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Theme

Isolation and screening of actinobacteria from different regions in the wilaya of Laghouat for valorization of lignocellulosic agri-food wastes

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موضوع البحث:

عزل و فحص الأكتينوباكتريا من مناطق مختلفة في ولاية الاغواط بغرض إستخدامها فى تثمين نفايات الأغذية الزراعية

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Full name: Harzallah

Title: Isolation and screening of actinobacteria from different regions in the wilaya of Laghouat for valorization of lignocellulosic agri-food wastes.

Abstract

Isolation of actinobacteria that produce cellulolytic enzymes is extremely important, given the increased demand for these enzymes in many industrial applications, particularly, in the valorization of lignocellulosic wastes for the production of biofule. 34 actinomycetes were isolated from four different region of Laghouat, using cellulose agar medium. Screening for the capacity of strains to degrade cellulose was undertaken on CMC agar medium with Congo red as a dye. The results indicate that 25 isolates showed positive activity, while 9 isolates were inactive. The actives actinomycetes were grouped according to the intensity of hydrolytic zones around the colonies on CMC agar, as: weak activity (5 isolates), moderate activity (9 isolates) and strong activity (11 isolates). The results of this study show that the soil of Laghouat region presents a very good reservoir of actinobacteria which is able to produce cellulolytic enzymes that is useful in many industries, especially bioethanol industry.

Key words: Isolation; Actinomycetes, Laghouat, valorization, Lignocellulosic wastes, Bioethanol.

Nom et prénom: Harzallah Halima.

Titre : Isolement des actinobactéries à partir des diffèrent régions de la Wilaya de Laghouat en vue de les utilisez dans la valorisation des déchets agroalimentaire lignocellulosique.

Résumé:

L'isolement des actinobacteria qui produisent des enzymes cellulolytiques est extrêmement important, surtout avec la demande croissante de ces enzymes dans de nombreuses industries, particulièrement dans la valorisation des déchets lignocellulosique pour la production des biocarburants. 34 actinomycetes ont été isolées à partir de quatre différentes régions de la wilaya de Laghouat, en utilisant le milieu cellulose agar. Le dépistage de la capacité des souches à dégrader la cellulose a été détecté sur le milieu carboxymethylcellulose agar avec l'utilisation de rouge Congo comme colorant. Les résultats indiquent que 25 isolats sont actifs, alors que 9 isolats sont inactifs. Les actinomycètes actifs ont été regroupés en fonction de l'intensité des zones hydrolytiques autour des colonies sur la gélose carboxymethylcellulose: faible activité (5 isolats), activité modérée (9 isolats) et forte activité (11 isolats).Les résultats de cette étude montrent que le sol de la région de Laghouat présente un très bon réservoir d'actinobactéries qui sont capables de produire des enzymes cellulolytiques utiles dans de nombreux secteurs, notamment celui du bioéthanol.

Mots clé : Isolement, Actinomycetes, Laghouat, Valorisation, Déchets lignocellulosique, Bioethanol.

العنوان:الاسم واللقب: حليمة حرزالله

العنوان:عزل و فحص الأكتينوباكتريا من مناطق مختلفة في ولاية الاغواط بغرض إستخدامها في تثمين نفايات الأغذية الزراعية

الملخص:

تعتبر عملية عزل الأكتينوبكتيريا التي تنتج إنزيمات تحلل السليلوز أمرا في غاية الأهمية؛ نظرا للطلب المتزايد على هذه الإنزيمات في العديد من المجالات الصناعية، و خاصة في تثمين النفايات الليغنوسليلوزية بغرض إنتاج الوقود البيولوجي. تم عزل 34 عزلة أكتينوميسيت من أربعة مناطق مختلفة في ولاية الأغواط بإستخدام وسط زرع يحتوي على السليلوز أجار. تم نقل العزلات المتحصل عليها من وسط الزرع إلى وسط يحتوي على الكربوكسيميثيل سليلوز أجار وذلك بغرض الكشف عن قدرتها على تحليل السليلوز بإستعمال صبغة الكونغو ككاشف لوني .كشفت النتائج المتحصل عليها عن وجود 25 عزلة أظهرت نشاط إيجابي؛ بينما أظهرت 9 عزلات نشاطا سلبيا. تم تصنيف الأكتينوميسات النشطة حسب شدة زوال اللون حول المستعمرات في الوسط إلى: نشاط ضعيف (5عزلات)؛ نشاط متوسط (9عزلات) و أخيرا نشاط قوي (11عزلة). أثبتت النتائج المتحصل عليها من هذه الدراسة أن تربة ولاية الأغواط عبارة عن خزان جيد للأكتينوبكتيريا التي لديها القدرة على إفراز إنزيمات لينوسليلوزية ذات قيمة صناعية كبيرة خاصة في مجال صناعة الإيثانول البيولوجي.

الكلمات المفتاحية: عزل , أكتينوميسيت , الأغواط,تثمين , النفايات الليغنوسلولوزية ,لإيثانول البيولوجي.

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I would like to express my gratitude to the members of the jury Mr. Boubrima and Mm.Ameur because they accepted to analyze and correct this work. Also I want to thank my supervisor Mr. Masoudi Omar who spent his precious time to guide me all the time to the right way in doing better job, my department and president of the department Mr.Ahmed Benchetouh and his vice-president Mme.Marfouaa Miriam, my teachers including: "Mr.Goudjal, Mr. Houicher, Mr.Adamou, Mr.Makoudi, Mr. Djoukhdem, Mr. Ben Hasine", Whom provides me well.

To all, I respect you all and many thanks for your assistance and encouragement.

Dedication

First of all, I would thank the" Great Allah" who helped me and guide me in my life.

I would like to dedicate this work to: my parents "my mom Zineb Abedelali, my dad Bachir Harzallah" may Allah protect them, who were encouraging me and proud of me all the time, I want to say that I love you so much and I'm so proud being your daughter.

To my precious husband "Maamar Mordjani" I want to say thank you for your support, you were always by my side I love you so much, also to my dear mother in low "Yamina Mordjani" my second mother may Allah always protect you.

My lovely sisters "Malika, Hadjer, Soltana, Fatima and of course to my little piece of heart my nephew Aissa ". My dear brothers: "Ibrahim, Khaled and Ahmed".

I would also thank my grandfather;" Hadj Mohamed Harzallah", who was giving me advisees and teaching me haw to stand up after each felling.

All my wonderful family may Allah always protects you.

To all: my teachers, my colleges, and my best friends "Masouda, Saiida, Houriya, Safa, Rihab, Nadya. Hadjer, Kawthar."

And finally, to myself I would say "the pain you felt yesterday was the strength you feel today".

Harzallah Halima

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List of abbreviations

(BEL) **:** Biodegradation Enzyme of Lignin.

(CAm) **:** Cellulose Agar medium.

(CE) **:** Cellulolytic enzymes.

(CHP) **:** Complex Heterogeneous Polysaccharides.

(CMC) **:** Carboxymethylcellulose Medium.

(CR) **:** Congo Red.

(CSAm) **:** Casein Starch Agar (CSA) medium.

(ECF) **:** Elementary Crystalline Fibrils.

(FAO) **:** The Food and Agriculture Organization.

(HSM) **:** Hexose Sugar Mannose.

(LCB) **:** Lignocellulose Biodegradation.

(LCC) **:** Lignin Carbohydrate Complexes.

(LCE) **:** Lignocellulolytic Enzymes.

(LCM) **:** Lignocellulosic Materials.

(LCP) **:** Lignocellulosic Products.

(LCW) **:** Lignocellulosic Wastes.

(LHE) **:** Lignocelluloses Hydrolyzing Enzymes.

(MSW) **:** Municipal Solide Wastes.

(pH) **:** Potentiel Hydrogène.

(PSX) **:** Pentose Sugar Xylose.

(SFS) **:** Simultaneous Fermentation and Saccharification.

(UN) **:** United Nations.

(UNEP) **:** United Nations Environment Programme.

(VFAs) **:** Volatile Fatty Acids.

GC-content **:** The percentage of nitrogenous bases on a DNA or RNA molecule that are either guanine or cytosine.

Introduction

Food loss or food waste is often used in scientific literature to identify materials intended for human consumption. The Food and Agriculture Organization **(FAO)** of the United Nations (UN) defined food loss as any change in the availability, edibility, wholesomeness or quality of edible material that prevents it from being consumed by people. The production of food wastes covers all the food life cycle: from agriculture phase, up to industrial manufacturing and processing, retail and household. **(UNEP; 2016).**

The increasing global population poses the food supply chain in serious problems especially with changes in consumption models. The food processing industry in the all over world is progressing at a very fast speed. Such an increasing industrialization can give rise to more waste that is ultimately left untreated due to lack of treatment options **(Sala et al; 2017).**

The huge amounts of bio- wastes produced by the agricultural sector create huge environmental, economic and social problems **(Arshadi et al; 2016).** Approximately 1.3 billion tons/year, i.e. one third of the food produced for human consumption, is wasted globally **(Kojima and Ishikawa; 2013).** It has been estimating that for each ton of food wastes there is an emission of about 2 tons of CO_2 which is the major cause associated with the problem of the global warming **(European Commission; 2010).**

Most of the wastes generated from the food industry are lignocellulosic. Wastes valorization practices have gained much attention lately as a means of a sustainable management.

Lignocellulolytic enzymes, including cellulase, hemicellulases and lignin, are potent enzymes produced by fungi, actinomycetes and the other bacteria, these enzymes could be exploited widely in various lignocelluloses based industries **(Prakash et al, 2013),** includes fossil fuel alternatives such as bioethanol, biogas, and fuel oil, or in production of food supplements such as prebiotics **(Uçkun Kiran et al, 2014; Yin et al, 2014).**

This study presents the method of isolation and screening of actinomycetes witch are aerobic Gram positive bacteria from different agricultural regions in the wilaya of Laghouat located in the south Algeria, these regions are (Hamda, Tadjrouna, Elbayda, Elgaicha), in order to discover their potential to produce lignocellulases enzymes involved in biomass degradation in purpose to use them in the valorization of lignocellulosic wastes (LCW).

