicated that both compost treatments stimulated the growth of *Vicia faba* and improved their mineral content, but CA compost provided better results than the CS. We conclude that inoculated compost had a main role in improving growth parameters and yield of *Vicia Faba* plant. Furthermore, the results obtained recommend its use as an alternative tool to stimulate plant growth and reduce chemical fertilizers.

Keywords: Compost, Vicia Faba L, Inoculated Compost, Streptomyces, plant growth promotion

1. Introduction

In recent times, the environment has been severely affected by the continuous application of pesticides and various synthetic fertilizers. The unrestricted use of various pesticides and inorganic fertilizers affects soil fertility, resulting in loss of productivity. Therefore, an alternative approach is needed to restore soil fertility. The ecological approach is an alternative method to reverse soil fertility and plant productivity by using various microorganisms. These microorganisms reduce the C:N ratio and improve soil productivity (Umsakul et al., 2010). In general, the composting process involves many steps using various microbial consortia, such as fungi, bacteria, and actinomycetes, which collectively participate in humus formation. Composting is an environmentally friendly method of reducing biodegradable organic waste into highly nutritious organic fertilizers (Al-Dhabi et al., 2019; Zeng et al., 2011). Microbial fertilizers are environmentally friendly, inexpensive, and non-bulky agricultural inputs that play an important role in plant nutrition as an additional and complementary factor to mineral nutrition (Chinthapalli et al., 2015; Mahajan & Gupta, 2009). Composted organic waste has been used to improve horticultural and agricultural soil to enhance crop growth and soil fertility. Biofertilizer has been defined by many authors, including (Rashid et al., 2016), who defined it as products containing living cells of different types of microorganisms which, when applied to seed, plant surface or soil, colonize plant rhizosphere or it interior and promote growth by increasing primary nutrients supply to the host plants. Therefore, research on fertilizers and plant nutrition should establish a viable relationship between environmental preservation and fertilizers.

Faba beans (*Vicia Faba*) are widely used for animal feed more than for human consumption (Shabuol & Obeidat, 2022; Vioque et al., 2012). Indeed, although faba bean contains some anti-nutritional compounds such as tannins, vicin and convicine (Crépon et al., 2010; Kumar Sinha & Kumar, 2018). it has a good nutritional value being rich in proteins, carbohydrates, fiber, vitamines and minerals (Ofuya & Akhidue, 2006). Faba bean also plays an important agronomic role in acting positively as a break crop in intensive cereal dominated crop rotations, also preventing soil erosion (Köpke & Nemecek, 2010). In Morocco, fava beans are widely used as a main meal or as an ingredient in various traditional recipes, in the form of fresh ripe seeds or dry seeds, the tender skin of bean pods was also used in certain specific dishes, thus diversifying the diet. In addition, it has been extensively cultivated as green manure as well as for feed and food.

Growth and productivity of the broad bean is affected by many factors, including chemical and organic fertilizers, where N P K fertilizer is necessary to increase production. Plant nutrition is

the most important factors affecting growth and yield. Broad bean is highly responsive to N fertilization and N is usually the most limiting essential nutrient for Broad bean growth. Thus, the use of organic fertilizer as a substitute for the chemical fertilizers improve the soil structure,increase roots efficiency on the absorption of water from soil, stimulate microorganisms activity in soil and thus improve plant growth and their quality (Nuaimi, 2011). The objective of this research is to evaluate the effect of the application of two compost treatments (the first one is inoculated with a consortium of *streptomyces sp,* the second compost is not inoculated) on a specie of *vicia faba L* plant, to compare agromorphological traits, seed quality and composition.

2. Material and methods

2.1. Plant cultivation

Faba bean (Vicia faba var. major Harz) cv. Aguadulce was cultivated at experimental station belonging to the Faculty of Science and Technology in Fes. The experimental design was a plot divided into three parts. Each part included three replicates of each treatment; three treatments were compared: Unfertilized control (T), a fertilized trial with an un-inoculated compost (CS) and a fertilized trial with a *Streptomyces sp* inoculated compost (CA).

2.2. Physical parameters

2.2.1. Germination percentage and Flowering pattern

Germination percentage (% G) was calculated according to the following equation: (Baysah et al., 2018)

$$\%\ of\ Germination\ = \frac{number\ of\ germinated\ seed}{number\ of\ total\ seeds\ planted} \times 100$$

Regarding the flowering pattern, from flowering onset, ten plants from each plot were randomly tagged and marked. The number of flowers was measured during flowering period, from first flower appearance to flowering end period, in order to determine following characteristics: Number of flowers/plant produced (Nfp) and total abscission/plant percentage (%abs) according to the equation:

$$\%\ of\ abscission/plant = \frac{(\text{number of flowers per plant} - number\ of\ mature\ pods\ per\ plant)}{(\text{number of flowers per plant})} \times 100$$

2.2.2. Vegetative growth

A random sample of 10 plants from each plot was taken at 42 days after sowing and following vegetative characters were recorded: plant length (Pl), number of branches (Nb), and number of pods per plant (Npp) as well as total plant fresh weight (Pw).

2.2.3. Pod quality

A random sample of 10 pods from each plot was taken and morphometric parameters was tested (weight (wp), length (Pol), width (Wtp) and thickness (Tp), number of seeds per pod (Nsp) as well as 100 seed weight) were recorded.

2.2.4. Seed quality

A random sample of 50 seeds from each treatment was taken and the following characters were measured: weight (Sw), length (Sl) , width (Swd) ,thickness (St) and the seed moisture content (MC) . The seed moisture content (MC) of tested cultivars (three replicates) was assessed based on wet weight (AACC, 2000). The seeds of each cultivar were ground by an electric coffee grinder and 2 g of each was weighed by an electronic balance .and oven-dried (130 ◦C for 3 h). The seed MC (%) was measured using following equation:

$$\text{MC (\%)} = \frac{\text{weight of wet seed} - \text{weight of dried seed}}{\text{weight of wet seed}} \times 100$$

2.3. Chlorophyl content

Chlorophyll content was measured according to (Arnon, 1949) method in which 100 mg of fresh leaves were crushed in 20 ml of 80% acetone and the extract was centrifuged for 10 min at 1000 rpm. Supernatant absorbance was recorded at 645 and 663 nm in a UV spectrophotometer. Total chlorophyll content (expressed as mg/g FW of each sample) was estimated according to (Arnon, 1949; Bansal et al., 1999; Chinthapalli et al., 2015) as follows:

$$\text{Total chlorophyll (mg/g}^{-1}) = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times V/1000 \times W$$

Where A = absorbance at the given wavelength, W = weight of fresh leaf sample, V = final volume of chlorophyll solution.

2.4. Biochemical parameters

Seeds were first dried and then ground into a powder and stored in a closed container at room temperature for future nutritional profiling. Maceration was carried out according to the protocol of (Nshimiyimana & He, 2010) with some modifications. 5 g of faba bean powder was then mixed with 20 ml of distilled water, incubated under stirring for 2 hours, and then filtered. This operation is repeated 3 times, and then the resulting solution was concentrated with a rotary evaporator. The final resulting extract is kept at 4 C for biochemical analysis.

2.4.1. Total flavonoids content determination

Determination of total flavonoids in extracts was performed according to the spectrophotometer method described by (Dehpour et al., 2009) , and results are expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g Dw).

2.4.2. Polyphenols content determination

Total phenolic content of the seed extract was determined by spectroscopy at 725 nm, based on a colorimetric reaction with Folin-Ciocalteu reagent, as described by (P. Saini et al., 2016). Results were expressed as gallic acid equivalent in mg/g on a dry matter basis(DM).

2.4.3. Condensed tannins content determination

The tannins are determined in extracts by the Bate Smith method as described by (Ribérau & Stonestreet, 1966; Wilhelmy et al., 2021). Analysis must be performed in parallel using two glass tubes, where only one tube is submitted to acidic hydrolysis. The condensed tannin content is calculated according to the following equation:

$$\text{Tannins (mg/gDM)} = (\text{tube B hydrolyzed} - \text{tubeA control}) \times 19.33$$

2.4.4. Hydrolysable tannins content determination

Hydrolysable tannins were determined according to the method described by (Willis, 1998; H. Zitouni et al., 2020). Results were expressed as tannic acid equivalent per dry matter (mg TAE/g DM).

2.4.5. Reducing sugar content determination

Reducing sugar content was measured by the DNS method using glucose as standard and expressed as mg glucose per g dry weight (mg G/g DM) (Miller, 1959).

2.4.6. Total sugar content determination

For better quantification of total soluble sugars, 100mg of faba bean powder is refluxed with 10ml of HCl (10N) for one hour, and then this solution is neutralized in 10ml of NaOh. Resulting solution is considered as an extract for the determination of total sugars according to the method of (DuBois et al., 1956). Results were expressed as glucose equivalent per dry matter (mg G/g DW).

2.4.7. Protein content determination

Protein content is estimated by the Lowry method (Hartree, 1972; Lowry et al., 1951). Before that, 100 mg of powder is refluxed for 30 minute with 10 mL of 0.5 N NaOH and filtered. 100 uL of filtat is diluted with water to 1 mL, then 5 mL of Lowry's reagent is added, mixed and incubated for 10 min at room temperature. Folin's phenolic reagent (0.5 ml of 1 N) is added and the solution is held for 30 min at room temperature after mixing. The absorbance is measured at 660 nm. Protein is reported in percent (%).

2.4.8. Lipid content determination

Seed powder (10 g) was packed in Whatman No Whatman No. 1. Cartridge was placed in a Soxhlet extraction flask and extracted with hexane (125 mL; 150 drops/min) by heating the contents at reflux for 6h to extract the lipids present. After recovery of lipids by repeated washings of the flask with hexane and drying at 105°C for 1 hour for traces of moisture and hexane evaporation (Rajendra et al., 2005). Lipid content (%) was calculated as follows:

$$the\ lipid\ content(\%) = \frac{\text{weight(flask + fat)} - \text{weight(flask)}}{\text{weight seed powder}} \times 100$$

2.4.9. Analysis of Micronutrients and macronutrients with Microwave Digest and ICP-MS Analysis

The seed samples were analysed by weighing 0.1 g of sample; adding 2.25 mL HNO_3 + 0.5 mL HCl + 2.25 mL water; and then placing the samples in a microwave oven, increasing the temperature to 200 °C for 20 min, and holding the samples at 200 °C according to the CEM procedure, Microwave Digestion of Feed Grains (Khazaei & Vandenberg, 2020). The analysis was conducted after digestion using a solution of 2.25% Nitric acid and 0.5% perchloric acid using an Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) in the Regional University Center of Interface (CURI) attached to the University Sidi Mohamed Ben Abdellah (USMBA) of Fe

3. Results and discussion

3.1. Physical parameters

3.1.1. Germination percentage and Flowering pattern

The following figure (Figure1) shows the effect of the two different compost compared to a control soil (T) on germination rate (%G) and abscission percentage (%Abs) of *Vicia Faba* plant. It can be seen that the application of the compost inoculated with *streptomyces* (CA) gave the highest germination rate (67.4%) and the lowest abscission percentage (42.3%). Thus, there is no great difference between the results obtained when applying the non-inoculated compost (CS) and the control soil (T).

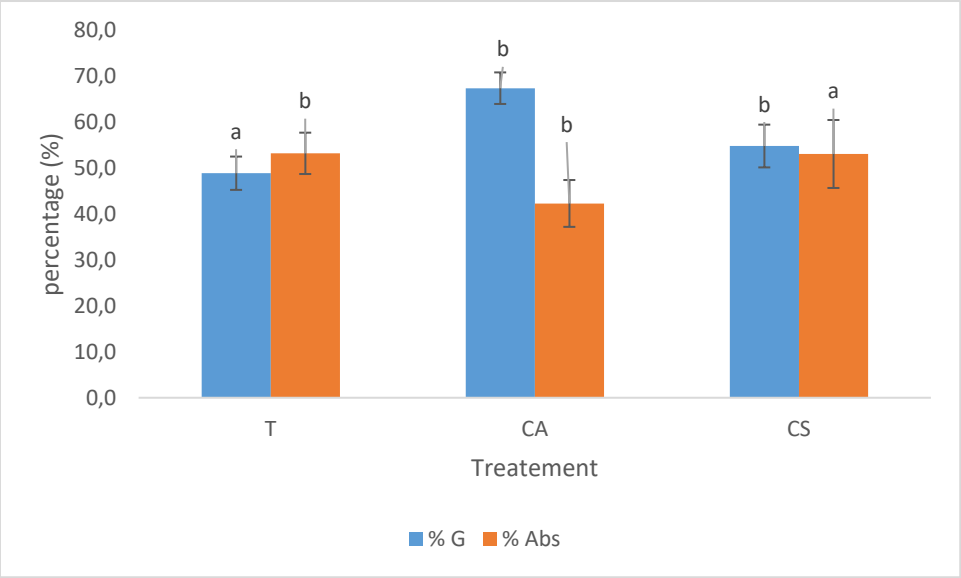

Figure 1: Effect of inoculated and un-inoculated compost on germination percentage (%G) et abscission percentage (%Abs) of *faba bean* plant

Our results of germination rate agree with those of (Abdelhamid et al., 2004) who reported values ranged from 71.1% to 81.6% when applying a biofertilizer, also (Chinthapalli, 2015) showed that the application of an organic fertilizer showed a high frequency of 92-96% germination compared to inorganic fertilizer. This can be explained according to (Zucconi, 1981), that germination percentage higher than 50% indicate that a compost is free of phytotoxins, which explains the good quality of the CA treatement.

3.1.2. Vegetative growth

The data in Table 1 show that the growth parameters of Vicia faba presented by plant height (Pl), number of branches (Nb), as well as total plant fresh weight (Pw), pod weight (wp), pod length (Pol), pod width (Wtp) and thickness (Tp), number of seeds per pod (Nsp), seed weight (Sw), length (Sl) , width (Swd) ,thickness (St) and 100-seed weight and seed moisture content (MC) were significantly improved when the soil was treated with the inoculated compost, while there were no significant differences between the treatment with the non-inoculated compost

and the control soil. Compared to the two fertilized experiments CA and CS, the unfertilized one (T) had a lower chlorophyll index. This can be explained according to (J.-H. Chen, 2006), that biofertilizers include different types of free-living microorganisms that have the ability to convert nutrients from the unavailable form to the available form and produce factors that stimulate plant growth through biological processes. One more reason why our inoculated compost (CA) gave better results. Thus, the significant results of the *vicia faba* plant vis-a-vis the two compost is explained by the fact that this latter are rich in organic matter, these organic matters are very promising because of their local availability as a source of multiple nutrients and their ability to improve soil characteristics (Khaliq et al., 2006b).

