Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-S2-006
S2-006

Isolation Characterization and Identification of a Pectinolytic *Streptomyces* Isolate

Alqahtani FS[1]*, Aly MM[1], Bokhari FM[1] and Alshehri WA[2]

[1]Department of Biology, College of Science, King Abdulaziz University, Saudi Arabia
[2]Department of Biology, College of Science, University of Jeddah, Saudi Arabia

Abstract

Agro-industrial residues are primarily composed of complex polysaccharides that strengthen microbial growth for the production of important industrial enzymes. Actinomycetes are efficient degraders of plant debris as they produce extracellular enzymes like, pectinase, cellulose and xylanase. Pectinases are enzymes that catalyze the hydrolysis of pectin (polygalacturonic acid) to galacturonic acid residues. This study involved the isolation of some bacteria for pectinase hydrolysis. The preliminary screening for pectinase production was done by well plate method. Of the twenty-one strains tested for pectin hydrolysis eighteen were very good producers of pectinase giving zones from 15 to 42 mm. Further screening of selected strains was done quantitatively by 3,5-dinitrosalicylic acid (DNS) method for pectinase production. Selection and identification of the most active isolate FMA18 was carried out. The strain FMA18 were identified based on their morphological, biochemical, and molecular characteristics. The result of 16s rRNA gene sequencing revealed that the isolate FMA18 was identified as *Streptomyces aurantiogriseus* with 99% similarity level.

Keywords: Pectin; Pectinase; *Streptomyces Aurantiogriseus*; Enzyme Activity; 16s rRNA

Correspondence to: Fatimah Sultan Alqahtani, Department of Biology, College of Science, King Abdulaziz University, Saudi Arabia; E-mail: fssmsq3337@hotmail.com

Citation: Alqahtani FS, Aly MM, Bokhari FM, et al. (2020) Isolation Characterization and Identification of a Pectinolytic *Streptomyces* Isolate. Prensa Med Argent, S2:006. DOI: https://doi.org/10.47275/0032-745X-S2-006.

Received: June 20, 2020; **Accepted:** July 30, 2020; **Published:** August 03, 2020

Introduction

Pectinase enzyme is commonly referred to a group of enzymes that play a role in pectin biodegradation [1]. Pectins are high molecular weight polysaccharides composing of α-1→4 linked D galacturonic acid residues with a few rhamnose residues in the main chain and arabinose, galactose and xylose on its side chain [2]. Pectinase enzymes are included three enzymes viz. poly galacturonase, pectine sterase, and pectate lyase based on their role on the pectin degradation [3]. In pickling industry, they are involved in softening process. In agricultural sector, they have widespread applications in purification of plant viruses, oil extraction, retting and degumming process, bio-scouring of cotton fiber. Pectinases are also used for extraction of pure DNA sample from plants, in maceration of plant tissue, isolation of protoplast from plant cells and in liquification and saccharification of biomass [4]. Pectinolytic microorganisms are widely spread in soil. Yeast and filamentous fungi are known to produce pectinases [5,6]. A wide variety of bacteria such as Pseudomonas, Xanthomonas, Erwinia and Bacillus [7,8] are known as pectinase enzyme producers. Actinomycete plays a major role in the plant residue degradation. Actinomycetes produce extracellular enzymes like cellulase, xylanase and pectinase as they are efficient degraders of plant debris. It belongs to a distinct class of gram-positive bacteria (Actinomycetales) of prokaryote [9]. Actinomycetes and particularly streptomycetes are the best-known enzyme producers [10].

Streptomyces sp. is salient one in soil ecology and present worldwide in soil. They are responsible for the characteristic earthy smell of soils [11]. They are considered as one of most significant bacteria, because of their capacity to develop the soil properties as well as producing several extracellular substances (enzymes) as secondary products [12,13]. The objective of the present study was aimed to isolation and screening of potent pectinase producing actinomycete isolates from the agricultural wastes and selection, characterization and identification of the most active pectinolytic bacterium.