Bibliography

I

Lignocellulosic wastes

1. Food wastes from agricultural industry

The huge amount of lignocellulosic wastes (LCW) produced by food industries affects municipal landfills because of FW high biodegradability, leachate and methane emissions (**Misi and Forster, 2002).** These wastes processing generally contain organic composition includes about 75% complex sugars and hemicelluloses, 9% cellulose and 5% lignin **(Kosseva, 2011).**

2. Source and composition of lignocellulosic products (LCP)

Lignocellulosic products (LCP) refer to plant biomass wastes that are composed of cellulose, hemicellulose, and lignin. They may be grouped into different categories such as wood residues (including sawdust and paper mill discards), grasses, waste paper, agricultural residues (including straw, Stover, peelings, cobs, stalks, nutshells, non food seeds, bagasse), domestic wastes (lignocelluloses garbage and sewage) **(Qi et al., 2005; Roig et al., 2006; Rodríguez et al., 2008).**

Lignocellulosic materials (LCM) may be described as one of the most promising natural abundant and renewable feedstock available for the enhancement and maintenance of industrial societies and critical to the development of a sustainable global economy **(Kumar et al, 2009).**

The composition of lignocelluloses depends on plant species, age and growth conditions. Distribution of cellulose, hemicelluloses and lignin as well as the content of the different sugars of the hemicelluloses varies significantly between different plants **(Table 1).**

Table 1: Composition of different lignocellulosic materials (**Jørgensen et al; 2007)**

Material	Glucose[a]	Xylose[b]	Arabinose[b]	Mannose[b]	Lignin	Reference
	[% of total dry weight]					
Hardwood						
Birch	38.2	18.5	–[c]	1.2	22.8	136
Willow	43.0	24.9	1.2	3.2	24.2	31
Softwood						
Spruce	43.4	4.9	1.1	12.0	28.1	34
Pine	46.4	8.8	2.4	11.7	29.4	137
Grasses (Poaceae)						
Wheat straw	38.2	21.2	2.5	0.3	23.4	137
Rice straw	34.2	24.5	n.d.[d]	n.d.[d]	11.9	137
Corn stover	35.6	18.9	2.9	0.3	12.3	136

[a]Glucose is mainly coming from cellulose.
[b]Xylose, arabinose and mannose make up hemicelluloses.
[c]Below detection limit.
[d]Not determined.

3. Structure of lignocellulosic wastes

➢ Cellulose

The most abundant constituent of the plant cell wall, is a homo-polysaccharide composed entirely of d-glucose linked together by β-1, 4-glucosidic bonds and with a degree of polymerization of up to 10 000 or higher (**Figure 1**)**.** The linear structure of the cellulose chain enables the formation of both intra- and intermolecular hydrogen bonds resulting in the aggregation of chains into elementary crystalline fibrils (ECF) of 36 cellulose chains. The structure of the elementary fibril is crystalline; however, some sources claim that the surface could be viewed as amorphous **(Ding et al, 2006).** The structure of cellulose along with the intermolecular hydrogen bonds gives cellulose high tensile strength, makes it insoluble in most solvents and is partly responsible for the resistance of cellulose against microbial degradation **(Ward et al, 1989).**

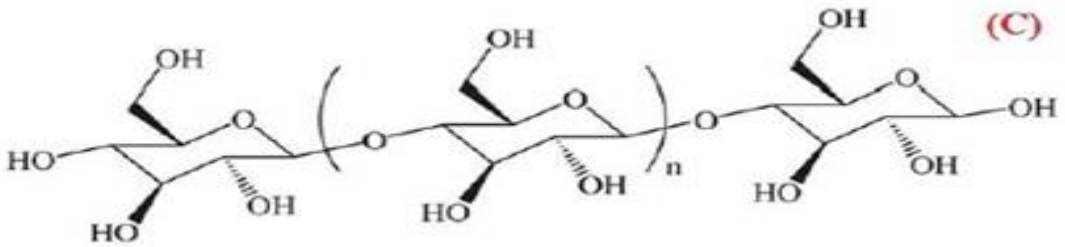

Figure 1: Chemical structure of the cellulose **(Iqbal et al; 2013).**

- **Hemicelluloses**

Are complex heterogeneous polysaccharides (CHP) composed of monomeric residues: d-glucose, d-galactose, d-mannose, d-xylose, l-arabinose, d-glucuronic acid and 4-*O*-methyl-d-glucuronic acid (**Figure 2**). Hemicelluloses have a degree of polymerization below 200, side chains and can be acetylated **(Kuhad et al, 1997).** Hemicelluloses are classified according to the main sugar in the backbone of the polymer, e.g. xylan (*β*-1,4-linked Xylose) or mannan (*β*-1,4-linked mannose). Plants belonging to the grass family (Poaceae), e.g. rice, wheat, oat and switch grass have hemicelluloses that are composed of mainly glucuronoarabinoxylans **(Carpita et al, 1996).** Due to these differences in hemicellulose composition, agricultural waste products like straw and corn Stover as well as hardwood materials are rich in the pentose sugar Xylose (PSX), whereas soft woods are rich in the hexose sugar mannose (HSM) (**Table 1**).

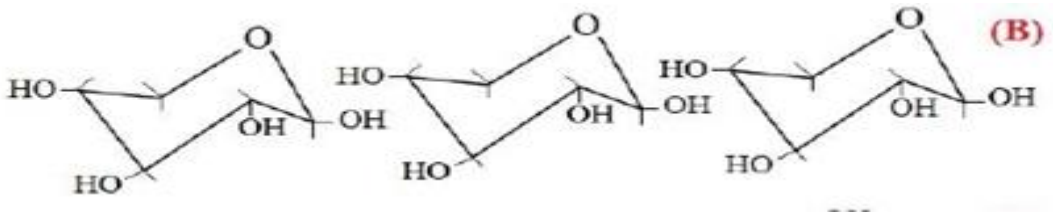

Figure 2: Chemical structure of Xylose unite of hemicellulose **(Iqbal et al; 2013).**

- **Lignin**

Is a complex network formed by polymerization of phenyl propane units and constitutes the most abundant non-polysaccharide fraction in lignocellulose. The three monomers in lignin are *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol and are joined through alkyl–aryl, alkyl–alkyl and aryl–aryl ether bonds (**Figure 3**). Furthermore, lignin is able to form covalent bonds to some hemicelluloses, e.g. benzyl ester bonds with the carboxyl group of 4-*O*-methyl-d-glucuronic acid in xylan. More stable ether bonds, also known as lignin carbohydrate complexes (LCC), can be formed between lignin and arabinose or galactose side groups in xylans and mannans **(Kuhad et al, 2011).**In general, herbaceous plants, such as grasses, have the lowest content of lignin, whereas soft woods have the highest lignin content (**Table 1**).

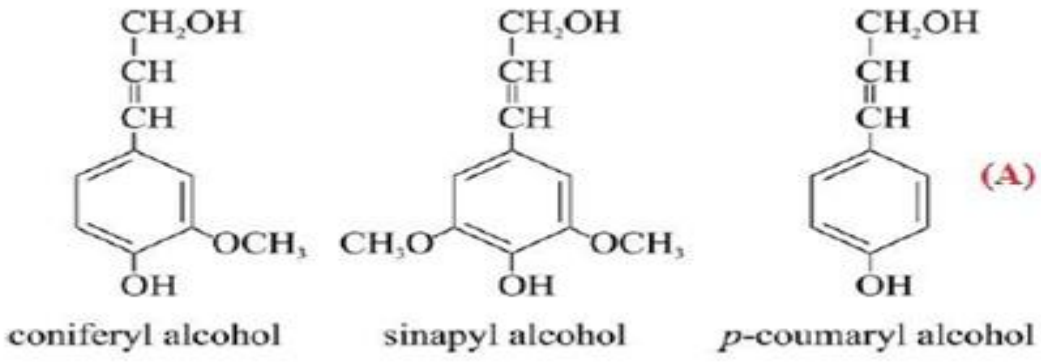

Figure 3: Chemical structure building blocks/unites of Lignin **(Iqbal et al; 2013).**

4. Lignocellulose biodegradation (LCB)

Lignocellulose is a complex substrate and its biodegradation is not dependent on environmental conditions alone, but also the degradative capacity of the microbial population **(Waldrop et al, 2000).** The composition of the microbial community charged with lignocellulose biodegradation determines the rate and extent thereof.

4.1. Biodegradation enzymes of cellulose and hemicelluloses

The efficient hydrolysis of cellulose requires the concerted action of at least three enzymes: (1) endoglucanases to randomly cleave intermonomer bonds; (2) exoglucanases to remove mono- and dimers from the end of the glucose chain; and (3) β-glucosidase to hydrolyze glucose dimers (**Figure 4**) **(Deobald &Crawford 1997; Tomme et al. 1995).**The concerted actions of these enzymes are required for complete hydrolysis and utilization of cellulose. The rate-limiting step is the ability of endoglucanases to reach amorphous regions within the crystalline matrix and create new chain ends, which exo cellobiohydrolases can attack. Although similar types of enzymes are required for hemicellulose hydrolysis, more enzymes are required for its complete degradation because of its greater complexity compared to cellulose. Of these, xylanase is the best studied reviewed by **(Kuhad et al. 1997).**

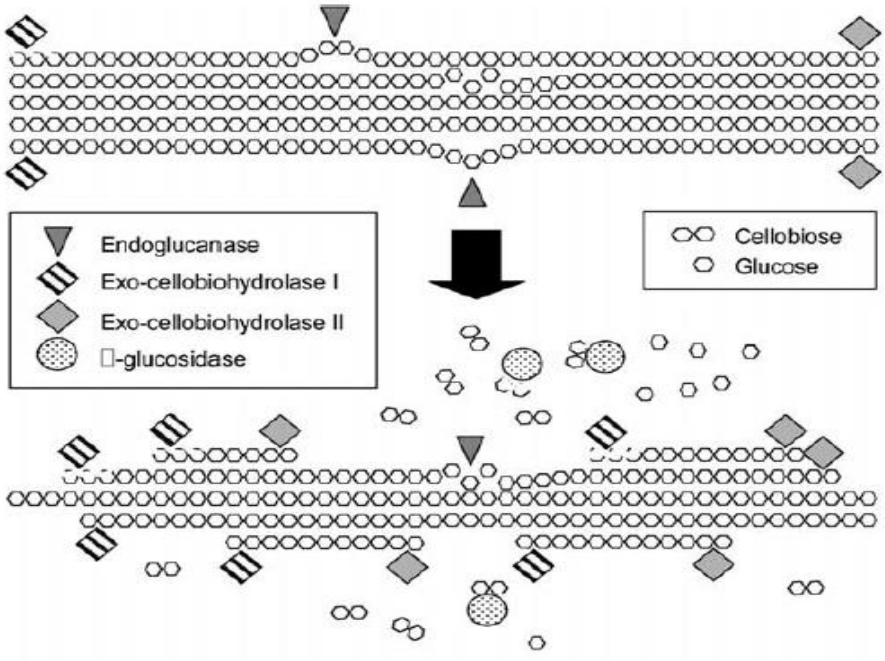

Figure 4: A simplified schematic representation of the process involved in complete enzymatic hydrolysis of a cellulose microfibril **(Malherbe.S & Cloete T.E; 2002)**