Table 1: Effect of the two compost treatement on the vegetative growth of *Vicia Faba* plants (mean ± SD).

		Treatement		
		T	CA	CS
Vegetative growth	Pl (cm)	94.8±4.44b	100.2±1.93a	95.6±2.57ab
	Nb (N/plant)	16.6±1.85a	15.4±1.82a	16.4±1.35a
	Npp (N/plant)	5.2±0.74b	7.8±0.72a	6.4±1.01b
	Pw (g/plant)	66.5±6.13b	96.38±5.71a	67.98±7.07b
Chlorophyll content	chlorophyl content (mg/gMF)	0.68±0.03c	1.034±0.07a	0.88±0.05b
Pod properties	Pol(mm)	18.2±1.63c	24.2±1.20a	21.6±0.48b
	Wtp(mm)	18.31±1.17b	20.03±0.76ab	20.13±0.73a
	Tp(mm)	13.35±1.37a	14.34±0.64a	14.42±1.03a
	Wp(g)	27.08±3.84b	35.62±4.13a	34.58±2.18a
	Nsp	5.2±1.01a	6.1±1.09a	5.3±1.2a
Seed properties	Sl(mm)	19.9±1.88b	23.12±1.05a	21.68±1.27ab
	Swd(mm)	14.45±2.13a	17.04±1.11a	15.66±1.57a
	St(mm)	8.69±0.99a	9.90±0.51a	8.978±0.81a
	Sw(g)	1.88±0.44a	2.54±0.24a	2.26±0.39a
	100 seed weight(g)	159.2±2.28c	192.5±1.65a	170.9±1.98b
	%MC	81.08±1.05a	79.26±1.04a	76.02±2.65a

T:unfertilized soil , CA : inoculated compost fertilization , CS : un-inoculated compost fertilization respectively). Different letters in column indicate significant differences according to the SNK test at $P \leq 0.05$.

Our results are consistent with those of (Bezabeh et al., 2021) who reported that the application of compost inoculated with efficient bacteria has increased significantly the grain yield of faba beans compared to traditional compost. Also (Jabeen, 2005) had noticed that plant height and dry weight/plant were significantly affected by the use of different bio-organic amendments. Organic matter holds great promise because of its local availability as a source of multiple nutrients and its ability to improve soil characteristics (Khaliq et al. 2006). (Cucci et al., 2019) has reported that an unfertilized (control) trial produced lower plants, less vigor, with lower

chlorophyll index, fewer shoots per plant, lower shoot dry biomass and fewer pods per plant and seeds per pod. Some research has suggested that integrated nutrient management strategies that involve inoculating grains with *Azotobacter sp.* and *Azospirillum sp.* in combination with fertilizers improve the growth of different crops (Namvar & Khandan, 2013; Piccinin et al., 2013; V. K. Saini et al., 2004).These results may be based on the ability of the microorganisms incoculated in the CA compost to produce certain growth regulating substances, capable of playing a prominent role in plant growth by stimulating photosynthesis, and translocation.

3.2. Biochemical parameters

3.2.1. Biochemical parameters

Data recorded in Table 2 show that total sugars, reducing sugars, total polyphenols, flavonoids and protein content were affected by the application of inoculated compost where it is noted that the values of these different biochemical parameters are elevated in the bean seeds fertilized with CA compost compared to the CS, which does not differ from the beans of the control soil.

On the other hand, the application of the inoculated CA compost decreased slightly the rate of condensed and hydrolyzable tannins as well as the rate of lipids.

Table 2: Chemical composition of faba been seeds (on dry weight basis).

	Treatement		
	T	CA	CS
Total sugar mgEQg/gDM	29.32±0.37a	30.13±0.31a	29.13±0.68a
Reducing sugar mgEQg/gDM	2.24±0.08b	3.90±0.38a	3.64±0.33a
Condensed tannins gEQTA/L = mgEQTA/g DM	6.83±0.66b	4.38±0.18a	6.06±0.66b
Hydrolyzable tanins mgEqTA/gDM	2.27±0.02c	1.44±0.17a	1.96±0.30b
Total polyphenol mgEqGA/gDM	1.52±0.34c	1.62±0.25a	1.6±0.26b
Flavonoides content mgEqQ/gDM	0.24±0.01b	0.34±0.03a	0.22±0.01b
Protein content (%)	29.28±0.80b	32.07±0.91a	31.59±0.87a
Lipid content (%)	3.2±0.12b	2.8±0.08a	3.23±0.21b

Values are averages with standard errors from triplicate experiments. Different letters within same line indicate significant differences at $p < 0.05$ (Tukey's HSD test).

For total soluble sugars and reducing sugars, our values are consistent and fall within the range found in research by several authors (El-Refai et al., 1988; A. Kumar et al., 2015a)who reported total sugars values ranging from 18 mg/g(A. Kumar et al., 2015b)to 40 mg/g (El-Refai et al., 1988) on dry weight basis. In addition, these same authors noted reducing sugars values ranging from 1.01 to 5 mg/g on dry weight basis.

Regarding the content of flavonoids, the results obtained are close to those found by (Boukhanouf et al., 2016) with a value of 0.167 mgQ/g DM and (Rahate et al., 2021) with a value ranging from 0.08 to 0.16 mgQ/g DM. In addition, for total polyphenols, our results are in agreement with those found by (Baginsky et al., 2013) who found a value of 1.067mgGA/g DM and (A. Kumar et al., 2015b) with a value of 1.4 mgGA/gDM. Our results for condensed tannin content agree with those of (Oomah et al., 2011) and are within the range found by this latter from 0.76 to 5.24 mgQ/g DW. For hydrolyzable tannins, the value found in the control beans agree with that found by (El-Sharawy et al., 2022) with values between 2.14 and 5.06 mgTA/gDM , on the other hand the results of the beans treated by both compost are slightly lower than the values previously cited. Regarding protein content, our results corroborate with those found by (A. K. Singh et al., 2014) (30 to 31%), (A. Kumar et al., 2015a) (26 to 32%) and (Khazaei & Vandenberg, 2020)(28 to 29%). The lipid content values of our beans are within the range of those reported by (Prabhu & Rajeswari, 2018)(2.30-3.21%) , but are higher than those found by (Martineau-Côté et al., 2022)(1.4%).

3.2.2. Analysis of Micronutrients and macronutrients with Microwave Digest and ICP-MS Analysis

The proximate Concentration of faba bean seed minerals is given in Table 3, Among the analyzed elements, B, Li, Cr, As, La, Hg, B, Be, Bi, Cd, Co, Cr, Mo, Ni, Pb, Sb, Se, Sn and Sr had values of zero. Elevated grain concentrations pose a potential threat to human health non-core carcinogens with no threshold.

Table 3: Concentration of faba bean seeds minerals (mg/g)

mg/g	T	CA	CS
K	12.93	14.7012	12.426
Mg	1.85444	1.96612	1.80084
Mn	0.0146	0.01488	0.01412
Na	0.502	0.4816	0.5284
P	5.8468	6.7532	6.6408
Al	0.01644	0.01276	0.01356
Ba	0.01236	0.00656	0.01328
Ca	2.81008	3.18356	2.82984
Fe	0.07112	0.07564	0.06
Zn	0.06444	0.069	0.06356

The results obtained showed that the potassium, phosphorus, calcium, iron, zinc and magnesium content in bean seeds from the soil treated with the inoculated compost is higher than the soil treated with the un-inoculated compost and the control. This may be due to the fact that chemical composition of Faba Bean seeds is highly dependent on genotypes/cultivars and

environmental conditions as well as agricultural management practices (Ivarsson & Neil, 2018; Meng et al., 2021; Micek et al., 2015; Pelagalli et al., 2020)). In turn, (Jarecki & Migut, 2022) reported that the content of some macronutrients and micronutrients in soybean seeds was dependent on inoculation and/or nitrogen fertilization, which can be applicable to *Vicia Faba* seeds too. Our results showed that faba beans seeds contained significant quantities (mg/g) of K (from 12.24 to 14.70), Mg (from 1.80 to 1.96), Na (from 0.48 to 0.52), P (from 5.84 to 6.75) and Ca (from 2.81 to 3.18). However, we note the presence of Mn, Al, Fe, Zn, and Ba with minimum values not exceeding 0.08 mg/g. Our results are consistent with those of multiple authors (Jarecki & Migut, 2022; Khazaei & Vandenberg, 2020; Meng et al., 2021; Sileshi et al., 2022), who obtained similar values to those mentioned in the present study. Faba Beans seeds are a good source of nutritional minerals (Cazzato et al., 2014; Meng et al., 2021), especially potassium, phosphorus, iron and zinc, while iron and zinc are essential for the subsistence and optimal physiological function of humans and livestock (Bailey et al., 2015; Meng et al., 2021).

PCA (principal component analysis), based on physicochemical properties, was used to assess the patterns of variation within vicia faba seeds from different composts applied to soil. Multivariate PCA analysis was performed by using R language as illustrated in Figure 2.

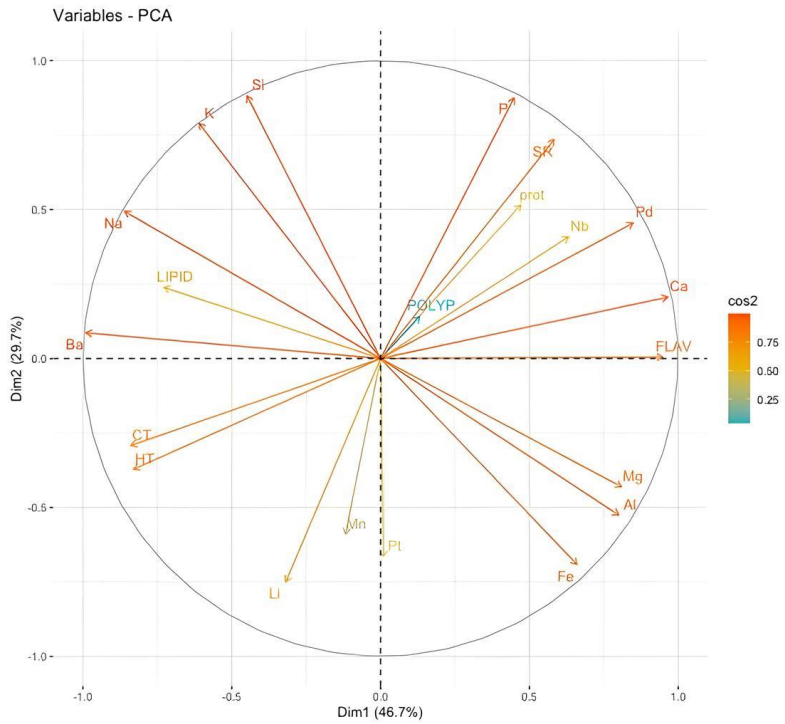

Figure 2: Principal Component Analysis: PCA biplot describing variation in chemical parameters of the three-vicia faba seeds (CA: treated with the inoculated compost, CS: treated with un-inoculated compost and T: control)

PCA (principal component analysis), based on physicochemical properties, was used to assess the patterns of variation within vicia faba seeds from different composts applied to soil. Multivariate PCA analysis was performed by using R language as illustrated in Figure 2. The first principal component (PC1) was important and accounted for 46.7% of the total variation and the second principal component (PC2) accounted for 29.7% of the variability. The Cosinus square of variables (Cos^2) represents the quality of representation of variables on the PCA chart.

PC1 was correlated with lipid, CT (condensed tannins), HT (hydrolyzed tannins), Na and Ba negatively as well as by flavonoids, polyphenols, protein, TS (total sugar) and Ca positively. PC2 was correlated with Mn, Fe, Al, Mg and Zn (negatively) and by reducing sugar, P and K (positively).

4. Conclusion

The main purpose of cultivating plants and vegetables is to provide such quality minerals and chemicals such as protein, phosphor, potassium and calcium for human nutrition. Many agrochemical and biochemical research reports that fertilizers are one of the most potent and rapid factors in affecting the chemical composition of plants and improving the quality of crops. Data collected in this study indicates that seeds produced by plants treated with streptomyces-inoculated compost are more healthy and well developed. In addition, it can be concluded that compost can provide essential nutrients for plant growth and development. Thus, the present research revealed that the integration of compost and in particular that inoculated with *Streptomyces* can improve sustainable soil fertility as well as crop yield, it is a promising approach for sustainable agriculture and can serve as an effective and alternative fertilizer to reduce chemical fertilizer consumption.

Chapitre V

Exploration and characterization of thermophilic bacteria in compost: isolation, characterization and identification of *Thermoactinomycetes Sacchari*

Exploration et caractérisation de bactéries thermophiles dans le compost : isolation, caractérisation et identification de *Thermoactinomycetes Sacchari*

Synthèse

Le présent chapitre est canalisé sur le compostage, un processus crucial pour la conversion des déchets organiques en un amendement précieux du sol. L'étude se concentre sur la compréhension du rôle des bactéries thermophiles et des actinomycètes dans le processus de compostage, ainsi que sur l'exploration de leurs applications potentielles en biotechnologie et en bioremédiation.

Pour parvenir à une compréhension de la dynamique microbienne du compostage, nous avons effectué un examen des populations bactériennes thermophiles et mésophiles présentes à différentes étapes du compostage. En utilisant des méthodes microbiennes standard, telles que le dénombrement bactérien, l'analyse de la température et de la croissance microbienne. L'étude apporte des informations sur l'abondance et le comportement des bactéries thermophiles et mésophiles tout au long du processus du compostage.

De plus, nous avons utilisé des milieux sélectifs pour isoler et identifier un actinomycète thermophile compétent à partir du compost. Ce processus d'isolement implique la collecte d'échantillons à différentes phases de compostage et leur soumission à une analyse microbienne. Les actinomycètes thermophiles sélectionnés sont cultivés dans une gamme de température (50°C, 55°C et 60°C), afin de caractériser les souches qui prospèrent dans ces conditions. Parmi les souches isolées, une attention particulière est accordée à la souche présentant la plus grande compétence à une température de 60°C. À travers sa caractérisation morphologique et biochimique, cette souche a été examinée plus en détail afin de mettre en évidence ses traits et capacités avantageuses. L'identification moléculaire pour déterminer la classification taxonomique spécifique de cette souche, révélant son identité en tant que *Thermoactinomycetes sacchari*.