4.2.Biodegradation enzyme of lignin (BEL)

Lignin degradation is an oxidative process and phenol oxidase is the key enzymes **(Kuhad et al. 1997; Leonowicz et al. 1999).** Of these, lignin peroxidases (LiP), manganese peroxidases (MnP) and laccases from especially white rot fungi (*P. chrysosporium*, *Pleurotus ostreatus* and *Trametes versicolor*) have been best studied. LiP and MnP oxidize the substrate by two consecutive one-electron oxidation steps with intermediate cation radical formation. Laccase has broad substrate specificity and oxidises phenols and lignin substructures with the formation of oxygen radicals. Other enzymes that participate in the lignin degradation processes are H_2O_2-producing enzymes and oxidoreductases, which can be located either intra- or extracellularly. Bacterial and fungal feruloyl and *p*-coumaroyl esterases are relatively novel enzymes capable of releasing feruloyl and *p*-coumaroyl and play an important role in biodegradation of recalcitrant cell walls in grasses **(Kuhad et al. 1997).** These enzymes act synergistically with xylanases to disrupt the hemicellulose-lignin association, without mineralization of the lignin **(Borneman et al. 1990; Fillingham et al. 1999; Tuor et al. 1995).** Therefore, hemicellulose degradation is required before efficient lignin removal can commence.

5. Industrial uses of lignocellulosic wastes

Many of these lignocellulosic wastes (LCW) could be valorized as animal feed, compost, or transformed into biomass-based energy fuel and a wide variability of industrial products, such as wood-based panels, bio-fertilizers, biofibers among others **(Santana-Meridas et al., 2012).**

5.1. Pretreatment technologies in transformation of lignocellulosic wastes

Due to their abundance and renewability, there has been a great deal of interest in utilizing LCW for the production and recovery of many value-added products **(Pandey et al., 2000; Das and Singh, 2004; Foyle et al., 2007).** The main goal of any pretreatment, therefore, is to alter or remove structural and compositional impediments to hydrolysis and subse-quent degradation processes in order to enhance digestibility, improve the rate of enzyme hydrolysis and increase yields of intended products **(Mosier et al., 2005; Hendriks and Zeeman, 2009).** These methods cause mechanical, physical chemical biological changes in the plant biomass in order to achieve the desired products.(**Figure 5**).

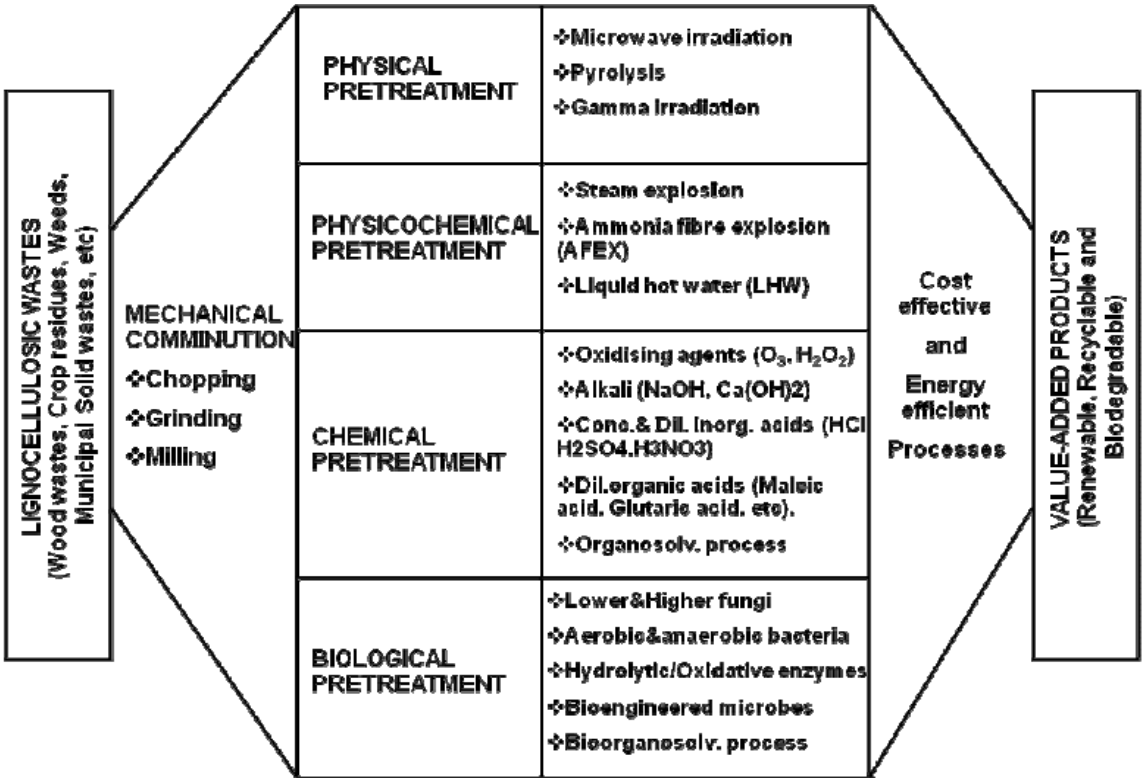

Figure 5: A summary of various methods used in the pretreatment of lignocellulosic wastes **(Godliving Y. S. Mtui; 2009).**

5.2. Importance of pretreatment in the biorefinery context

There is an extensive variety of lignocellulosic materials (LCM), ranging from grasses, softwoods, and hardwoods, which also have different physical and chemical properties. For this reason there is interdependence between pretreatment the type of substrate, and the way it is eventually processed.

6. Biofuels generated from (LCW)

Conversion of LCW to bio-fuels (BF) provides the best economically feasible and conflict-free second generation renewable alternatives **(Rubin, 2008).** Significant advances have been made towards bioconversion of plant biomass wastes into bioethanol, biodiesel, biohydrogen, biogas (methane), (**Figure 6**).

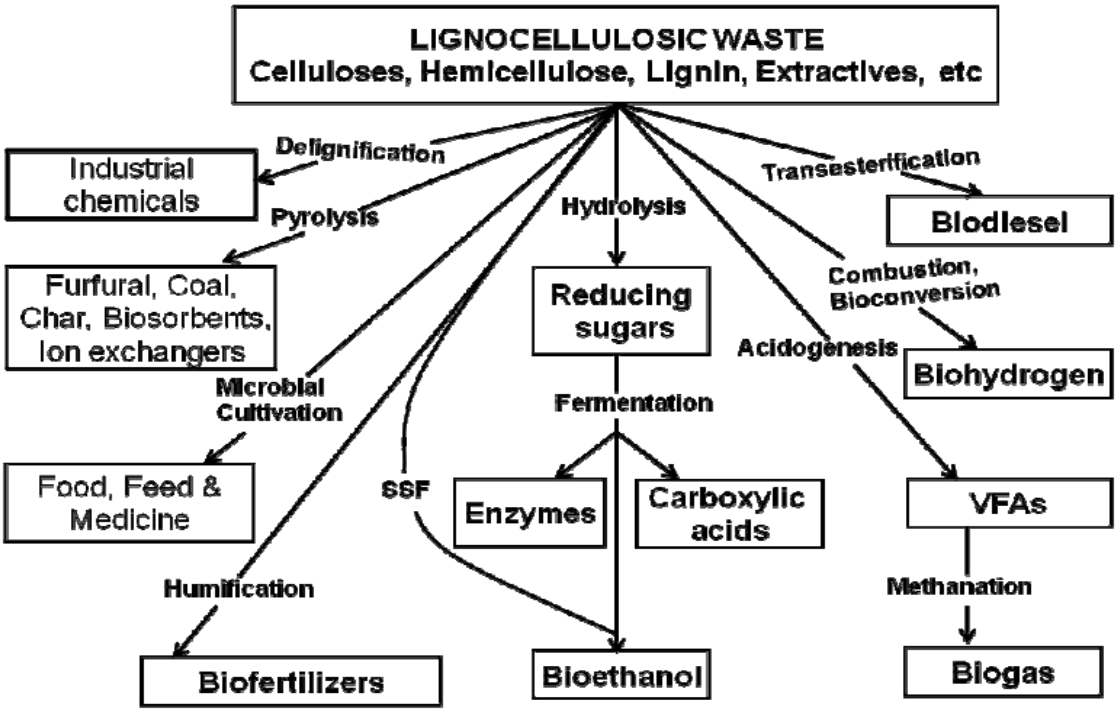

Figure 6: products from lignocellulosic wastes (SSF=simultaneous fermentation and saccharification, VFAs = volatile fatty acids) **(Godliving Y. S. Mtui; 2009).**

6.1. Bioethanol

Production of ethanol from sugars or starch from sugarcane and cereals, respectively, impacts negatively on the economics of the process, thus making ethanol more expensive compared with fossil fuels. Hence, the technology development focus for the production of ethanol has shifted towards the utilization of residual lignocellulosic materials to lower production costs **(Howard et al., 2003).** Currently, research and development of saccharification and fermentation technologies that convert LCW to reducing sugars and ethanol, respectively, in eco-friendly and profitable manner have picked tempo with breakthrough results being reported **(Lin and Tanaka, 2006; Prasad et al., 2007; Patel et al, 2007; Pasha et al., 2007; Tahezaden and Karimi, 2007; Sánchez and Cardona, 2008).** Ethanol yield of 6 - 21% has been obtained through fermentation of agricultural and municipal residues **(Akin-osanaiye et al., 2005; Mtui and Nakamura, 2005; Sjöde et al, 2007; Li et al., 2007; Cara et al., 2008; Sørensen et al, 2008).** While microaeration enhances productivity of bioethanol from (LCW) using ethanologenic E.coli **(Okuda et al, 2007).** Simultaneous saccharification and fermentation (SSF) using recombinant Saccharomyces cereviasiae result to as high as 62% of the theoretical value **(Itoha et al, 2003).** The principal benefits of performing the enzymatic hydrolysis together with the fermentation, instead of in a separate step after the hydrolysis, are the cofermentation of both hexoses and pentoses during SSF, reduced end-product inhibition of the enzymatic hydrolysis and the reduced investment costs **(Kádár et al, 2004; Olofsson et al, 2008).** (**Figure7**).