En plus de l'étude des populations microbiennes et de l'isolement d'un actinomycète thermophile, le chapitre explore la succession microbienne qui se produit pendant le compostage. Il révèle un schéma cyclique, commençant par une prédominance des bactéries mésophiles aux premières étapes et évoluant progressivement vers une prévalence des bactéries thermophiles à mesure que le compost mûrit. Cette compréhension de la succession microbienne offre des informations précieuses sur le processus de compostage et met en

évidence la nature dynamique des communautés microbiennes en réponse aux changements environnementaux.

Le chapitre 4 souligne également l'importance des conditions de compostage dans la formation des populations bactériennes et met en évidence le potentiel d'isolement de souches précieuses à partir de l'environnement du compost. L'isolement de *Thermoactinomycetes sacchari*, un actinomycète thermophile peu étudié, souligne davantage l'importance de futures recherches sur ses caractéristiques et ses applications potentielles en biotechnologie et en bio remédiation. À travers son exploration approfondie de la microbiologie du compostage, ce chapitre contribue au développement de pratiques durables de gestion des déchets. En dévoilant les dynamiques complexes des bactéries thermophiles, des actinomycètes et de la succession microbienne pendant le compostage. cette recherche ouvre la voie à des approches innovantes pour utiliser le compostage dans diverses applications industrielles.

Exploration and characterization of thermophilic bacteria in compost: isolation, characterization and identification of *Thermoactinomycetes Sacchari*

This part of the work was published in the journal "Sci Afr" (Q1 - IF: 3.33).

Abstract

Composting is a crucial process for converting organic waste into a beneficial soil amendment. During this process, a diverse community of micro-organisms, including thermophilic bacteria and actinomycetes, plays an essential role in organic matter decomposition. These micro-organisms are capable of thriving at high temperatures, and have therefore received increasing attention due to their potential industrial applications such as biotechnology and bioremediation.The aim of the present study was to enumerate thermophilic and mesophilic bacteria in the various composting phases and to isolate competent thermophilic actinomycetes from this ecological niche. During the composting process, samples were taken during all the temperature fluctuations that accompanied the process. Isolation, characterization and classical identification of competent thermophilic actinomycetes were carried out using selective media. Ten thermophilic strains were isolated and grown at three temperatures namely at 50°C, 55°C and 60°C. Strains able to grow at this temperature range were characterized morphologically and biochemically, while the most competent strain growing at 60°C was subjected to molecular identification to verify its taxonomic position.The use of a controlled bioreactor in composting process can standardize the physico-chemical and nutritional conditions to ensure composting result reproducibility. Its use in this study has considerably reduced the composting cycle.

This study highlights the role that certain strains can play in the success of composting process, particularly those that are always present in the active composting population. These strains, such as *Thermoactinomycetes sacchari*, open up several issues of research into composting and other valuable applications of microorganisms. Thanks to its resistance to high osmotic pressures (20-60g/L), a wide range of hydronium potentials (pH 5-10) and high temperatures, *Thermoactinomycetes sacchari* strain is a valuable source of enzymes that can be exploited under the most stringent industrial conditions. This strain could be the focus of research into molecules produced by these microorganisms with antioxidant and anticancer activities.

Keywords: Composting , Bacterial enumeration, Thermophilic bacteria, Actinomycetes , Thermoactinomycetes sacchari

1. Introduction

Given that Morocco is one belong to MENA countries groups (Middle East and North Africa), its population growth generates a massive production of organic solid waste by industry, households and agriculture (Hemidat et al., 2022). Organic matter management is a major problem for the development of cities especially in a context where there is a tendency to create smart cities. The integration of several environmental issues in a circular economy strategy forms a means of combating solid agro-industrial and agrarian pollution. In this perspective, the Moroccan state, like other countries under other skies, has initiated emergency procedures to resolve the dilemma of solid waste management in urban and rural areas. In urban areas, the National Household Pollution Abatement Plan (PNDM) has successfully solved a significant fraction of solid waste with activated sludge refraction is a challenge for city managers (Sadik et al., 2023). In rural areas, composting is one of the most recommended solutions for managing solid waste from agricultural activity. The Generation Green project (2020-2030), highlights that composting is one of the pillars of agricultural development in Morocco. This technique thus opens up ways of using urban and rural biomass to create added value (Chaher et al., 2020; Manu et al., 2017). This is a rational way to bio-convert organic solid waste into a hygienic and valuable product (Asadu et al., 2019; S. Zhang et al., 2019). Composting reduce more than 30% of organic matter volume that ends up in already overloaded landfills (Chaher et al., 2021).

One of the handicaps that hinder composting technology development in Morocco is the lack of expertise and data on sector (Persiani et al., 2021). This raises the question of qualification and technology transfer to Morocco. With this in mind, we found it useful to channel biotechnology into environmental management through composting well adapted to local context and specific characteristic of organic solid waste in Morocco. Composting is still a very difficult microbiological phenomenon to manage with usual means and therefore requires an interdisciplinary understanding of the process. Composting can be harmful to the environment in terms of the choice of materials to be treated or during their transport to the composting site, gas emissions during the degradation and recovery of the finished product (Nordahl et al., 2023). To develop a stable and reproducible one, we adopted a fermentation engineering strategy focusing on microbial strains competent quality in composting and having biotechnological skills well solicited under controlled conditions (bioreactor). According to the bibliography (Gousia et al., 2023), actinomycetes form the hard core of the composting process and online research is the most convenient way to select competent strains capable of boosting composting in a reproducible way.

The conventional process, harnesses the power of naturally existing microorganisms to transform a blend of organic materials into a product that resembles humus (Bamdad et al., 2022; Udume et al., 2023; Zhu, 2004). This process has several benefits, including pathogen destruction, conversion of unstable ammonia nitrogen into stable organic forms, and reduction in waste volume (Núñez et al., 2022). The microorganisms that thrive on composting substrates reflect the progression and effectiveness of the composting method (El Fels et al., 2014). Through their metabolic pathways, these microorganisms cause notable modifications in the physical and chemical properties of the composting material (Game et al., 2018), leading to modifications in the microbial community structure. Extensive focus has been dedicated to investigating the microorganisms thriving in composting materials and their behavior as a community. Numerous researchers have observed a clear progression of microbial succession from mesophilic to thermophilic bacteria during the initial stages of composting, as well as during the subsequent temperature decline in later stages (De Bertoldi et al., 1983; Grgić et al., 2019; Shelly et al., 2022; Sun et al., 2022). There is no doubt that temperature influences the succession of microorganisms during composting, making it one of the primary influencing factors., and it is relatively easy to establish microbial succession from mesophilic to thermophilic, or conversely from thermophilic to mesophilic. Compost microenvironment can serve as a potential resource for screening potent strains such as thermophilic microorganisms that have shown many advantages in industrial applications, e.g., relatively higher temperatures can increase hydrolysis rates and improve degradation rates as well as reduce costs. Thus, The aim of this study is to generate an organic compost and to monitor mesophilic and thermophilic bacterial population of this compost in order to isolate and characterize a thermophilic actinomycete species present in this ecologic niche.The intended outcomes are to isolate a catalyzing strain that ensures the success of composting via its metabolic capacities.

2. Material and methods

2.1. Composting materials

Table 1 presents the properties of the composting materials used in this experiment. The initial compost mixture consisted of 40% vegetable waste, 10% fruit waste, 30% dead tree leaves, and 20% sawdust. These materials were thoroughly blended using a mechanized mixer and then loaded into a specially designed mechanical bioreactor with dimensions of 85 cm × 42 cm × 42 cm (length × width × height). To ensure uniformity, the bioreactor is equipped with motorized propeller axes for effective agitation (refer to Figure 1). Table 1 provides an overview of the initial characteristics of the compost mixture, including its moisture content, pH, and C/N ratio. These parameters were in line with the recommended starting conditions for promoting efficient composting, as suggested by previous studies (Rynk et al., 1992; Saludes et al., 2008).

Table 1: Composition and properties of the initial mixture

Composition	%Volume	C:N
Vegetable waste	40	19
Fruit waste	10	20
Dead tree leaves	30	54
Sawdust	20	641
Total C:N	154 :1	
Moisture content	50%	
pH	8.1	

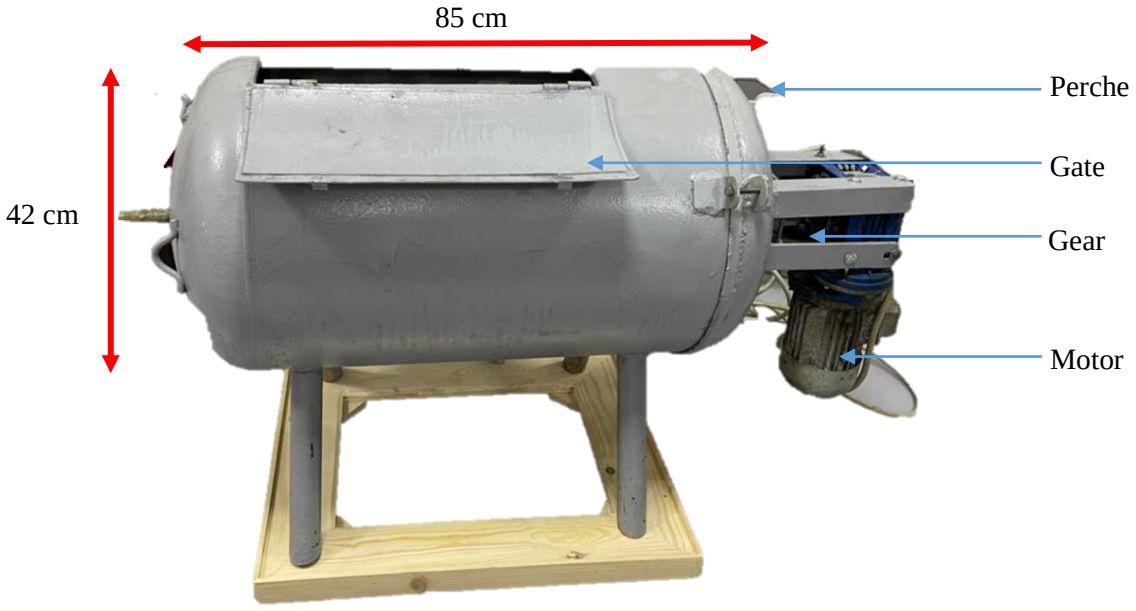

Figure 1: Photo of the mechanical bioreactor used in composting process

2.2. Evolution of bacteriological population

The bacteriological population in compost samples was determined using the agar plate dilution method (Iwabuchi & Miyatake, 2001). One gram of compost sample was mixed with 99 ml of sterile water and homogenized for 5 minutes at 8000 rpm. The resulting suspension was then diluted in sterile physiological water (NaCl 9‰) through a series of dilutions. Subsequently, 0.1 ml of the diluted suspension was spread onto prepared agar petri dishes. Tryptic soy agar medium was utilized for bacterial growth, with mesophilic bacteria cultured at 30 °C and thermophilic bacteria at 60 °C (Saludes et al., 2008). After an incubation period of 3 days for mesophilic bacteria and 2 days for thermophilic bacteria, the colonies were counted (Saludes et al., 2008). The microbial population was quantified by expressing the number of microorganisms as colony-forming units (CFU) per unit of dry compost weight. Enumeration was conducted at each stage of composting, including the mesophilic, thermophilic, and cooling phases (Fourti et al., 2008; Makan, 2021).

2.3. Isolation of thermophilic actinomycete strains

Thermophilic Actinobacteria were isolated using the method outlined by Williams et al. (1993) on International Streptomyces Project (ISP2) agar medium. The agar plates were subjected to incubation at a temperature of 45 °C for a period of 14 days. Actinomycete colonies were identified by visually assessing their cultural and morphological characteristics, which were further confirmed through microscopic examination. To select the high-temperature resistant strains, purified isolates were streaked on solid ISP2 plates and incubated at 45 °C for 4 days to observe their growth, following the protocol described by (L. Zhang et al., 2022). Subsequently for selected strains were grown at three temperature values of 50, 55 and 60˚C, respectively. Strains capable of growing on different temperature gradients were characterized morphologically and biochemically, and the most competent strain growing at a temperature of 60˚C was selected for molecular identification.

2.4. . Morphological and biochemical characterizations of selected strains

Cultural characteristics, including colony appearance, substrate mycelium color, and pigment production, were assessed according to the method outlined by (Ismail Saadoun et al., 2007; Reddy et al., 2010) following the guidelines provided by the International Streptomyces Project (ISP) (S. Selim, 2016; Shirling & Gottlieb, 1966). Initially, bacterial isolates were subjected to Gram staining and examined under light microscopy. Subsequently, physiological tests were performed on nutrient agar plates for a duration of 1-3 days, which included assessing growth in the presence of various NaCl concentrations (2%, 4%, 6%, and 8% w/v) at 45 °C and at

different pH levels (5, 8, 9, and 10) at 45 °C. Also,to confirm the taxonomic position at the genus level, physiological and biochemical characteristics were employed, including the strain's capability to degrade various organic substrates and assimilate different carbon sources. These criteria were assessed to support the potential classification at the species level and aid in the overall taxonomic identification process (Beroigui et al., 2023). The works of (El-Naggar & El-Shweihy, 2020; Lechevalier & Lechevalier, 1965; Pridham & Gottlieb, 1948; Shirling & Gottlieb, 1966) were referenced for this purpose. The obtained results were then compared with established criteria provided in the Bergey's Manual of Systematic Bacteriology (Locci, R. et al, 1989; Parte et al., 2012), Nonomura's key (Nonomura, 1974), Pridham and Gottlieb's key (Pridham & Gottlieb, 1948), and Pridham and Lyons' classification(Pridham & Lyons, 1965).

2.5. Scanning electron microscopy (SEM) for the most thermophilic strain

The shape and size of spores are determining criteria in the classification of Actinomycetes (Beroigui et al., 2023). To investigate these criteria, we employed scanning electron microscopy (SEM) to analyze the spore morphology of the most thermophilic strain (Beroigui et al., 2023). The spore specimens were coated with a layer of gold and observed under high vacuum conditions using a computerized scanning electron microscope (JEOL JSM-IT500HR) (Beroigui et al., 2023) at the University Regional Center of Interface (CURI), which is affiliated with the University Sidi Mohamed Ben Abdellah.