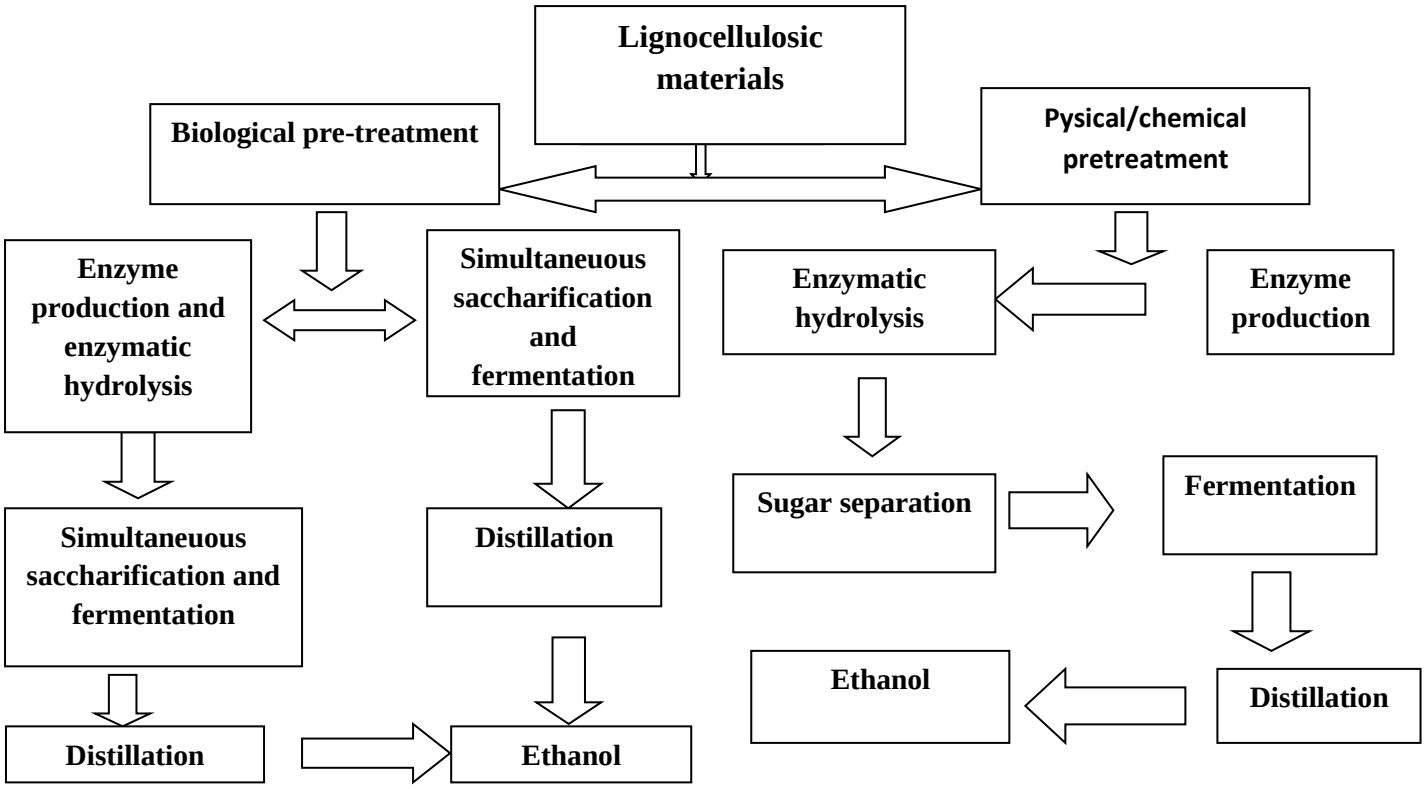

Figure 7: Generalized schematic representation of lignocellulosic materials bio-conversion into ethanol **(Iqbal et al; 2013)**

Life cycle assessment (LCA) shows that bio-ethanol from LCW results to reductions in resource use and global warming **(Blottnitz et al, 2007).** The long-term benefits of using waste residues as lignocellulosic feed stocks will be to introduce a sustainable solid waste management strategy for a number of lignocellulosic waste materials; contribute to the mitigation in greenhouse gases through sustained carbon and nutrient recycling; reduce the potential for water, air, and soil contamination associated with the land application of organic waste materials; and to broaden the feedstock source of raw materials for the bio-ethanol production industry **(Champagne, 2007).**

II

Generalities of actinomycetes

Full name: Harzallah
Title: Isolation and screening of actinobacteria from different regions in the wilaya of Laghouat for valorization of lignocellulosic agri-food wastes

Abstract
Isolation of actinobacteria that produce cellulolytic enzymes is extremely important, given the increased demand for these enzymes in many industrial applications, particularly, in the valorization of lignocellulosic wastes for the production of biofule. 34 actinomycetes were isolated from four different region of Laghouat, using cellulose agar medium. Screening for the capacity of strains to degrade cellulose was undertaken on CMC agar medium with Congo red as a dye. The results indicate that 25 isolates showed positive activity, while 9 isolates were inactive. The actives actinomycetes were grouped according to the intensity of hydrolytic zones around the colonies on CMC agar, as: weak activity (5 isolates), moderate activity (9 isolates) and strong activity (11 isolates). The results of this study show that the soil of Laghouat region presents a very good reservoir of actinobacteria which is able to produce cellulolytic enzymes that is useful in many industries, especially bioethanol industry.

Key words: Isolation; Actinomycetes, Laghouat, valorization, Lignocellulosic wastes, Bioethanol.

Nom et prénom: Harzallah Halima.

Titre : Isolement des actinobactéries à partir des diffèrent régions de la Wilaya de Laghouat en vue de les utilisez dans la valorisation des déchets agroalimentaire lignocellulosique.

Résumé:

L'isolement des actinobacteria qui produisent des enzymes cellulolytiques est extrêmement important, surtout avec la demande croissante de ces enzymes dans de nombreuses industries, particulièrement dans la valorisation des déchets lignocellulosique pour la production des biocarburants. 34 actinomycetes ont été isolées à partir de quatre différentes régions de la wilaya de Laghouat, en utilisant le milieu cellulose agar. Le dépistage de la capacité des souches à dégrader la cellulose a été détecté sur le milieu carboxymethylcellulose agar avec l'utilisation de rouge Congo comme colorant. Les résultats indiquent que 25 isolats sont actifs, alors que 9 isolats sont inactifs. Les actinomycètes actifs ont été regroupés en fonction de l'intensité des zones hydrolytiques autour des colonies sur la gélose carboxymethylcellulose: faible activité (5 isolats), activité modérée (9 isolats) et forte activité (11 isolats).Les résultats de cette étude montrent que le sol de la région de Laghouat présente un très bon réservoir d'actinobactéries qui sont capables de produire des enzymes cellulolytiques utiles dans de nombreux secteurs, notamment celui du bioéthanol.

Mots clé : Isolement, Actinomycetes, Laghouat, Valorisation, Déchets lignocellulosique, Bioethanol

الاسم واللقب: حليمة حرزالله

العنوان: عزل و فحص الأكتينوباكتريا من مناطق مختلفة في ولاية الاغواط بغرض إستخدامها في تثمين نفايات الأغذية الزراعية

الملخص:

تعتبر عملية عزل الأكتينوبكتيريا التي تنتج إنزيمات تحلل السليلوز أمرا في غاية الأهمية؛ نظرا للطلب المتزايد على هذه الإنزيمات في العديد من المجالات الصناعية، و خاصة في تثمين النفايات اللينوسليلوزية بغرض إنتاج الوقود البيولوجي. تم عزل 34 عزلة أكتينوميسيت من أربعة مناطق مختلفة في ولاية الأغواط بإستخدام وسط زرع يحتوي على السليلوز أجار. تم نقل العزلات المتحصل عليها من وسط الزرع إلى وسط يحتوي على الكربوكسيميثيل سليلوز أجار وذلك بغرض الكشف عن قدرتها على تحليل السليلوز بإستعمال صبغة الكونغو ككاشف لوني .كشفت النتائج المتحصل عليها عن وجود 25 عزلة أظهرت نشاط إيجابي؛ بينما أظهرت 9 عزلات نشاطا سلبيا. تم تصنيف الأكتينوميسات النشطة حسب شدة زوال اللون حول المستعمرات في الوسط إلى: نشاط ضعيف (5عزلات)؛ نشاط متوسط (9عزلات) و أخيرا نشاط قوي (11عزلة). أثبتت النتائج المتحصل عليها من هذه الدراسة أن تربة ولاية الأغواط عبارة عن خزان جيد للأكتينوبكتيريا التي لديها القدرة على إفراز إنزيمات لينوسليلوزية ذات قيمة صناعية كبيرة خاصة في مجال صناعة الإيثانول البيولوجي.

الكلمات المفتاحية: عزل , أكتينوميسيت , الأغواط,تثمين , النفايات اللينولسلولوزية , , لإيثانول

البيولوجي.

1. Identification

Actinomycetes, a separate taxonomic group within domain bacteria, are members of the order Actinomycetales **(Chaudhary et al, 2013).** They are Gram positive bacteria, primarily aerobic and spore formers, with high G+C content **(Jeffrey, 2008).** As their name reflects (in Greek, "*atkis*" means ray and "*mykes*" means fungus), they share some morphological features with fungi **(Das et al, 2012).** They show filamentous growth, producing aerial or substratemycelium. Actinomycetes are responsible for earthy smell of the soil **(Chaudhary et al, 2013).** They are ubiquitous in nature, found both in terrestrial and aquatic habitats **(Das et al, 2014).** They belong to both mesophilic and thermophilic groups **(Wilson, 1992),** which broaden the range of habitats inhabited by them. Actinomycetes are known to produce an extensive range of bioactive compounds including various enzymes having multiple biotechnological applications. Lignocellulolytic enzymes (LCE), one of the potent enzymes produced by actinomycetes (**Table 2**), can be exploited widely in various lignocelluloses based industries **(Prakash et al, 2013).** Lignocellulases are hydrolytic enzymes capable of degrading tough lignocellulosein the plant biomass and include cellulases, hemicellulases, and lignolytic enzymes **(Mtui, 2012).**

Lignocellulolytic Actinomycetes (LCA) have been discussed along with description of their lignocellulases enzyme systems involved in biomass degradation.

Table 2: Summarized classification of genera to which lignocellulose-degrading actinomycetes have been assigned.

Genus [a]	Circonscriptions [b]
Streptomvces (T)	Chains of arthrospores borne on aerial hyphae; wall chemotype 1.
Micrornonospora	Aerial mycelium absent: single spores on substrate hyphae; wall chemotype II.
Microbispora (T)	Longitudinal pairs of spores on aerial hyphae; wall chemotype Ill (madurose present).
Thermornonospora (T)	Single heat-sensitive aleuriospores on aerial hyphae; wall chemotype III (madurose absent).
A ctinornadura	Aerial hyphae bear chains of arthrospores; wall chemotype III (madurose usually present).
Pseudonocardia (T)	Aerial hyphae bear chains of arthrospores; spores also produced on substrate hyphae; wall chemotype IV; mycolic acids absent.
Saccharomonospora (T)	Single spores on aerial hyphae; wall chemotype IV; mycolic acids absent.
Nocardia	Substrate hyphae fragment into bacillary and coccoid elements; aerial hyphae may bear chains of spores; wall chemotype IV; mycolic acids present.
Rhodococcus	Cocci or short rods which may develop into filaments or branched hyphae; aerial hyphae absent or rudimentary; wall chemotype IV; mycolic acids present. (T) denotes genera which contain thermophilic strains with

a (T) denotes genera which contain thermophilic strains with activity against lignocellulose.

b Wall chemotype as defined by Lechevalier and Lechevalier.

2. Ecology

Soil is the primary reservoir of degradative actinomycetes and lignocellulose one of the most abundant carbon sources available to support growth in this environment. Nutrients are not uniformly distributed in soil and so it is presumed that routine recovery of actinomycetes from soil samples in numbers exceeding 10 s per gram is attributed to the survival of spores and possibly mycelial fragments. These will have resulted from previous colonisation of organic material and dissemination by air, water and arthropods **(Williams et al, 1982).**

Actinomycetes are also commonly encountered in aquatic environments but here their contribution to nutrient recycling, even in sediments, is debatable. In any case, their capacity for sporulation and dispersal is such that there are few environmental niches which do not contain a diverse population of actinomycetes in active or dormant form. Aerobic conditions and neutral to alkaline pH are general prerequisites for the growth of saprophytic actinomycetes. The former is related to water content in soil and while acidophilic streptomycetes can be recovered from acid soils **(Khan and Williams, 1975).** There is no evidence that they are particularly active against lignocellulose. One important ecosystem where anaerobic degradation of lignocellulose by prokaryotes predominates is the rumen. Although there have been reports of anaerobic cellulolytic micromonosporas **(Maluszyhska and Janota-Bassalik, 1974); (Hungate, 1946),** their identity as actinomycetes requires confirmation.

3. Isolation

Adaptation of standard procedures for the recovery of actinomycetes by incorporation of lignocellulose or related substrates in enrichment and isolation media is the usual approach. Suppression of bacterial and fungal growth is desirable and in the case of the latter, is routinely achieved by including cycloheximide in the medium. Bacterial growth can rarely be excluded but its interference with actinomycete recovery can be minimised in a number of ways. These include heat treatment of samples at temperatures which actinomycete spores can survive and the use of an Andersen sampler and sedimentation chamber in which suspension of particles in air favours recovery of actinomycete spores **(Lacey and Dutkiewicz, 1976).** The latter approach is particularly useful for isolating species with relatively complex nutritional requirements from substrates where heat-resistant Bacillus spp. predominate. Dilution plating on standard actinomycete isolation media with or without lignocellulose tends to yield mainly streptomycetes. This does not necessarily indicate that they are predominant but could be a reflection of the emphasis

placed on this group when isolation media are formulated. In some cases, highly selective isolation media have been developed for specific taxa and later used to isolate Micromonospora, Thermomonospora and Actinomadura strains with activity against grass lignocellulose **(Carthy and Broda, 1984).** A number of strategies have been used to obtain isolates active against lignocelluloses. In most cases, the primary objective is to select strains for further study but some studies have been directed towards enumeration of cellulolytic and xylanolytic actinomycetes in natural substrates **(Godden and Penninckx, 1984) ;(Hankin et al, 1976).** The simplest approach is to directly observe clear zones around colonies on isolation media containing insoluble xylan or cellulose preparations. In this way, xylanolytic strains of Strept tomyces **(lizuka, and Kawaminami, 1969)** and cellulolytic strains of Streptomyces **(Crawford, 1978),** Thermomonospora **(Stutzenberge et al, 1970),** and Micrornonospora **(Sandrak, 1977)** have been selected. Other workers have preferred to screen pools of purified isolates without any prior indication of activity on isolation plates **(Godden and Penninckx ,1984) (Sreenath et al, 1978).**

4. Enzyme degrading cellulose:

Cellulose is the most abundant biological compound on terrestrial and aquatic ecosystem and is the main component of plant biomass **(Shankar *et al*., 2011).** It is the dominant waste material from agricultural industry in the form of stalks, stems and husk, there has been great interest in utilizing cellulose as an energy resource and feed **(Balachandrababu *et al*, 2012).**

Cellulose is commonly degraded by cellulase. Cellulolytic enzyme system consists of three major components such as endoglucanases, exoglucanases and ß-glucosidases.

Cellulases have a potential to use in biotechnology and industry such as, starch processing, alcoholic beverage, malting and brewing, clarify of juice, pulp bleaching, and animal feed **(Sreeja *et al*, 2013).** (**Table3**).

Table 3: Temperature and pH relationships of actinomycete cellulases.

Species	Enzyme activity ~	Optima for Production	Optima for Activity	Thermostability (half-life)	Reference
Stm. Lividans	Endoglucanase βglucosidases	34 37 °C 31°C	55°C 40 °C pH 6.5-7	30 min at 60 °C < 10 min at 40 °C	(Kluepfel et al, 1986) (Moldoveanu, Kluepfel, 1983).
Stm. flavogriseus	Endoglucanase Cellulase βglucosidases	30°C, pH 7	50 ° C, pH 6.0-7.5 40 45°C pH 5.3-6.0 40°C,pH 6.5-7.5	2 h at 40 ° C 2 h at 40 °C 2 h at 40 °C < 10 rain at 40 ° C	(Ishaque and Kluepfel, 1980);(MacKenzie et al, 1984).
Stm.albogriseus Strn.nitrosporeus Mim.rnelanospora	Endoglucanase Cellulase	25-35 °C, pH 6.7-7.2	45-55 ° C, pH 5.0 6.0	> 24 h at 50 ° C t < 24 h at 50 ° C	(VanZyl, 1985).
Thm. Curvata	Endoglucanase Cellulase	45 55°C, pH 8.0	65°C, pH 6.0-6.5	> 60 min at 70 ° C	(Stutzenberger, 1972) ;(Stutzenberge andLupo, 1986);(Stutzenberger,1972)
Thermomonospora sp.	Endoglucanase Cellulase βglucosidases	55°C, pH 7.4	70°C,pH 6.0 65°C,pH 7.0 55°C,pH 6.5	24 h at. 65°C 4 h at 65°C < 1 h at 55°C	HS_gerdal et al,1980

4.1. Cellulases

Cellulolytic enzymes (CE) are a group of glycosyl hydrolases classified into different families depending on their sequence homologies. The mechanisms of action and substrate specificities vary among different cellulases, but they are generally divided into exoglucanases (EC 3.2.1.74), endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21) **(del-Pulgar, Saadeddin, 2014); (Sadhu.S, Maiti.T.K, 2013).** Exoglucanases act on reducing or nonreducing ends of cellulose chains releasing glucose units, whereas endoglucanases hydrolyse β-1,4-glycosidic bonds randomly inside the cellulose chains releasing dextrans of variable lengths **(Kuhad et al, 2011).** Cellobiohydrolases cleave glycosidic bonds at nonreducing ends and release cellobiose units **(Lynd et al, 2002).** These enzymes are particularly important in hydrolysing crystalline cellulose because of their processivity **(del-Pulgar, Saadeddin, 2014).** β-glucosidases enzymes take part in hydrolysis of cellobiose units to monomeric glucose **(Kuhad et al, 2011).** Complete hydrolysis of cellulose involves synergistic effect of all these enzymes, showing synergy between endoglucanases and exoglucanases (endo-exo synergy), exoglucanases acting on the reducing and nonreducing ends (exo-exo synergy), between cellobiohydrolases and β-glucosidases, and between catalytic and carbohydrate binding domains **(Lynd et al, 2002). (Figure8**) shows schematic presentation of enzymatic hydrolysis of cellulose polymer.