2.6. . Phylogenetic Analysis for the most thermophilic strain

The identification of the most competent isolate was carried out through 16S DNA-based sequence analysis. Actinomycete DNA was extracted from pure isolates using the method described by Saito and Miura (1963). Partial fragments of the 16S rRNA gene were amplified using polymerase chain reaction (PCR) with the forward primer p27f (5'-AGAGTTTGATCCTGGCTCAG') (L. Zhang et al., 2022, p. 022) and the reverse primer p1492r (5'-TACGGCTACCTTGTTACGACTT'). The PCR reaction was conducted in a total volume of 100 µL, including Taq polymerase, deoxyribonucleotide 5'-triphosphate (dNTPs), forward and reverse primers, template DNA, MgCl2, PCR buffer, and water (El-Naggar & El-Shweihy, 2020). The thermal profile for PCR involved denaturation, annealing, and extension steps. The amplified PCR products were analyzed on a 1.0% agarose gel, purified after excision from the gel (Vikram et al., 2012). The resulting partial 16S rRNA gene sequences (1300 bp) showed close similarity to published strains within the genus Thermoactinomycetes, and the species-level identification was validated using the Alignment Search Tool (BLAST) server provided by the National Center for Biotechnology Information (NCBI) (Beroigui et al., 2023).

A phylogenetic tree was constructed using the maximum likelihood algorithm implemented in MEGA11: Molecular Evolutionary Genetics Analysis version 11. The tree was bootstrapped with 1000 replicates to evaluate the statistical support for the inferred evolutionary relationships (Tamura et al., 2021).

3. Results and discussions

3.1. Evolution of bacteriological population

The findings presented in Figure 2 demonstrate distinct microbial dynamics throughout the various stages of composting. Initially, the mesophilic bacteria dominate the composting process, as they are well-suited to metabolize the easily decomposable materials. As a consequence of their metabolic activity, the temperature of the compost rises, creating an environment favorable for the growth and proliferation of thermophilic bacteria. This leads to a shift in the microbial community composition, with thermophilic bacteria becoming the predominant group during the second phase. However, as the composting process advances and the easily degradable materials are depleted, the temperature begins to decrease. This decline in temperature causes a decline in the population of thermophilic bacteria and creates conditions suitable for the reemergence of mesophilic bacteria. Consequently, the mesophilic bacteria experience a significant resurgence during the cooling phase of composting. At this stage, there is a nearly equal presence of both mesophilic and thermophilic bacteria, indicating a dynamic balance between the two groups. Overall, the results highlight the dynamic nature of microbial succession during composting, with a shift from mesophilic to thermophilic bacteria followed by a subsequent reestablishment of mesophilic bacteria as the composting process progresses.

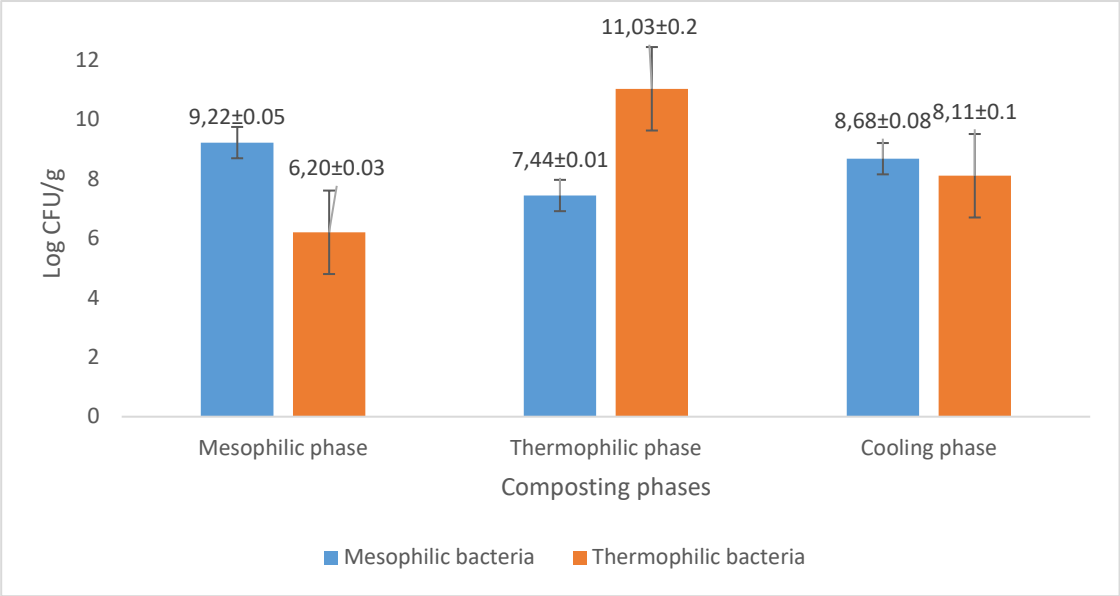

Figure 2: Changes in bacterial population during the composting process

The current findings demonstrate a cyclic transition of microorganisms during the composting process, progressing from mesophilic bacteria to thermophilic bacteria. These findings align with previous research done by several authors (Chroni et al., 2009; Hassen et al., 2001; Ma et al., 2003; Saludes et al., 2008; Shelly et al., 2022; Sun et al., 2022; R. Wang et al., 2016) who have highlighted the same succession of microorganisms, also (Saludes et al., 2008) have reported the presence of fungi during the mesophilic phase and (Chang et al., 2021; Ma et al., 2003; Nemet et al., 2021) have reported thermophilic actinomycetes presence also during the thermophilic phase.

Studies have been conducted in the same direction to qualitatively and quantitatively identify the composition of mesophilic and thermophilic fractions of the microbial population (Biyada et al., 2021), as they aim to explore microbial biodiversity in the composting process. In our case, the selection of a highly competent strain in composting appears to be a higher priority in developing a controlled process.

3.2. Isolation of thermophilic actinomycete strain

Ten isolates exhibiting thermophilic properties were isolated from the compost samples.. Among them, 6 strains exhibited growth at 50˚C, while 2 strains showed growth at 55˚C. Interestingly, only one strain demonstrated the ability to grow at the highest temperature of 60˚C. To gain a comprehensive understanding of the thermotolerant strains, a thorough characterization was conducted, including morphological, biochemical, and enzymatic analyses. These analyses offered valuable understandings regarding the distinctive

characteristics and capacities of the six thermotolerant strains. However, due to their exceptional performance, the most competent strain was selected for further molecular identification.

3.3. Morphological and biochemical characterization of the selected strains

All strains are gram positive (Figure 3a) ,it shows almost the same aspects on different tested media with slight differentiation, the aerial mycelium was white and abundant, spores had a smooth surface and no pigment formation was reported (Figure 3b).

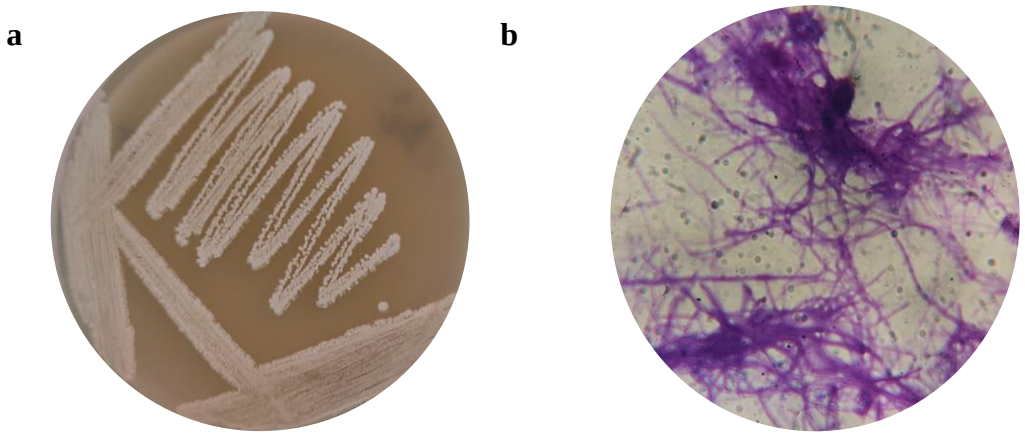

Figure 3: photos showing the aspects of the isolate a) on ISP agar medium b) under electronic micscope (Gx100)

Despite the absence of 16S rRNA data for a comprehensive analysis, we employed morphological characteristics and biochemical tests to discern notable distinctions among the isolated strains. To validate our findings, we compared the results with established criteria from Bergey's Manual of Systematic Bacteriology (Beroigui et al., 2023). The observed traits of the isolated strains strongly suggest their classification within the genera Thermoactinomyces, Streptomyces, and Thermonospora. To further elucidate their taxonomic position, we conducted a thorough examination of published descriptions of similar taxa in Bergey's Manual and utilized the Nonamura classification key. As a result, we propose the potential presence of six distinct species among the isolated strains. Detailed information regarding strains characterizations are found in Tables 2 and 3.

Table2: Cultural and morphological characteristics of the thermophilic isolates

	ISP2			ISP3			ISP4			ISP6		
	Gr	SM	DP	Gr	SM	DP	Gr	SM	DP	Gr	SM	DP
Thermoactinomycetes sacchari	good	cream white	no	good	white	No	moderate	cream white	no	moderate	white	no
Thermoactinomycetes vulgaris	good	yellow white	no	good	white	No	good	white	no	moderate	white	no
Thermoactinomycetes thalpophilus	good	white	no	good	white	No	moderate	white	no	moderate	white	no
Thermonospora fusca	good	white	no	good	white	No	moderate	white	no	moderate	white	no
Streptomyces thermovulgaris	good	grey	no	good	grey	No	moderate	white grey	no	moderate	white grey	no
Streptomyces thermoviolaceus	good	grey	no	good	grey	No	moderate	grey	no	moderate	grey	no

Gr:Growth; SM; Subsrate Mycelium; DP:Diffusible pigment

Table3: Characterization of biochemical and physiological traits in the thermophilic isolates

	Thermoactinomyc etes sacchari	*Thermoactinomyc etes vulgaris*	*Thermoactinomyc etes thalpophilus*	*Thermonospora fusca*	*Streptomyces thermovulgaris*	*Streptomyces thermoviolaceus*
Physiological and biochemical properties: Hydrolysis of :						
Starch	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+
Chitin	-	-	-	-	-	-
Cellulose	+	-	-	-	-	-
Unilin	-	-	-	-	-	-
Xylan	+	-	+	-	-	-
Casein	+	+	+	+	+	-
Catalase test	+	+	+	+	+	+
Carbon sources utilization :						
Glucose	+	+	+	+	+	+
Fructose	+	+	-	-	+	+
Maltose	+	-	-	+	+	+
Lactose	+	-	-	-	-	-
Saccharose	-	-	-	-	+	+
Arabinose	+	+	-	+	-	+
Mannitol	+	+	+	-	+	+
Mannose	+	-	+	-	+	+
Xylose	-	-	-	+	+	-
Sorbitol	+	+	-	-	+	+
Inositol	-	-	-	-	-	+
Growth at different temperature (˚C):						
45	+	+	+	+	+	+
50	+	+	+	+	+	+
55	+	+	-	-	-	-
60	+	-	-	-	-	-
Growth at different pH values						
5	±	±	±	±	±	±
8	+	+	+	+	+	+
9	+	±	-	-	-	-
10	±	-	-	-	-	-
Growth at different concentration of NaCl (g/l)						
20	+	+	+	+	+	+
40	+	±	±	-	-	-
60	±	-	-	-	-	-
80	-	-	-	-	-	-

The presented data when compared to those of others authors (Bergey, 1994; ElSharayidi et al., 2020; Mutschlechner et al., 2021; Parte et al., 2012; Petrova & Vlahov, 2007; Yoon et al., 2005) showed that the most thermophilic strain is close to *Thermoactinomyces Sacchari* and *Laceyella sacchari.* Subsequently, in order to ensure the identity of our strain, molecular identification was performed.

To understand the ecophysiological causes responsible for the selection of our collection of thermophilic actinomycete strains, we compared our results to those obtained from various substrates and under different bioclimatic conditions. Regarding the *Thermoactinomycetes sacchari strain (*synonym*: Laceyella sacchari*), it has been found in ecological niches where extremely harsh conditions prevail, such as deserts and volcanic rocks. This confirms its genetic adaptation to these environments characterized by high temperatures (Seyed Shirazi & Hamedi, 2023). Under such conditions, this strain has been associated with *Thermoactinomycetes sp*. and has been selected for its potential antibiotic production.

Among the isolated and identified strains in our collection, the strains *Thermoactinomyces vulgaris* and *Laceyella sacchari* (in combination) have also been mentioned by (C.-H. Yang et al., 2009) and (Giambra et al., 2021), who obtained them from compost. This is consistent with our findings, the niches studied in these two recent studies are composting soil and Compost-Bedded Dairy Cows, which highlights the genetic adaptation of these strains to ecologically rich niches containing solid animal waste. A study similar to ours, conducted by (Xiao et al., 2011), also succeeded in isolating *Laceyella sacchari* from compost composed of kitchen waste, leaves, grass, and small branches. This explains the abundance of our identified strain in such environments. This result suggests that the substrate has an effect on the genetics of microorganisms. A limestone soil amended with olive pomace mixed with remnants from a two-phase olive oil extraction process selected a population of actinomycetes that greatly differs from ours (Cuesta et al., 2012). A study conducted by (Giambra et al., 2021) over a period ranging from 2017 to 2019, involving 5 samples, found that the strains *Thermoactinomyces sacchari* (synonym *Laceyella sacchari*) and *Thermoactinomyces vulgaris* were present in the compost microorganism consortium with a very high frequency (0.8 to 0.33 for *Laceyella sacchari* and 0.8 to 0.43 for *Thermoactinomyces vulgaris*). These two strains were consistently associated with *Bacillus licheniformis*, which is a key component of the composting process.These findings confirm the substrate's effect on the selection of a specific genetic heritage. Additionally, this well-known identified strain (*Thermoactinomycetes sacchari;*

synonym: Laceyella sacchari) has also been found in a landfill site in Thailand, exhibiting the ability to degrade polyester-based plastics (Sriyapai et al., 2018). These results underscore the extraordinary metabolic capabilities of this strain.

3.4. Scanning electron microscopy (SEM) Analysis of the most thermophilic strain

Scanning electron microscope (SEM) analysis revealed notable characteristics of the spores. They exhibited smooth surfaces and had a spherical or cylindrical shape. The spores were arranged in elongated chains, with each chain containing 6 to 12 spores. The mature spores were observed to have a diameter of approximately 1 µm (Figure 4).

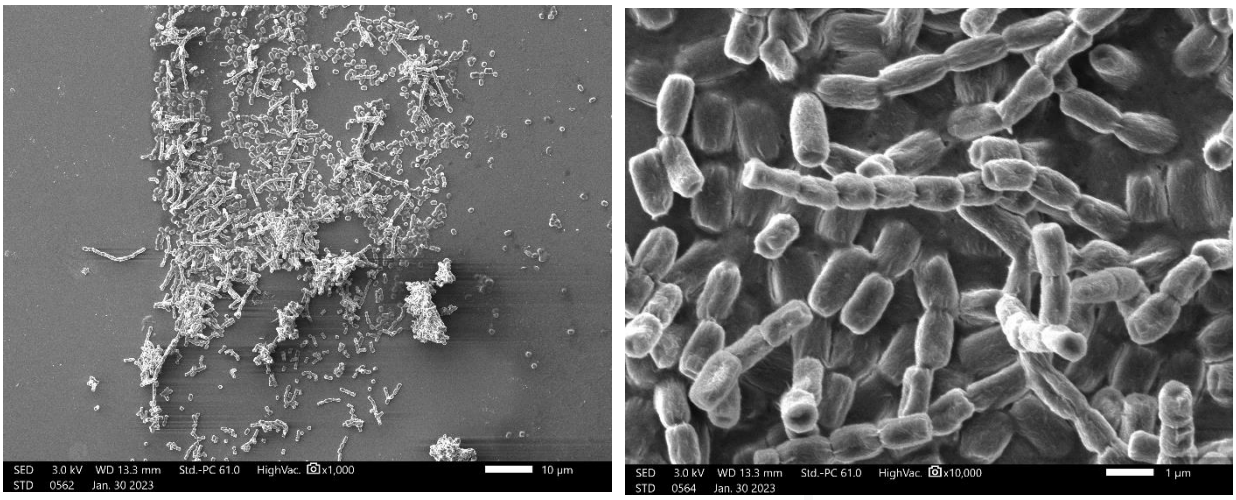

Figure 4: Scanning Electron Microscope (SEM) Image of *Thermoactinomyces sacchari* OBEF23

The morphological characterization of actinomycetes holds significant importance in species identification, particularly in fields like medicine and industry (Kurtböke, 2022). However, in educational contexts, educators face challenges in visually representing the morphological features of these microorganisms due to limited available data on their varied morphology, leading some researchers to rely on electron microscopy for identification support (Mahdi et al., 2022).

3.5. PCR and Sequence Analyses for the most thermophilic strain

To validate the identification of the strain, the genomic DNA was subjected to PCR amplification of the 16S rDNA region. The resulting amplified product was then analyzed using a 1.0% agarose gel (Figure 5).. The marker bands (1) From top to bottom can be described as follows: Band 1: 2000 bp, 50 ng/µl, Band 2: 1200bp, 30 ng/µl, Band 3: 800 bp, 20ng/µl, Band 4: 400 bp, 10ng/µl, Band 5: 200 bp, 5ng/µl

In the gel analysis, lane 2 represents the negative control while lane 3 corresponds to the positive control. Lanes 4 and 5 display the results obtained for the isolate. Notably, the BOEF23 strain

exhibited a PCR product with the anticipated size of 1300 bp. Further analysis of the 1300 bp amplicon through sequence analysis will enable us to accurately identify the isolate.

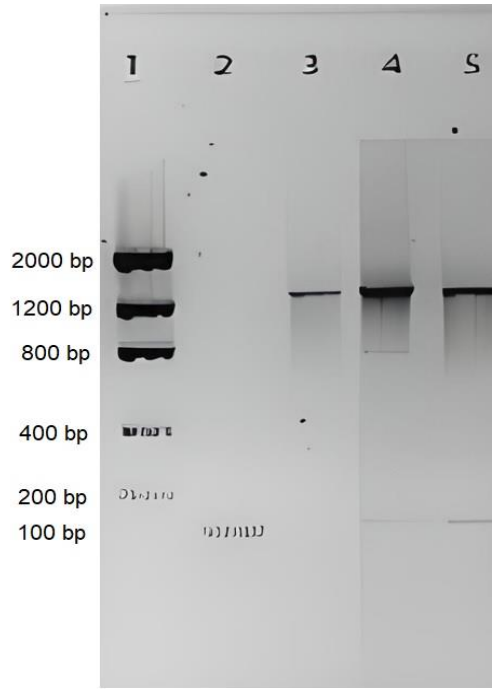

Figure 5: Gel electrophoresis analysis of amplified 16s rdna product for BOEF23 strain ; lane1: molecular size marker, 2: negative control; 3: positive control; 4 and 5: amplified 16s rdna product

3.6. Phylogenetic Analysis

The obtained strain was subjected to genomic analysis of its 16S rRNA gene sequences to determine its identification and confirmation. Through comparison with similar matches in the GenBank database, it was found that the strain exhibited a close relationship (99% similarity) with Laceyella Sacchari strain DSM 43356, which belongs to the family Thermoactinomyces. Additionally, it showed a 95% identity with *Laceyella sediminis* RHA1 and *Laceyella tengchongensis* YIM 10002, both of which also belong to the Thermoactinomycetes family. A phylogenetic tree was constructed using maximum likelihood algorithms based on the nearly complete 16S rRNA gene sequences (Beroigui et al., 2023) (Figure 6). The strain, identified as *Thermoactinomycetes Sacchari* (also known as *Laceyella Sacchari*), has been registered in the Culture Collection of the Functional Ecology and Environmental Engineering Laboratory at Fst-Fez. The deposited strain is assigned the entry code BOEF23 and has been published under the accession number OQ923793 in NCBI.



Figure 6: The phylogram of *Thermoactinomycetes Sacchari syn Laceyella Sacchari* BOEF23 and its comparison to other species using 16S rDNA sequences.

The taxonomic position derived from our study is consistent with findings obtained in multiple previous studies. (J. H. Yoon & Park, 2000), in their contribution, utilizing a molecular tool distinctly different from ours, found that the *Laceyella sacchari* strain is closely related phylogenetically to the two strains, *Thermoactinomyces putidus* and *Thermoactinomyces vulgaris*. Similarly, (Goda et al., 2020; Yao et al., 2014) obtained alignment results akin to our taxonomic position, albeit using different primers than ours. In strong agreement with our work, (Panosyan, 2019) obtained results that perfectly harmonize with ours using the same molecular tool. The investigation conducted by (Giambra et al., 2021) on compost samples corroborated the stability of the taxonomic position of our strain within a consortium of thermoactinomyces.

4. Conclusion

The isolated competent strains (*Streptomyces thermovulgaris, Streptomyces thermoviolaceus, Thermoactinomyces sacchari, Thermoactinomyces vulgaris, Thermoactinomyces thalpophilus, and Thermonospora fusca*) exhibit significant metabolic capabilities that account for their presence in compost. *Thermoactinomyces sacchari* strain presents several promising avenues for applied valorization. Mastering these strains will enable effective control of the composting system, where their utilization could enhance the performance of biological and biotechnological applications sought by industries. This is due to their ability to function in processes at elevated temperatures, thereby reducing the risks of contamination by other disruptive microorganisms. The taxonomic position reveals that *Thermoactinomyces sacchari* (Synonym: *Laceyella sacchari*) resides within an evolutionary cluster comprising highly competent strains in their degradative system. This confirms the genetic adaptation of this strain to the ecological niche associated with the composting process, particularly in conjunction with consortium elements. The isolation of *Thermoactinomyces sacchari* highlights the potential for promoting the growth of proficient thermophilic actinomycetes, which can have a crucial role in various industrial processes. This study also lays the groundwork for further research aimed at enhancing organic matter decomposition and achieving exceptionally high-quality compost. This work opens up several research prospects that hold priority for Morocco, such as formulating a microbial consortium to enhance and expedite the composting process, which is part of the development plans in several African countries. Furthermore, *Thermoactinomyces sacchari*, with its significant hydrolytic capabilities (protease, amylase, cellulase), could play a role in producing biotechnologically relevant enzymes.

Chapitre VI

Production, purification et caractérisation de l'inulinase de Streptomyces anulatus

Production, purification et caractérisation de l'inulinase de *Streptomyces anulatus*

Synthèse

Le présent chapitre se concentre sur l'inulinase, une enzyme qui catalyse la conversion de l'inuline en D-fructose. Bien qu'il soit possible d'extraire cette enzyme des plantes, il est difficile d'en obtenir de grandes quantités, ce qui entraîne des coûts de production élevés. Par conséquent, l'utilisation d'inulinase microbienne présente un potentiel considérable pour les besoins industriels.

Au cours de la dernière décennie, peu de rapports ont été publiés sur les inulinases actinobactériennes, notamment sur la purification et la caractérisation du processus d'extraction de l'inulinase. Cette étude a pour objectif de sélectionner des souches d'actinomycètes présentant une activité inulinase élevée à partir du sol. Pour identifier les bactéries productrices d'inulinase, un milieu Czapex Dox modifié, contenant 1% de poudre d'inuline, a été utilisé. L'isolat le plus efficace s'est révélé être *Streptomyces sp*. EFBO8. Les méthodes d'identification morphologique et génotypique ont confirmé que cette souche était Streptomyces anulatus, et sa séquence nucléotidique a été déposée dans GenBank sous le numéro d'accès OQ073700.

Dans le but d'optimiser la production d'inulinase, des cinétiques ont été réalisées en utilisant la souche *Streptomyces anulatus*, qui s'est avérée être la plus productive avec une valeur de 24, 024 EU/ml. L'enzyme a ensuite été purifiée à partir du filtrat de culture par précipitation avec du sulfate d'ammonium ($(NH_4)2SO_4$), suivie d'une séparation par chromatographie sur colonne Sephadex (G50). La protéine purifiée présentait une masse moléculaire de 3331,83 Da.

L'inuline est un polyfructane végétal composé de chaînes linéaires de résidus de fructose liés à une molécule de saccharose terminale. Elle est utilisée comme réserve de glucides de stockage et se trouve dans les organes souterrains de plusieurs plantes telles que le topinambour, la chicorée et le dahlia. Étant donné que l'inuline est abondamment disponible dans la nature et que sa composition principale est le fructose, elle peut être utilisée comme substrat pour la production de fructo-oligosaccharides et de sirop de fructose élevé dans les industries alimentaires (boissons) et pharmaceutiques.

La méthode traditionnelle d'extraction du fructose à partir de l'inuline consiste en une hydrolyse acide, mais cela entraîne une dégradation facile du fructose à pH bas, ce qui conduit à la décoloration de l'hydrolysat d'inuline et à la formation de sous-produits tels que le difructose anhydride. Une autre méthode utilise des sources microbiennes qui sécrètent une grande

quantité d'inulinase et peuvent cliver le fructose de l'inuline en une seule réaction avec des rendements allant jusqu'à 95% de fructose. L'exo-inulinase clive principalement les unités terminales de fructose de l'inuline pour produire du fructose, tandis que les liaisons internes de l'inuline sont hydrolysées par l'endoinulinase en inulo-oligosaccharides.

De nombreux microorganismes tels que *Aspergillus niger*, *Kluyveromyces marxianus*, *Bacillus smithii* et *Kluyveromyces sp*. ont été signalés comme sources d'inulinase, une enzyme catalysant la dégradation de l'inuline. Ainsi, les microorganismes sont considérés comme les meilleures sources d'inulinase en raison de leur facilité de culture, de leur modifiabilité génétique, de leur rendement élevé et de leur caractère généralement inducible et extracellulaire. Cependant, il existe peu de rapports sur les inulinases provenant de streptomyces. Il est bien connu que plusieurs espèces de streptomyces produisent une grande variété de métabolites bioactifs utiles, tels que les antibiotiques, les insecticides et diverses enzymes industrielles.

En tenant compte des applications potentielles des inulinases, cette étude vise à sélectionner des souches performantes d'actinomycètes produisant de l'inulinase. Ainsi, l'étude analyse la cinétique de production de l'inulinase de la souche *Streptomyces anulatus*, qui s'est avérée être la plus compétente parmi une collection de souches isolées d'un sol irrigué par les eaux usées d'une huilerie d'olive. Par conséquent, la purification et la caractérisation de l'inulinase ont été réalisées.

En conclusion, cette étude met en évidence l'importance de l'inulinase dans la production de fructose et d'autres produits dérivés de l'inuline. Elle souligne également le potentiel des actinomycètes, en particulier de la souche *Streptomyces anulatus*, en tant que source d'inulinase. La purification et la caractérisation de l'inulinase de cette souche permettront d'améliorer les processus industriels utilisant cette enzyme.

Production, purification et caractérisation de l'inulinase de *Streptomyces anulatus*

This part of the work was published in the journal "JBM" (Q2 - IF: 2.65).