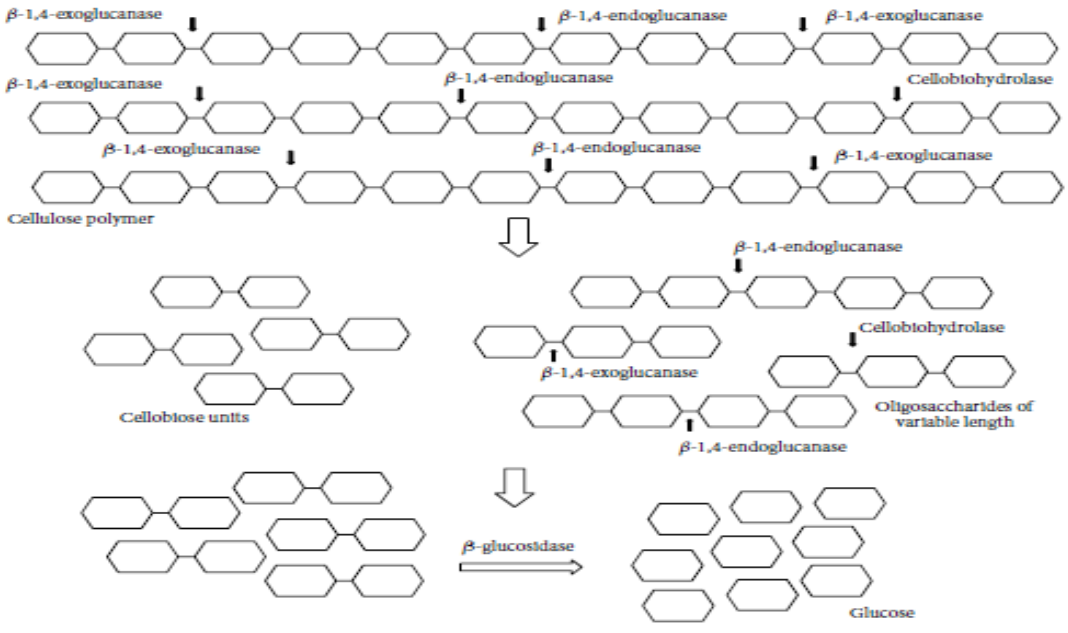

Figure 8: Scheme of cellulose hydrolysis **(Saini et al; 2015)**

Materials and methods

1. Sampling:

In order to isolate the cellulose degrading bacteria, a total of 4 soil samples were collected in January 2019 from different agricultural area located wilaya of Laghouat (south of Algeria): Hamda were the palm agriculture is widely spread (Exploitation of Mr.Ben Brahim; Bayda (Exploitation that interest in vegetables agriculture); El Gaicha (Exploitation that produce cereals) and Tadjruna were the cereals agriculture took place (Figure 9), The study area was sub-divided into four regions; A, B, C and D.; using the Pochon Tardieu technique **(Pochon J et al, 1962)** (Figure 10). the first five centimeters of the surface layer of the soil were removed from each of the subdivisions using large sterile spatula then with a small sterile spatula 100 g of sub-samples were collected from the layer subjacent (between 5 and 15 cm of depth) (Figure 11).

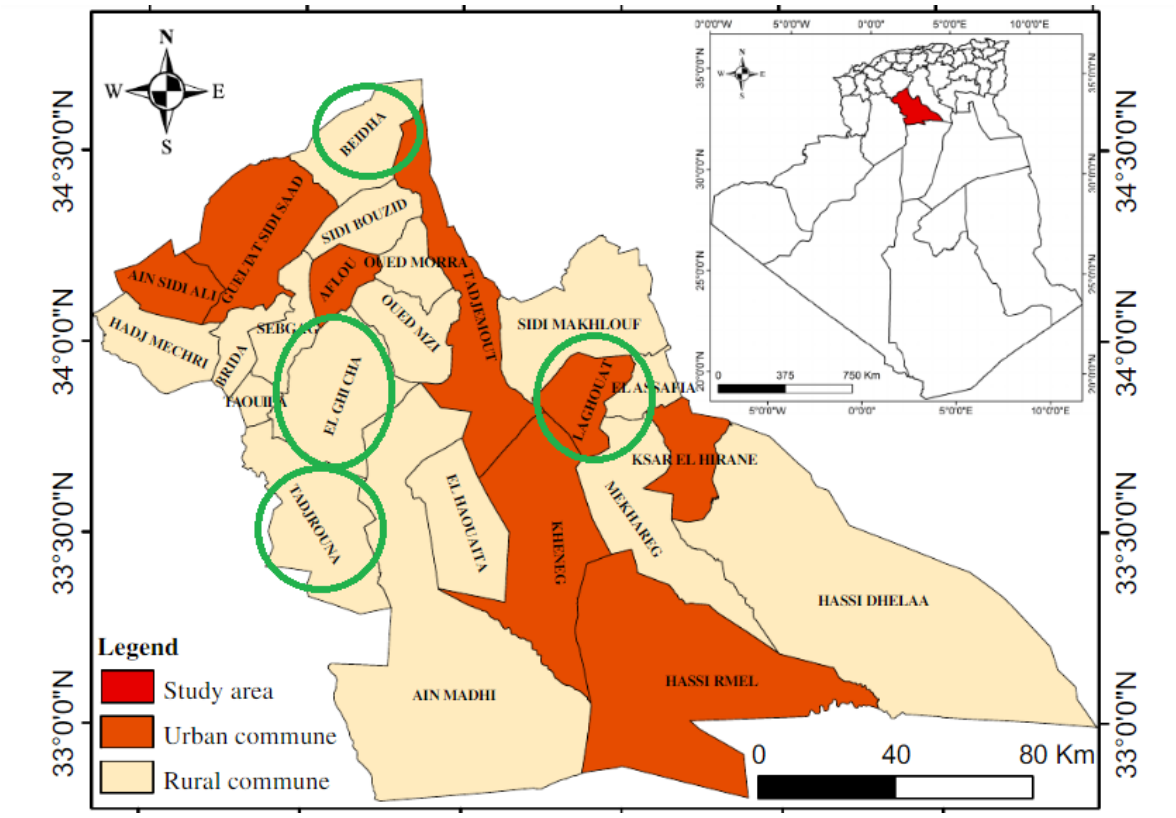

Figure 9: Map of the wilaya of Laghouat shows the sampling region indicated with green circles (Google maps).

Figure 10: Sub-divided region using the Pochon Tardieu technique (Personal picture).

Figure 11: sub-samples collected from the layer subjacent (between 5 and 15 cm of depth) (Personal picture).

In every region, the sub-samples were mixed to make a composite sample. The samples were separately packed in sterile bags and carried under aseptic conditions to the laboratory (Figure 12).

The samples were separately air dried on the benches for 3days at room temperature. This was done to prevent growth of other bacterial flora **(Adamu et al, 2017)** (Figure 13).

Figure 12: Sub-samples mixed to make a composite sample (Personal picture). .

Figure 13: The samples air dried separately For 3days at room temperature (Personal picture). .

2. Isolation of actinomycetes:

From each of the composite samples, 10 g of soil sample was separately added to 90 ml distilled water and shaken vigorously at room temperature, using an orbital shaker at 200 rpm for 10 min. The test flasks were considered as stock solution for the soil samples. Aseptically, 1 ml aliquot from the stock solution was transferred to a test tube containing 9 ml of sterile distilled water and was mixed well. From these test tubes, 1 ml of aliquot was again transferred and mixed with another 9 ml of distilled water to make 10^{-3} dilution factor. Similarly, dilutions up to 10^{-4} were made using serial dilution technique for all soil samples **(Sujatha et al, 2017)** (Figure 14).

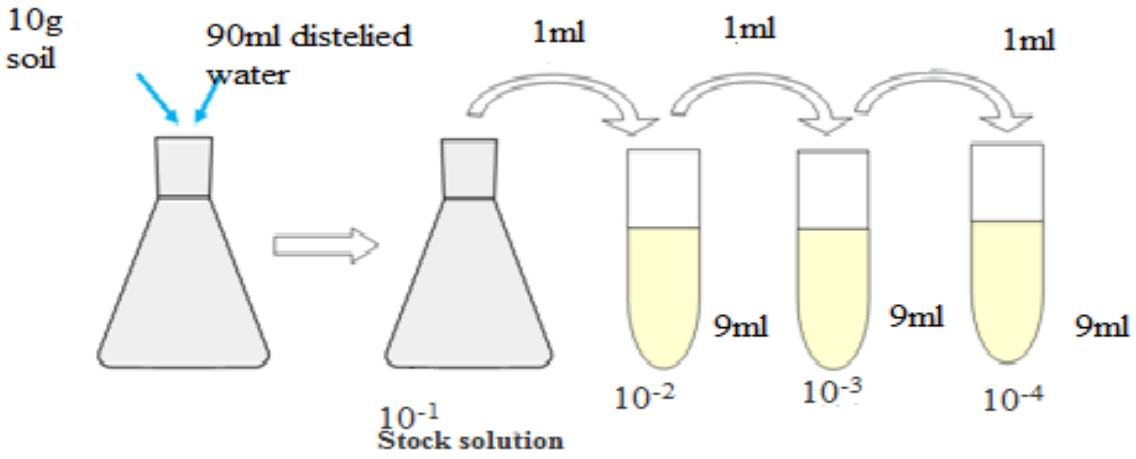

Figure 14: Scheme of the serial dilution technique.

About 0.5ml of each dilution was spread, using spread plate technique by sterile glass spreader on the surface of the cellulose agar media (CAm): cellulose, 10.0; $NaNO_3$, 1.2; KH_2PO_4, 3.0; K_2HPO_4, 6.0; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.05; $MnSO_4 \cdot 7H_2O$,0.01; $ZnSO_4 \cdot 7H_2O$, 0.001; pH 7.0 **(Houfani et al, 2017)** (Figure 15).

In order to avoid fungi contamination, the media (CA) was supplemented with 50 µg/ml of cycloheximide. The plats were incubated at 30 °C for 2 weeks.

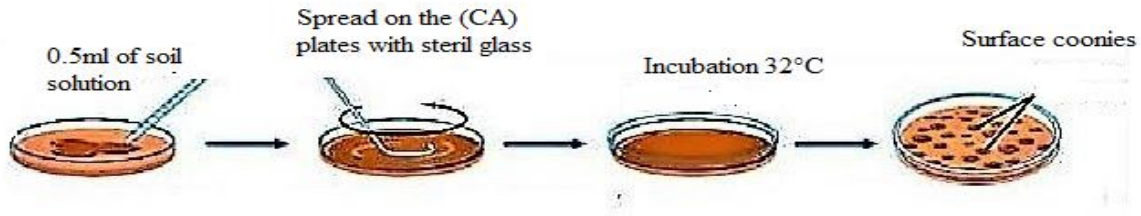

Figure 15: Scheme of the spread plate technique.

After incubation, many actinomycete isolates were obtained therefore some strains were selected (Purified) for further study and it were distinguished from other microbial colonies by characteristics such as tough, leathery colonies which are partially submerged into the agar **(Yaminisudha et al, 2015).**

3. Purification of Actinobacteria:

In order to obtain a pure culture, colonies with suspected actinomycetes morphology were sub-cultured on Casein Starch agar (CSA) medium plates containing in (g/l): Starch: 10,Casein: 0.3, (KNO_3): 2, (NaCl): 2, (K_2HPO_4): 2, ($MgSO_4 \cdot 7H_2O$): 0.05, ($CaCO_3$):0.02, ($FeSO_4$): 0.01.

The mediums were supplemented with (50µg/mL) of cycloheximide to prevent the growth of fungi **(Houfani et al, 2017).** Plates were in incubation under aerobic condition 32°C for 2weeks with daily observation.

4. Screening for cellulolytic activity:

All isolates were screened for the ability to cleave amorphous cellulose on minimal medium agar with 1% of carboxymethylcellulose as the only carbon and energy source (CMC), containing (g/L): CMC, 10.0; $NaNO_3$, 1.2; KH_2PO_4, 3.0; K_2HPO_4, 6.0; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.05; $MnSO_4 \cdot 7H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, 0.001; pH 7.0 **(El-Naggar et al. 2014).**

The pure cultures of actinomycetes (Figure16) were individually spot inoculated from (CSA) medium with almost equal distance and transferred in (CMC) agar plates by sterile

glass (Figure17). The plates were in incubation for 2weeks at 30 ± 2°C until significant growth was recorded **(Gautam *et al*, 2012).**

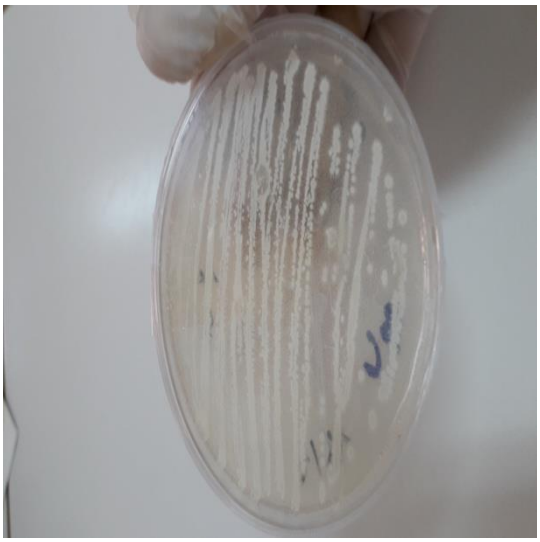

Figure 16: Pure culture of actinomycetes strains (Personal picture).

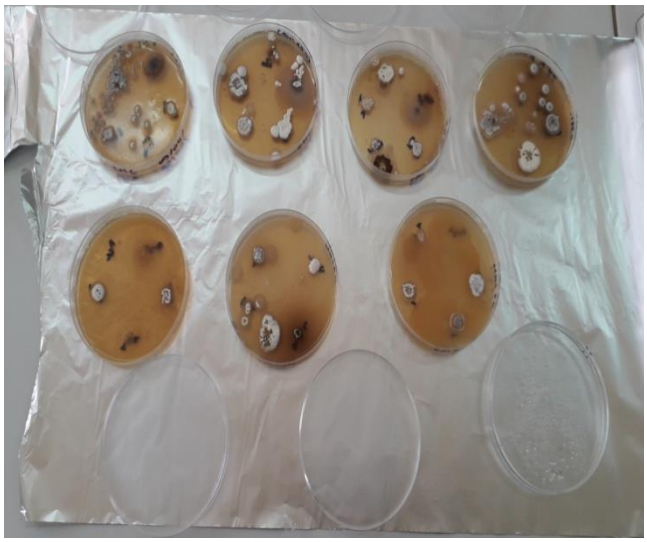

Figure 17: Inoculated Actinomycete strain from (CSA) to (CMC) agar plates after incubation 2w/30 ± 2°C (Personal picture).

Confirmation of cellulose-degrading ability of bacterial isolates was performed by the Congo red essay **(Hendricks et al, 2016).**

To indicate the CMCase activity, the Petri plates were flooded with the Congo red solution (0.1%w/v) for 15minutes (Figure18).

The Congo red solution was discarded, and the plates were washed with 1 M NaCl solution allowed to stand for 15– 20 min.

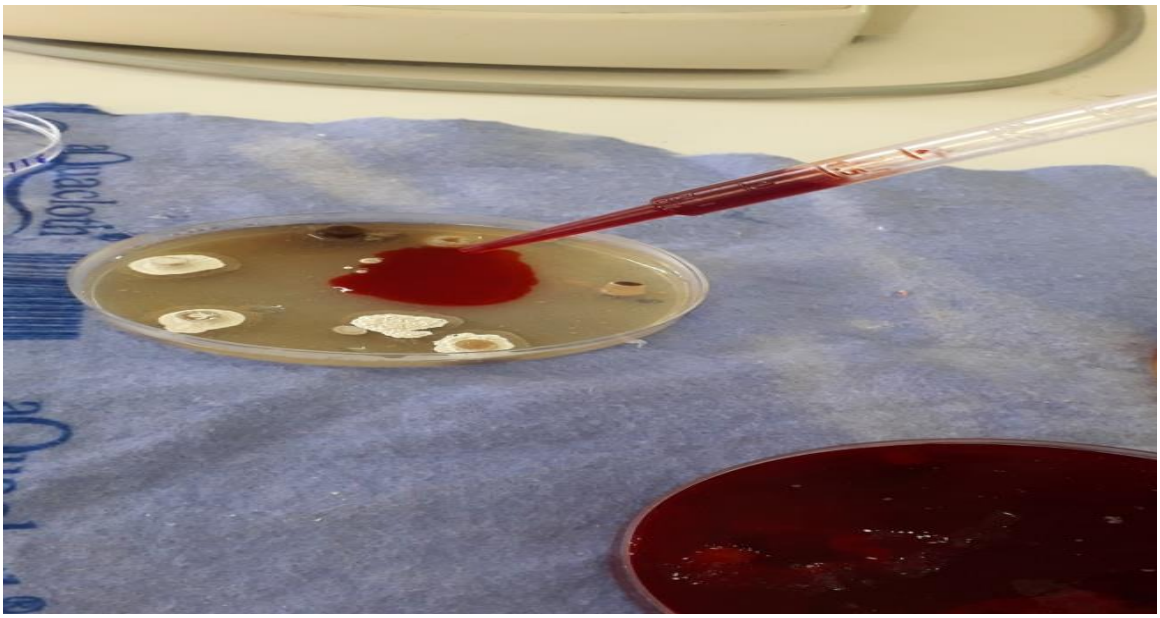

Figure 18: Petri plates flooded with Congo red (0.1%w/v).

Congo red was used as a dye in the colorimetric assay. The detection of the cellulolytic activity is achieved by staining undigested CMC in plate regions which were not exposed to cellulolytic activity, while areas exposed to cellulase give clear halos surrounding the source of the enzyme **(Shaikh et al, 2013)**. The symbols **(-): negative activity; (+) : weak activity; (++) : moderate activity; (+++) : strong activity** were used to indicate the degree of discoloration.

Results and discussion

1. Results of isolation of actinomycetes isolates

The results of isolation of actinomycetes from different region of Laghouat, after 14 days of incubation, were represented in **(Table 4).**

Table 4: Results of isolation of actinobacteria from different agricultural soil samples collected from different region of Laghouat.

	Isolates recover from each dilution			
Regions	10^{-2}	10^{-3}	10^{-4}	isolates number
Hamda	3	3	3	9
Tadjrouna	3	3	3	9
Elgaicha	3	3	3	9
Elbayda	4	2	1	7
Total	13	11	10	34

In this study, 36 strains of actinomycetes were isolated from four agricultural soils located in different regions of the wilaya of Laghouat, in fact, 9 isolates were obtained from three different regions: Hamda; Elgaicha and Tadjrouna, while, 7 isolates were obtained from Elbayda region (Figure 19) and (Figure 20).

Figure 19: Colonies obtained from CA medium.

Figure 20: Purified strains in CSA medium.

The exploitation of new habitat, such as the soil of Laghouat region, is important for the discovery of new taxa of actinomycete that can produce new useful bioactive substances and enzymes as reported by **Houfani et al, (2017),**

The diversity of actinomycetes varies from one soil to another, and it's influenced by several physicochemical parameters such as temperature, pH, salinity, organic matter, cultivation, aeration and moisture content **Zanane et al, (2018),** the geographic location would surly influenced the number and types of actinomycetes that particularly presents in each of the sampling regions and that explains the difference in the number of actinomycetes recovered from each region of Laghouat in (Table 4).

Screening for cellulase activity:

Screenings of actinomycetes strains for their cellulase activity were carried out on carboxyl methyl cellulase agar (CMC agar), using the Colorimetric assay with the Congo red as dye (Figure 21). The obtained results are presented in (**Table 5**).