Abstract:

Inulinase is an enzyme that catalyzes inulin to D-fructose. This enzyme can be extracted from plants but it is difficult to obtain it in large quantities, so its production cost is high. Therefore, microbial inulinase has great potential for industrial needs. In the last decade, there have been very few reports on actinobacterial inulinases, especially on purification and characterization of inulinase process extraction. This study aims to select actinomycetes that possess inulinase activity from the soil. To screen inulinase-producing bacteria, modified Czapex Dox agar supplemented with 1% inulin powder was used. To optimize inulinase production, kinetics were performed by using *Streptomyces anulatus* strain, which proved to be most productive with a value of 24,024 EU/ml. The enzyme was purified from the culture filtrate by precipitation with $(NH_4)2SO_4$, followed by column chromatography Sephadex (G50), the purified enzyme has a molecular mass of 3331.83Da

Keywords: Actinomycete, *Streptomyces anulatus*, Characterization, Inulinase activity Production and Enzyme Purification

1. Introduction

Inulin is a plant polyfructan consisting of linear chains of fructose residues bound to a terminal sucrose molecule (Vandamme & Derycke, 1983). It constitutes a storage carbohydrate reserve and accumulates in underground organ of several plants such as Jerusalem artichoke (*Helianthus tuberosus*), chicory (*Cichorium intibus*) and dahlia (*Dahlia pintana*) (Trojanova et al., 2004). Since it is abundantly available in nature and its main composition is fructose, it can be used as a substrate for the production of fructo-oligosaccharides and high-fructose syrup for food, pharmaceutical and beverage industries (Laowklom et al., 2012a). Fructose can be extracted by acid hydrolysis of inulin, but fructose is easily degraded at low pH and the process leads to discolouration of the inulin hydrolysate and by-products formation in difructose anhydrides form (Barthomeuf et al., 1991; Chi et al., 2009; de Araujo Ribeiro et al., 2021; A. D. Sharma & Gill, 2007; Vandamme & Derycke, 1983). Another method uses microbial sources, which secrete a large amount of inulinase and can cleave fructose from inulin, in a one-step catalysed reaction with yields up to 95% fructose (Das et al., 2019; Mohammadi et al., 2019). Exo-inulinase produces fructose as the main product by cleaving terminal units of fructose from inulin whereas the inner linkages of inulin are hydrolyzed by endoinulinase into inulo-oligosaccharides (Chi et al., 2009; Das et al., 2019). Inulinase is a valuable enzyme used principally for fructose production (Atzler et al., 2020; Ricca et al., 2009), ultra-high fructose syrup (Lima et al., 2011; Rawat et al., 2021) and ethanol production (Das et al., 2019; Ge & Zhang, 2005).

There are reports of inulinase, an enzyme that catalyzes inulin, from many microorganisms including *Aspergillus niger* (Awdaa, 2010; Nakamura et al., 2001; Yedahalli et al., 2016), *Kluyveromyces marxianus* (R. S. Singh et al., 2018; Y. Zhang et al., 2019), *Bacillus smithii*(W. Gao et al., 2008), *Kluyveromyces sp*(Cruz-Guerrero et al., 2006). Therefore, the best sources of inulinase are microorganisms, since they are easy to grow, genetically manipulated, have a high yield and are usually inducible and extracellular by nature. However, there are only a few reports on inulinases from streptomyces. It is well known that several streptomyces species produce a wide variety of useful bioactive metabolites (Donald et al., 2022), including antibiotics, insecticides and various industrial enzymes of interest.

Taking into consideration the potential applications of inulinases, the present study target the selection actinomycete strains producing unilinase. Thus, this study analyse production kinetics of *Streptomyces anulatus* strain which proved to be the most competent among a collection of

strains isolated from a soil irrigated by olive mill waste water and consequently, its inulinase purification and characterization were done.

2. Materials and methodes

2.1. Microorganisms used

Streptomyces used in this study were isolated from the from a soil, irrigated by olive mill waste water, in botany garden belonging to faculty of science and technology of Fez.

2.2 Inulinolytics strains selection and isolation

Strains were subcultured on the medium Czapex-dox-containing (gl^{-1}): $NaNO_3$, 3.0; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5 ; $FeSO_4 \cdot 7H_2O$ and 1% inulin (w/v). pH was adjusted to 5.0 and then incubated at 37°C for 3 days. Petri dishes replicate have been treated with lugol's solution for 3 to 5 minutes and washed with distilled water to remove excess Lugol's iodine. The clear zone around colonies, indicate inulin hydrolysis (Das et al., 2019).

2.2. Morphological and biochemical characterization of isolates

Cultural characteristics such as colony and substrate mycelium colour and pigment production was carried out (Reddy et al., 2010) following the directions given by the International Streptomyces Project (ISP) (Shirling & Gottlieb, 1966).

Physiological and biochemical criteria such as the ability to degrade gelatin, casein, starch, cellulose substrates by the various Streptomyces strains were used for genus taxonomic position confirmation. The utilization of different carbon sources , utilization of amino acids were studied in order to reach a possible classification to species (El-Naggar & El-Shweihy, 2020; Lechevalier & Lechevalier, 1965; Pridham & Gottlieb, 1948; Shirling & Gottlieb, 1968) and the results were compared with valid criteria reported in the Bergey's Manual of Systematic Bacteriology (Locci, R. et al, 1989),Nonomura key (Nonomura, 1974) Pridham and Gottlieb key (Pridham & Gottlieb, 1948) and Pridham and Lyons classification (Pridham & Lyons, 1965). After the quantitave study of inulinase activity, the molecular study will be performed for the most efficient strain to ensure its identification.

2.1. Screening of strains

To screen a best-producing strain of inulinase, a preculture has been prepared in inoculating each strain in a liquid medium, ISP3, and this suspension is then used in as a culture. This culture (10%, V/V) was inoculated into Czapex-dox basal medium supplemented with 1% inulin, and incubated under agitation at 200 rpm at 30°C for 3 days. After this, mycelium was removed by centrifugation at 8000 rpm for 10 min and the supernatant culture was tested for the inulinase activity as described by (A. D. Sharma & Gill, 2007). The reaction mixture contains 50 µl of sample enzyme, 55 µl buffer sodium phosphate 50 mM pH 6.0 and 95 µl of inulin at 1 % suspension in a buffer sodium phosphate 50 mM in suspension in a phosphate

buffer sodium 50 mM pH 6.0 was incubated at 55 °C for 20 minutes (Laowklom et al., 2012b). After that, the reaction mixture was kept in an ice bath for 5 min to inactivate the enzyme. The reducing sugars produced were determined by the DNS method (Miller, 1959). One unit of inulinase was defined as the amount of enzyme that produces one micromole reducing sugar per minute under tested conditions (X. Li et al., 2021).

2.2. Scanning electron microscopy (SEM) for the most effective *Streptomyces* strain EFBO8

The shape and size of spores are determining criteria in the classification of streptomyces. In this perspective, we analyzed the morphology of the spores of the strain EFBO8 under scanning electron microscope (SEM) to identify the criteria. The gold coated spores specimens were examined in high vacuum mode of the under high vacuum of the computerized scanning electron microscope using a scanning electron microscope (JEOL JSM-IT500HR) at the University Regional Center of Interface (CURI) attached to the University Sidi Mohamed Ben Abdellah.

2.3. DNA extraction and molecular identification of the most effective *Streptomyces* strain

In order to support the characterization of the most performing strain EFBO8 '*Streptomyces anulatus'* and to be sure of its identity, we performed a molecular identification.

According to the method described by Sambrook (Sambrook, 1989), the total genomic DNA of the strain was prepared. The PCR reaction was carried out using (Noura & Sarah, 2011) method. The amplification of 16S rRNA was carried out via polymerase chain reaction (PCR) using universal primer designed to amplify the full length of the gene. The reverse primer was 1492r (5′-TACGGYTACCTTGT TACGACTT-3′) and the forward primer was 27f (5′-AGAGTTTGATCMTGCCTCAG-3′). The PCR reaction was conducted in a total volume of 100 µL that contains 0.5 µL Taq polymerase, 10 µL deoxyribonucleotide 5′- triphosphate (dNTP's) (250 mM), 4 µL of both forward and reverse primers of 10 pmol, 1 µL template DNA 50 ng, 3.5 µL MgCl2 (25 Mm), 10 µL PCR buffer, up to 100 µL with water. The resulting partial 16S rRNA (1450 bp) gene sequences were closely related to the published strains under the genus Streptomyces, and the species-level affiliation of our sequence was validated using the Alignment Search Tool (BLAST) server from National Center for Biotechnology Information (NCBI). A phylogenetic tree was created using the 16S rRNA gene sequence of active strains by maximum likelihood algorithms using 1000 bootstraps replicates in MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura et al., 2021).

2.4. Inulinase production Kinetics

After selection of the best inulinase producing strain, a kinetic each twelve hours for 6 days was conducted for the latter according to the same protocol previously cited (A. D. Sharma & Gill, 2007) in order to quantify inulinase produced amount as a function of time.

2.5. Enzyme purification

At the maximal phase of inulinase production, cells were separated by centrifugation at 20000 rpm for 20min at C° and the clear supernatant (crude extract) was used as source of enzyme for purification. Obtained inulinase were partially purified by salting with ammonium sulfate (quality Enzyme) at a concentration of saturation (70%), and mixture it has been left for up to 2 hours. Formed precipitate was obtained by centrifugation at 6000 rpm. Then, the precipitated enzyme was dissolved in a phosphate buffer (pH7, 0.05 mM) and dialyzed against the same buffer for 18 hours.

2.6. Precipitation with ethanol

Precipitation with ethanol was carried out according to (Golunski et al., 2011) method with slight modifications . Before precipitation, enzyme extract was centrifuged at 2800 × g for 20 min at 5°C to remove particles from fermentation broth. The precipitate was discarded and supernatant was used for enzyme concentration. A chilled aqueous ethanol solution was added dropwise to an aliquot of crude enzyme extract at a flow rate of 9,2 ml/min . The extract solution was kept at 5°C throughout ethanol addition and under constant stirring. After ethanol solution addition, resulting precipitate was centrifuged at 9000 × g for 15 min at 5°C. The precipitate was collected and dissolved in sodium acetate buffer (pH 4.8, 100 mM)

2.7. Gel Filtration using Sephadex G50

Gel retardation chromatography on Sephadex g-50 column (L=25xØ2.5) has increased significantly, enzyme purity. For each assay, elution was performed with a phosphate buffer (pH 7, 0.05 mM). Fractions were collected at a flow rate (mL/min) in the form of aliquots (10mL) separately in cleaned and dried tubes.

2.8. Enzyme assay

Inulinase activity in each fraction was measured as described above (A. D. Sharma & Gill, 2007).

2.9. Molecular weight determination

The molecular weight of the purified enzyme was determined by size exclusion chromatography using Sephadex G-50 column with the dimensions of (Ø1.6 × 50 cm. Elution was performed using 0.05 M phosphate buffer (pH 7). Standards proteins of molecular weights

ranging from 1500 Da to 5000 Da were used. The graph was plotted between log10 of molecular weight and the elution volume and the molecular weight of the enzyme was determined using this straight line graph.

3. Results

3.1. Isolation and selection of inulinolytic strains

In the preliminary test, sixteen strains out of 34 showed positive inulinase activity, which was revealed by clear halo formation around colonies after treatment with lugol solution. Lugol solution. Figure 1 shows three positive inulinase activities against a negative one.

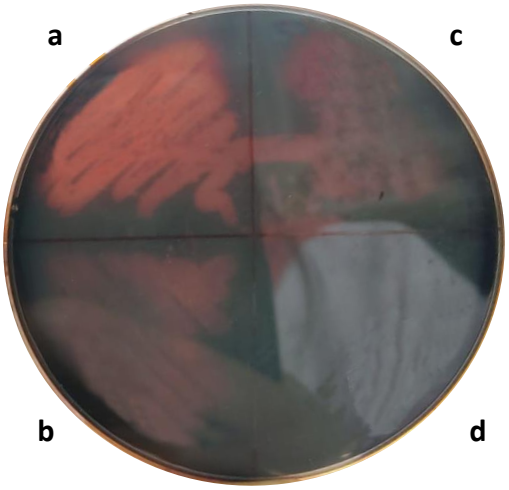

Figure 1: Inulinase activity of isolates a: *Streptomyces anulatus*; b: *Streptomyces griseus*; c: *Streptomyces felleus* and d: *Streptomyces sp*.

3.2. Strain screening

After strains qualitative screening, a quantitative inulinase assay was performed. The amount of enzyme that releases 1 µmol of fructose per minute from inulin is defined as one unit of inulinase activity. The figure below (Figure 2) shows inulin assay results.

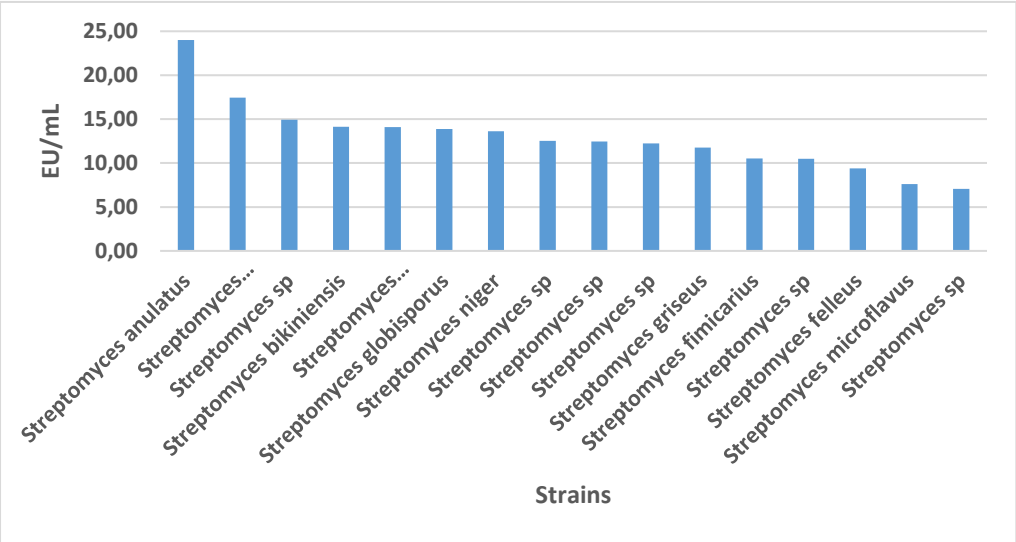

Figure 2: Inulinase activity of competent *Streptomyces* strains

Streptomyces anulatus , according to figure 1, proved to be the most potent and showed the highest value of enzyme unit based on an enzyme unit (EU) of inulinase activity 24.02 EU/ml. While the *Streptomyces sp* was the weakest. *Streptomyces anulatus* was therefore selected for a kinetic study to determine the time of maximum inulinase production.

3.3. Morphological and biochemical characterization of isolates

Since 16S rRNA data are not yet available for systematic works, we could establish clear differences among the isolated strains using morphological characteristics and biochemical tests, and comparing the obtained results with valid criteria reported in the Bergey's Manual of Systematic Bacteriology (Locci, R. et al, 1989). After a quantitative measurement of the inulinase activity, a molecular study will be performed for the most efficient strain. The characteristics of the isolated strains confirm their belonging to the genus Streptomyces. A study of the published descriptions of comparable taxa in the bergeys manual, the nonamura classification key suggest sixteen species, table 1 and 2 present the results of cultural, morpholgical and biochemical characterizations of the isolates.

Table1: Cultural and morphological characteristics of the inulinolytic isolates

	Growth	**Substrate Mycelium**	**diffusible pigment**	**Growth**	**Substrate Mycelium**	**diffusible pigment**	**Growth**	**Substrate Mycelium**	**diffusible pigment**	**Growth**	**Substrate Mycelium**	**diffusible pigment**	**Growth**	**Substrate Mycelium**	**diffusible pigment**
Streptomyces anulatus	G	WG	N	G	WG	N	G	WG	N	G	WG	N	G	WG	N
Streptomyces olivochromogenes	G	W	Lr	G	W	Lr	G	W	Lr	G	W	N	G	W	N
Streptomyces sp	G	W	N	M	W	N	G	W	N	G	W	N	M	W	N
Streptomyces bikiniensis	G	DG	N	G	DG	N	G	Gr	N	G	DG	N	G	DG	N
Streptomyces cinnamonensis	M	BG	N	G	BG	N	G	BG	N	M	BG	N	G	BG	N
Streptomyces globisporus	G	YG	N	M	YW	N	M	YW	N	G	YG	N	M	YG	N
Streptomyces niger	G	W	Grn	G	W	Grn	G	W	Grn	M	W	N	M	W	N
Streptomyces sp	G	W	N	M	W	N	G	W	N	G	W	N	M	W	N
Streptomyces sp	G	W	N	M	W	N	G	W	N	G	W	N	M	W	N
Streptomyces sp	M	W	N	G	W	N	G	W	N	G	W	N	M	W	N
Streptomyces griseus	M	WG	N	G	WG	N	G	WG	N	G	WG	N	G	WG	N
Streptomyces fimicarius	G	WB	N	G	WB	N	G	WB	N	G	WB	N	G	WB	N
Streptomyces sp	G	W	N	G	W	N	G	W	N	G	W	N	G	W	N
Streptomyces felleus	M	B	N	G	B	B	M	B	N	M	B	N	M	B	N
streptomyces microflavus	M	WB	N	G	WB	B	G	WB	B	M	WB	N	G	WB	N
Streptomyces sp	G	W	N	G	WG	N	G	W	N	G	W	N	G	W	N

G :good ; M : moderate ; W:white; WG : white grey ; Gr: Grey; DG: dark grey; B: brown ;WB: white brown;
Grn: green ; Lr: light red ; N: none

Table2: Biochemical and physiological characteristics of the inulinolytic isolates.

	Streptomyces anulatus	*Streptomyces olivochromogenes*	*Streptomyces sp*	*Streptomyces bikiniensis*	*Streptomyces cinnamonensis*	*Streptomyces globisporus*	*Streptomyces niger*	*Streptomyces sp*	*Streptomyces sp*	*Streptomyces sp*	*Streptomyces ariseus*	*Streptomyces fimicarius*	*Streptomyces sp*	*Streptomyces felleus*	*streptomyces microflavus*	*Streptomyces sp*
Physiological and biochemical properties: Hydrolysis of :																
Starch	+	+	-	-	+	-	+	-	-	+	-	-	-	+	+	-
Cellulose	+	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-
Casein	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-
Gelatin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Unilin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Utilization of carbon sources																
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Saccharose	-	+	+	+	+	-	+	+	+	+	-	+	+		+	+
Maltose	-	+	-	+	+	-	-	-	-	-	-	-	-	+	-	-
Mannose	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+
Xylose	+	+	-	-	+	+	+	-	-	-	-	-	-	+	+	-
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	-	-	+	-	-	-	+	+	+	-	-	+	-	-	-	-
Citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Utilization of amino acids																
Histidine	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
Methionine	-	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Asparagine	+	-	+	+	+	+	+	+	+	-	+	+	-	-	+	+
Proline	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at different temperature (˚C):																
30-37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	+	-	+	+	-	+	+	+	+	-	+	-	+	+	+	+
47	-	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-
50	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at different pH values																
3-4	-	-	+	+	+	+	+	+	+		+	+	-	+	-	-
5-9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10-11	-	-	+	-	+	-	-	-	-	+	+	-	-	-	+	+
13	-	-	+	-	-	-	-	-	-	+	+	-	-	-	+	+
Growth at different concentration of NaCl (%)																
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	-	+	+	+	+	-	+	+	-	+	+	+	+	-	+
8	+	-	-	+	+	+	-	+	+	-	+	-	+	+	-	+

3.4. Scanning electron microscopy (SEM) for the most effective Streptomyces strain

Observation under SEM analysis showed that spores have a smooth surfaces, were spherical or cylindrical, arranged in long chains and each chain contains 6-14 spores Mature spores have a diameter of 1 µm (Figure 3).

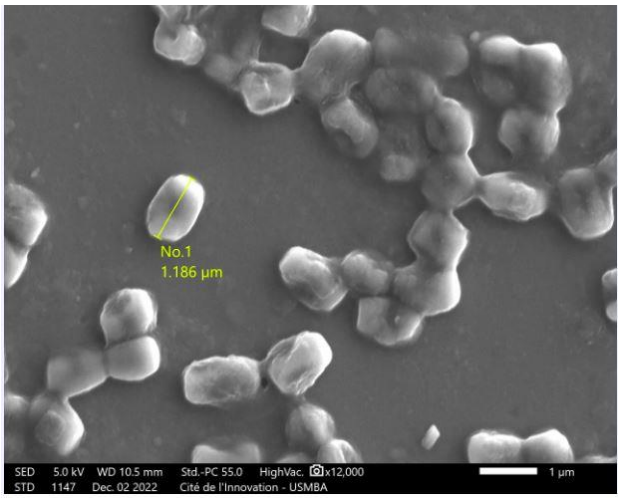

Figure 3: Spores of *Streptomyces anulatus* EFBO8 observed under Scanning Electron Microscope (JEOL JSM-IT500HR)

3.5. Molecular identification of the effective Streptomyces strain

The obtained strain was further identified and confirmed as Streptomyces species based on genomic analysis of their 16S rRNA gene sequences. Comparison of 16S rRNA gene sequences (ranging from 1450 to 1518 nucleotides) gene sequences with their similar matches in the GenBank verified that the obtained strain was closely related (99.93%) with 9 species under the genus Streptomyces and 100% identical to *Streptomyces anulatus*, strain NBRC 12861 published under the accession number AB184199. The nearly complete 16S rRNA gene sequences were analyzed in a phylogenetic tree using maximum likelihood algorithms. (Figure 3). The strain (*Streptomyces anulatus*) was deposited in the Culture Collection of the laboratory Functional Ecology and Environmental Engineering Fst-Fez. The entry code of the EFBO8 culture collection is assigned to the deposited strain and published under the accession number OQ073700 in NCBI.

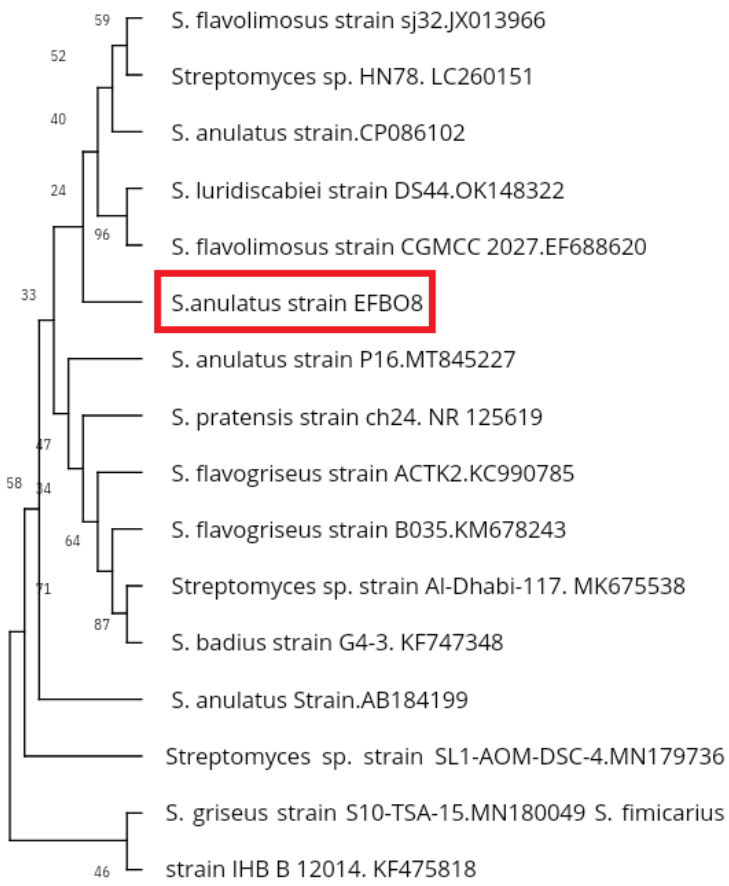

Figure 4: The phylogram of *Streptomyces anulatus EFBO8* compared to other Streptomyces species based on 16S rDNA sequences.

3.6. **Inulinase production kinetics**

The following figure (figure 5) shows inulinase activity, assessed by EU/ml, kinetic where assay were performed every 12 hours.

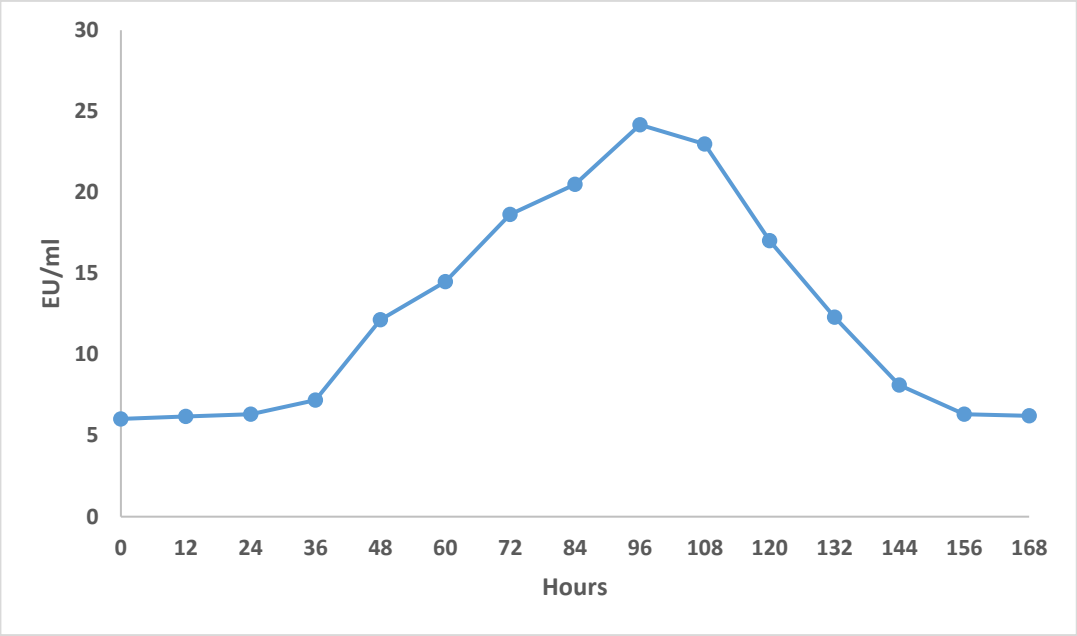

Figure 5: *Streptomyces anulatus* Inulinase activity kinetic

Kinetic showed that constitutive activity of inulin hydrolysis was detected from 0 to 24h. after that we noticed an inducible inulin hydrolysis from 36 to144h. After this period, constitutive activity of inulin hydrolysis pops up a second time (156-168h). Maximum production of inulinase activity was reached at 96h (24.17 U/ml) and then enzyme activity level decreased until it stabilized at a value of 6.032 U/ml.

3.7. Purification of enzyme

The supernatants obtained from ammonium sulphate precipitates were loaded into a Sephadex G-50 column.

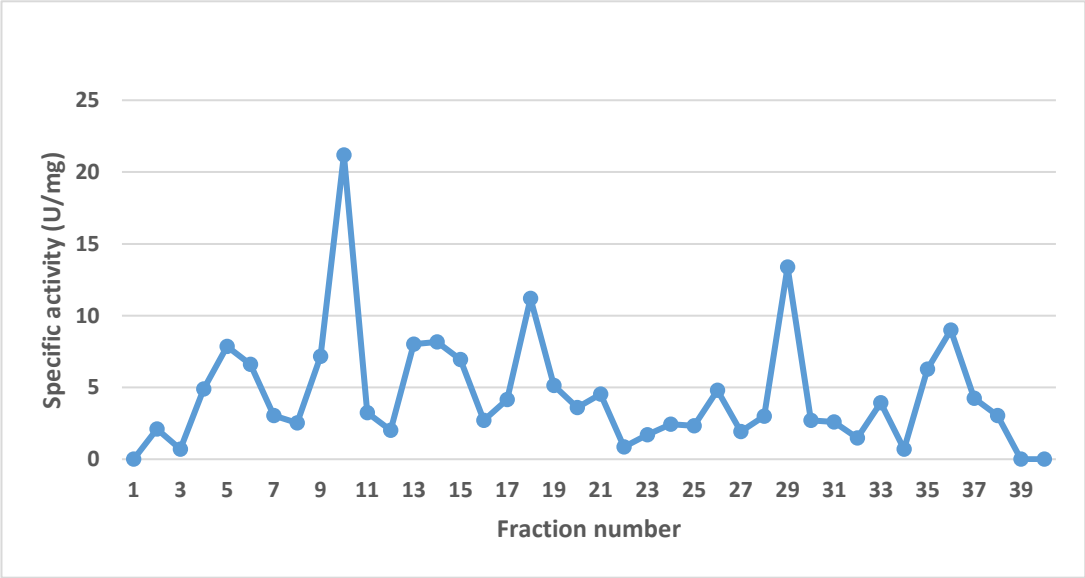

Figure 6: Elution profile of Sephadex G-50 for exo-inulinase purification.

Results showed the presence of four peaks for unilinase specific activity in fractions number 10, 18, 29 and 36 and the maximum value of this specific activity (21.19 U/mg) is recorded in the 10th fraction.

3.8. Precipitation with ethanol

The results of the enzymatic tests of the enzyme precipitated with ethanol are shown in Figure 7.