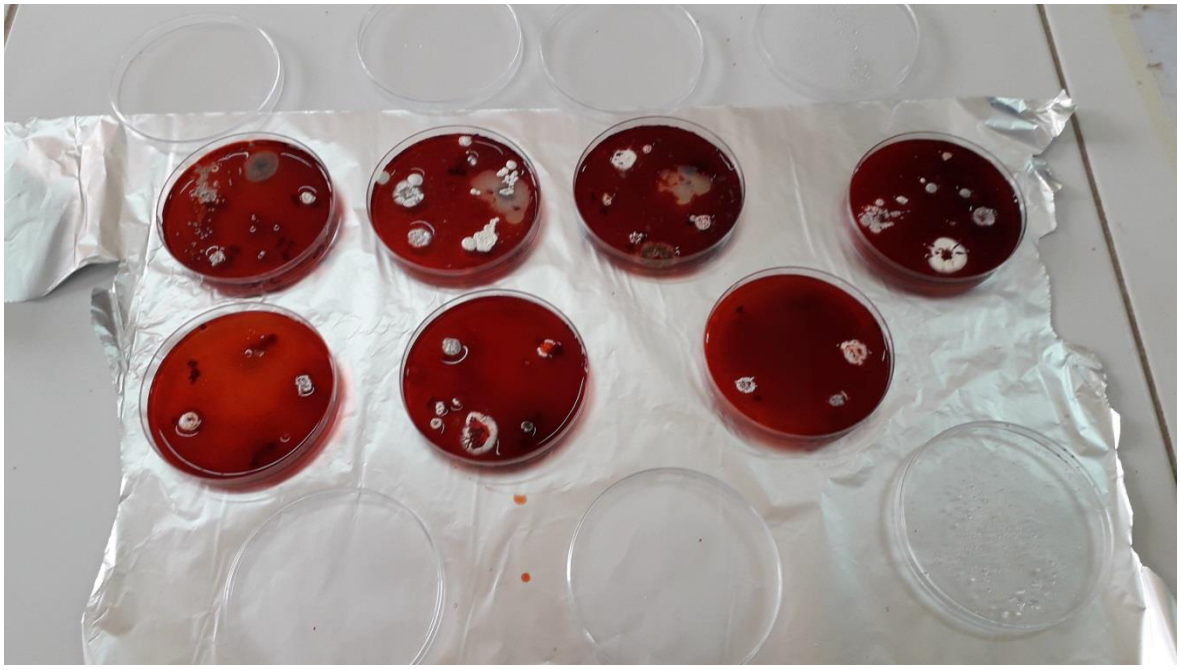

Figure 21: Results of colorimetric essay by Congo red.

Table 5: Results of colorimetric essay of actinomycetes strain.

Region	Cod of the colonies	Degree of discoloration zone
Hamda	A1	++
	A2	+++
	A3	+
	A4	++
	A5	+++
	A6	++
	A7	_
	A8	++
	A9	_
Elbayda	B1	_
	B2	_
	B3	+++
	B4	_
	B5	_
	B6	++
	B7	_
Elgaicha	C1	++
	C2	+++
	C3	+++
	C4	+++
	C5	+
	C6	_
	C7	_
	C8	+
	C9	++
Tadjrouna	Tc1	+++
	Tc2	+++
	Tc3	++
	Tc4	+++
	Tc5	+++
	Tc6	+++
	Tc7	+
	Tc8	+
	Tc9	++

(-): negative; (+) : weak activity; (++) : moderate activity; (+++) : strong activity

The results of colorimetric essay, by Congo red , indicated that 25 isolates (74%), from 34 isolates, showed positive activity (Figure 22), while 9 isolates (26%) were inactive (negative activity) (**Table 5**).

The actinomycetes bacteria is widespread in nature and they are able to degrade several complex molecules, such as cellulose, chitin, keratin, by the secretion of different kind of enzymes (cellulase, chitinase and keratinase) **(Peristiwati et al, 2018).**

The appearance of the clear zone around the colony when the Congo red solution was added was strong evidence that the actinomycetes produced cellulase in order to degrade cellulose **(Lisdiyanti et al., 2012).** The detection of the cellulolytic activity in these cases is achieved by staining of undigested CMC in the plate regions which were not exposed to cellulolytic activity, while areas exposed to cellulase give clear halos surrounding the source of the enzyme (Figure 22).

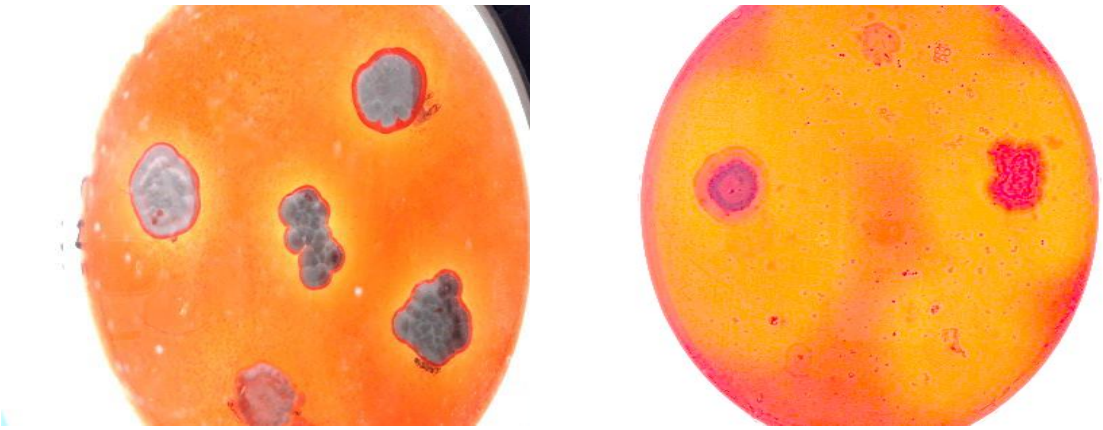

Figure 22: Actinomycetes strain on CMC agar shows clear zone around the colonies when Congo red was added.

According to **Matthews et al, 2006,** negative activity that was detected by some strains of actinomycetes is because of the presence of dense layer of water in the surface of cellulose, which may hinder diffusion of cellulase.

Few studies were undertaken on the purification of the cellulolytic bacteria isolated from Algerian environmental sources, **Houfani et al, 2017,** screened the diversity of bacteria collected from Algerian compost, 115 isolates (68%), from 170 obtained strains, showed endocellulase activity on agar plates.

The actinomycetes strains obtained from Laghouat soil were grouped according to the intensity of hydrolytic zones around the colonies as:

- Weak activity: 5 isolates (15%).
- Moderate activity: 9 isolates (27%).
- Strong activity: 11 isolates (32%).
- Negative: 9 isolates(26%). (**Figure 23**).

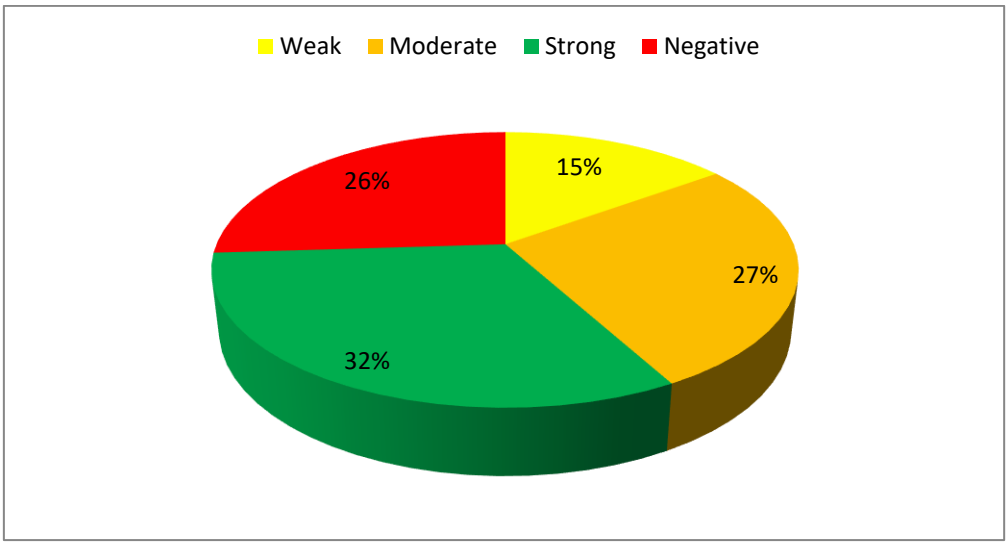

Figure 23: Percentage indicates the intensity of hydrolytic zones around the colonies of actinomycetes isolates.

In this study, the results were based only on the colorimetric assay, because the materials were not available to go farther on the extract, purification and study of the enzyme activity.

Screening for extracellular cellulase production by bacteria and fungi is often done on agar plates containing CMC as substrate, since; the degradation of crystalline cellulose is very slow **(Dashtban et al, 2009)**.

Das et al, (2010), indicated that the biosynthesis of cellulase enzymes, which is an inducible enzyme, can be affected by nature of substrate used in the culture media, in their experiment, they used different carbon sources to studied their affect on the cellulase production, such as: filter paper, CMC, dry leaves, hay, saw dust and coir fiber, they found that carboxymethylcellulose (CMC) give the maximum production of the enzyme.

That proved that the utilization of (CMC) in this study as carbon source is best for cellulase production.

According to the obtained results, we can conclude that the soil of Laghouat region is a high potential source of actinobacteria that can be an excellent source for the cellulase enzymes, the extraction of this enzyme is quite difficult for this stage but the benefits of

extraction of cellulase enzyme open the door for several utilization especially in the valorization of (LCW) produced by the agricultural and food industries, into various production such as bioethanol industries.

Conclusion

Lignocellulose is a generic term used to describe plant biomass. It is the most abundant renewable carbon resource in the world and is mainly composed of lignin, cellulose and hemicelluloses. Most of the food and food processing industry wastes are lignocellulosic in nature with a global estimate of up to 1.3 billion tons/year this huge amount of wastes needs to be valorized in order to reduce the environmental impact. One of the best solutions for these wastes is to be used as biofule by biological treatment; actinomycetes are an important source of lignocelluloses hydrolyzing enzymes in converting lignocellulosic wastes into ethanol.

34 actinomycetes strains were isolated, using the culture media Casein Starch Agar media (CSA), from four different samples, which were collected from four regions of Laghouat (south of Algeria): Hamda, Bayda, Tadjrouna, and Elgaicha.

Screening for the capacity of strains to produce cellulase enzymes was carried out on CMC agar medium, which contained carboxymethylcellulose as the only carbon source. Congo red was used as a dye in the colorimetric assay. The detection of the cellulolytic activity in these cases is achieved by staining undigested CMC in plate regions which were not exposed to cellulolytic activity, while areas exposed to cellulase give clear halos surrounding the source of the enzyme. The results indicated that 25 isolates (74%), from 34 isolates, showed positive activity, while 9 isolates (26%) were inactive.

The actives actinomycetes were grouped according to the intensity of hydrolytic zones around the colonies on CMC agar, as: weak activity (5 isolates), moderate activity (9 isolates) and strong activity (11 isolates).

According to the obtained results, we can consider the soil of Laghouat as a good source for strains that produces cellulose enzymes.

Cellulase enzyme is considered as potent enzymes used in several lignocellulosic based industries such as the valorization of lignocellulosic wastes into biofuel.

The results obtained from this study could be used as point of start for more studies, like extraction of the enzyme and the analyses by more testes.

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