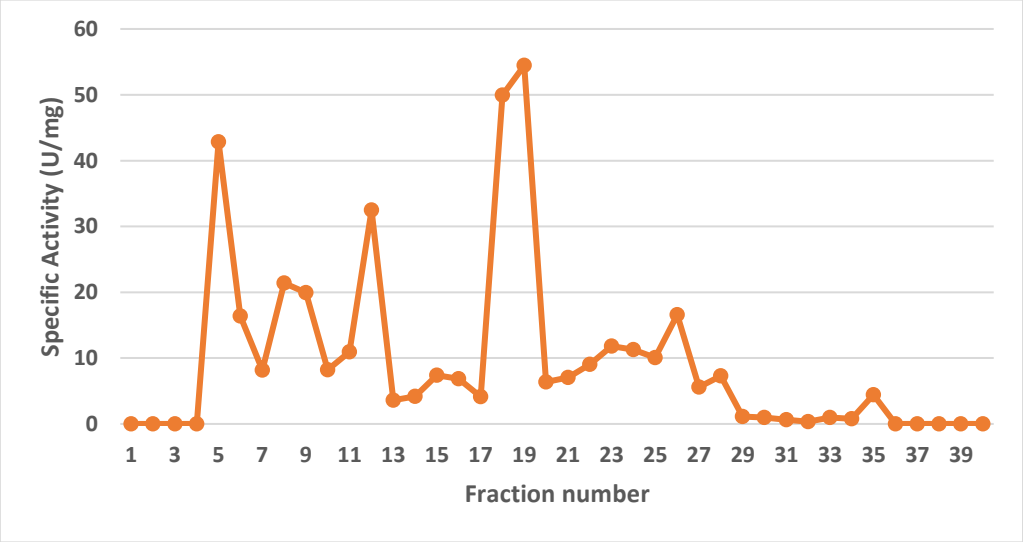

Figure 7: Elution profile of Sephadex G-50 for the precipitate with ethanol

Result of elution profile of enzyme after precipitation with ethanol shows the elution of three active fractions, the first is located at the 5th fraction, the second at the 12th fraction and the third at the 19th fraction with values of 42.84 U/ml, 32.74 U/mg and 54.45 U/ml respectively.

3.9. Determination of molecular weight

There is a huge variation in molecular weight of the same protein when purified by different methoses. The molecular weight of purified inulinase from actinomycete strain and determined by size exclusion chromatography was approximately 3331.83 Da, whereas the functionally similar enzyme purified from the same strain with ethanol precipitation has a molecular weight of 3691.4 Da (Figure 8). A total of 40 fractions were collected and for polymers revelation presence we used using 3,5-dinitrosalicylic acid DNS. This protocol allows colorimetric determination of sugar. The aim of this experiment is obtaining a calibration curve that links molecular weight with retention time. The link between the molecular weight and retention time is represented as the following equation: $Rt = aMW + b$

Where:

- Rt: retention time;
- Mw: Molecular weight.

The equation of the calibration curve is:

$$y = -0.0041x + 3.9361 \text{ and } R^2=0.99$$

The presence of the polymers was detected using 3,5-dinitrosalicylic acid protocol. While the intensity of the peak was evaluated using the absorbance reading of each fraction at 540 nm. After calibration curve establishment, we performed an exclusion chromatography of our two purified enzymes. Table 3 shows the results obtained according to the sampling interval.

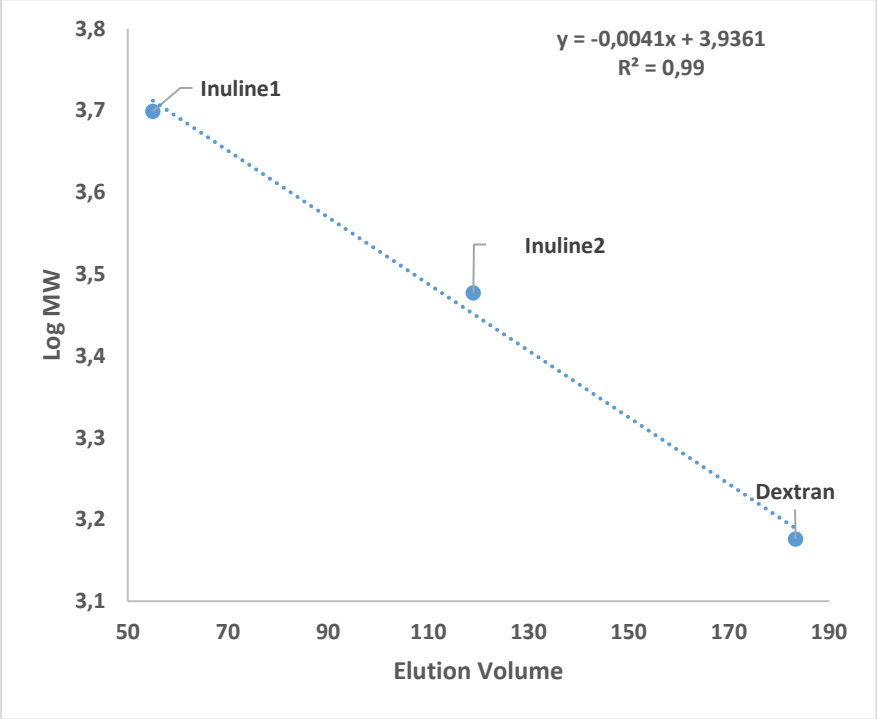

Figure 8: Plot of Log MW vs elution volume to calculate the molecular weight of purified inuline

The extracellular enzyme was eluted out at 101 ml and thus the corresponding Log10 MW was equal to 3,48 giving a native molecular weight of ~ 3331,83Da and the purified enzyme after precipitation with ethanol was eluted out at 82,5 ml and thus the corresponding Log10 MW was equal to 3,59 giving a native molecular weight of ~3961,4Da

Table 3: Molecular weight of the different enzymes

	Molecular weight (Da)
Standard Inuline 1	5000
Standard Inuline 2	3000
Standard Dextran	1500
Extracellular purified enzym	3331.83
Purified enzyme with Ethanol precipitation	3961.4

4. Discussion

The results of our qualitative screening are comparable with those found by with those found by (Wannasutta et al., 2019), which found that among 18 strains of actiomycetes, one strain identified as *Streptomyces gancidicus* possesses height inulinase activity.

The finding of the inulinase production kinetics are similar to those (Ali & Khalid, 2020), having found that in *R. oligosporus*, maximum production of inulin hydrolysis is reached after 72h with a value of 30 U/mL. Also according to (W.-D. Lu et al., 2014) , maximum inulin hydrolyzing activity (24 U/mL) for *Nocardiopsis sp*, was achieved at 60h, and thereafter the level of enzyme slightly decline. Thus, the alternation between a constitutive enzyme system and an inducible system is already tacitly mentioned in these aforementioned works.

The enzyme purification results confirms that obtained by (R. S. Singh et al., 2018) who also found in *Penicillium oxalicum* different peaks of inulinase showing a multilude of isoforms of exo-inulinase. Our results are consistent with those of (A. D. Sharma & Gill, 2007) who found a specific activity of 38 U/mg in streptomyces sp. Also, Comparison of unilinase activity values before and after precipitation of the enzyme with ethanol allowed us to deduce that the unilinase specific activity increase after precipitation. This suggests that precipitation may promote enzyme concentration by precipitation process. Our results are similar to those of (Golunski et al., 2011) which report that it was possible to obtain a 5.5-fold purification of crude extract with 86.1% yield, after ethanol precipitation followed by ultrafiltration.

In this study, *Streptomyces anulatus* was found to be the most efficient for the production of inulinase with a value of 24.7 U/ml. A study of the production of inulinase was carried out, in order to optimise the enzyme production time, a kinetic of the production was performed. Later, the enzyme was purified and its molecular weight was found to be 3331.83 Da. After precipitation of the enzyme with ethanol, the enzyme gave a molecular weight of 3951.83 U/ml.

These results show that *Streptomyces anulatus* strain has a viable potential that can be exploited in several fields.

5. Conclusion

In this study, *Streptomyces anulatus* was found to be the most efficient for the production of inulinase with a value of 24.7 U/ml. A study of the production of inulinase was carried out, in order to optimise the enzyme production time, a kinetic of the production was performed. Later, the enzyme was purified and its molecular weight was found to be 3331.83 Da. After precipitation of the enzyme with ethanol, the enzyme gave a molecular weight of 3951.83 U/ml. These results show that *Streptomyces anulatus* strain has a viable potential that can be exploited in several fields.

Conclusion générale et perspectives

Cette thèse met en évidence l'importance des actinomycètes dans différents aspects de l'agriculture et de la biotechnologie. Le chapitre 1 souligne que l'utilisation des eaux résiduaires de l'industrie oléicole dans le traitement des sols cultivés avec différentes variétés de menthe permet de sélectionner des souches d'actinomycètes compétentes pour la dégradation de différentes sources de carbone et d'énergie. Cette approche ouvre la voie à de nouvelles recherches visant à découvrir de nouvelles molécules aux propriétés intéressantes, notamment dans le domaine agronomique.

Le chapitre 2 a mis en évidence le potentiel des actinomycètes en tant que source d'enzymes et de métabolites bioactifs. Les résultats montrent que les souches d'actinomycètes isolées à partir des sols irrigués avec les eaux résiduaires de l'industrie oléicole ont une activité enzymatique élevée et un bon pouvoir antimicrobien. Ces actinomycètes représentent une ressource inexploitée et leur potentiel biotechnologique peut contribuer au développement de nouveaux processus industriels dans les domaines de la pharmacie, de l'agriculture et en particulier du compostage.

Le chapitre 3 a souligné en évidence l'importance de l'utilisation du compost, en particulier du compost inoculé avec des streptomyces, pour améliorer la fertilité du sol et le rendement des cultures. Les résultats indiquent que les graines produites par les plantes traitées avec le compost inoculé sont plus saines et mieux développées, et que le compost peut fournir les éléments nutritifs essentiels pour la croissance et le développement des plantes. Cette approche offre une alternative prometteuse et durable aux engrais chimiques, contribuant ainsi à l'agriculture durable.

Le chapitre 4 met en évidence l'importance des actinomycètes thermophiles dans le processus de compostage. Les résultats montrent que l'isolement de *Thermoactinomycetes sacchari* ouvre la voie à l'utilisation de ces actinomycètes compétents dans divers processus industriels. Cette étude fournit également des informations sur les communautés microbiennes présentes dans le compost et peut servir de base pour optimiser les conditions de compostage afin de maximiser la dégradation des déchets organiques et obtenir un compost de haute qualité.

Le dernier chapitre (chapitre 5) met en évidence le potentiel des actinomycètes, en particulier de la souche *Streptomyces anulatus*, dans la production d'inulinase. Les résultats montrent que cette souche présente une activité élevée d'inulinase, ce qui ouvre la voie à son exploitation dans différents domaines.

Dans l'ensemble, cette thèse démontre l'importance des actinomycètes dans l'amélioration de la fertilité des sols, la production d'enzymes et de métabolites bioactifs, ainsi que dans le développement de nouvelles approches biotechnologiques. Ces résultats offrent de nouvelles perspectives pour une agriculture durable, une gestion efficace des déchets organiques et le développement de nouveaux processus industriels. Il reste encore beaucoup à explorer et à approfondir dans le domaine des actinomycètes, notamment en ce qui concerne leur diversité, leurs capacités enzymatiques et leurs applications potentielles. Les résultats de cette thèse soulignent la nécessité de poursuivre la recherche sur les actinomycètes afin d'exploiter pleinement leur potentiel biotechnologique. Les actinomycètes peuvent jouer un rôle clé dans le développement de solutions durables pour l'agriculture, telles que la production de compost de haute qualité et la réduction de la dépendance aux engrais chimiques. Leur capacité à produire des enzymes et des métabolites bioactifs ouvre également des perspectives prometteuses dans des domaines tels que la pharmacie, l'agroalimentaire et la valorisation des déchets organiques.

Ce projet de thèse met en évidence l'importance des actinomycètes en tant que ressource précieuse et inexploitée dans divers domaines. Les résultats obtenus fournissent des informations précieuses sur leur potentiel biotechnologique et soulignent la nécessité de poursuivre la recherche dans ce domaine. En exploitant pleinement le potentiel des actinomycètes, nous pourrions contribuer à des pratiques agricoles durables, à la réduction de l'utilisation de produits chimiques et à la promotion d'une économie circulaire basée sur la valorisation des déchets. En alignant ses objectifs avec ceux du Plan Maroc Vert (GMP 2008-2020), cette recherche contribue à la promotion de pratiques respectueuses de l'environnement, à la valorisation des ressources organiques et à la construction d'un secteur agricole plus résilient et compétitif au Maroc.

La présente contribution converge avec les recommandations des politiques agraires africaines (Agenda 2063 : L'Afrique que nous voulons), qui visent à développer une agriculture durable qui ne nuit pas à l'environnement. Le compostage contrôlé pourrait apporter des éléments de réponse à la question de la gestion correcte et optimale de l'énergie et de la biomasse. Les valeurs ajoutées des connaissances scientifiques générées par ce travail pourraient également améliorer le cadre de vie des agriculteurs et des citoyens en Afrique. Les résultats innovants de notre recherche peuvent également contribuer à la réussite du développement et de la généralisation de l'économie circulaire au Maroc en tant que pays africain bien engagé dans

l'économie de cluster qui rassemble les universités, le secteur privé et les associations pour aller vers un développement agricole durable.

Les perspectives de ce travail sont très ramifiées vu la complexité de la thématique, ainsi plusieurs travaux de recherches futures peuvent être planifiés pour continuer la recherche et dévelopement sur le compostage contrôllé au Maroc et sous d'autre cieux. Le nouveau concept des travaux futures va se baser sur un principe qui concentre les efforts sur plusieurs pistes à savoir :

-Au niveau ingenieurie biologique, plusieurs travaux scientifiques et techniques seront canalisées dans une visée économique et rationnelle pour créer des entreprises spécialisées dans le domaine.

-Au niveau théorique, les résultats scientifiques obtenus pourraient faire l'objet de travaux de masters et de thèses pour comprendre les mécanismes moléculaires et biochimiques des micro-organismes impliqués dans le compostage afin de l'optimiser et le réaliser à échelle indistruelle.

-Au niveau économique, des études d'ingenieurie économique des projets exploitables en agriculture afin de déterminer avec précision les charges économiques des projets de valorisation des connaissances scientifiques et techniques de notre projet de thèse.

-Au niveau de politique de gestion de l'agriculture, des études secterielles du compostage pourraient expliquer et recommender plusieurs procédés de compostage bien adaptées au conditions locales et régionales.

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