Material and Methods

Sample Collection

Samples were collected from several sites e.g., agriculture and vegetable waste dump soil, in Jeddah, Saudi Arabia. Soil samples are taken with the help of sterile spatula, in sterile plastic bags the samples were brought to Microbiology laboratory for further processing.

Isolation of Actinomycetes

One gram of pretreated soil samples was suspended in 100 ml sterile distilled water then incubated in shaker incubator at 28°C with shaking at 120 rpm for 30 min. Mixtures were allowed to settle then serial ten-fold dilutions were prepared. 0.1 ml was taken from each dilution and spread evenly over the surface of starch casein nitrate agar (SCNA) plates (in triplicate) with sterile L-shaped glass rod, and incubated at 28°C for 10 days [14,15]. The selected colonies

were purified by repeated streaking. Streptomyces-like colonies were selected and screened for their ability to produce pectinase enzyme on a specified medium [16].

Screening for Pectinase-Producing Streptomyces

Screening of pectinase producing actinomycetes was carried out in Pectin agar medium was prepared with (in g/L): NaNO3 1.0, KCl 1.0, K2HPO4 1.0, MgSO4 0.5, yeast extract 0.5, pectin 10 and agar 20 with pH adjusted to 7.0 [17]. at 30°C for 5-7 days of incubation. After incubation, the colonies showing clear zones upon flooding with iodine-potassium iodide solution were selected as pectinase producers [18].

Characterization of the Most Active *Streptomyces* Isolates

Streptomyces colonies that showed the largest clear zone (>1cm, the distance from the edge of the colony to the rim of the clear zone) were characterized morphologically and physiologically according to the International Streptomyces Project (ISP) and as described by Saadoun et al. [19,20].

Pectinase Activity Assay

Pectinase activity was assayed by the colori-metric method of Miller [21]. Briefly, 0.5ml of cell free supernatant was incubated with 0.5ml of pectin in 0.1M acetate buffer with pH 6.0 and the reaction mixture was incubated at 40°C for 10 minutes in static condition. After adding 1ml of DNS reagent, the mixture was boiled for 5 min at 90°C. The reaction was stopped by adding 1ml of Rochelle's salt. Then the mixture was diluted by adding 2ml of de-ionized water. The absorbance was measured Spectrophotometrically at 590 nm. A standard graph was generated using standard D- Galacturonic acid solution. One unit of Pectinase activity was defined as the amount of enzyme which liberated 1µm D- Galacturonic acid per min.

Results and Discussion

Sampling and Screening

There are only a few reports about production of pectinases by actinomycetes. In the current study we aimed to find potential pectinolytic actinomycetes. For this purpose, we collected agriculture and vegetable dump waste soil as the source of potential organisms. out of 21 actinomycetes isolated from agricultural wastes and 18 isolates were found to be pectinase positive through appearance of clear zone on the pectin agar medium. Among them, strain FMA18, being the highest pectinase producer (Figure 1).

The isolate FMA18 was chosen as the most active pectin-degrading isolated. It grew well on both starch casein nitrate agar and Pectin agar medium. When grew on the selective medium, it exhibited a 38 mm clear zone diameter on pectin agar medium (Figure 2).

Characterization and Identification of Streptomyces Isolate

Characterization of isolate FMA18 based on the morphological, physiological, biochemical and light and scanning electron microscopic was carried out. Figure 3 showed the selected bacterial isolate under microscope. It was Gram positive with aerial and substrate mycelia. The aerial mycelium mass of isolate FMA18 was peige in color with flexuous spore chains. Spores were oval shaped and had a smooth surface. The selected isolate grows well and hydrolyze different carbon sources like starch, pectin, Protein, gelatin and citrate in addition to lactose. The isolate FMA18 was resistant to different antibiotics as penicillin (Table 1).

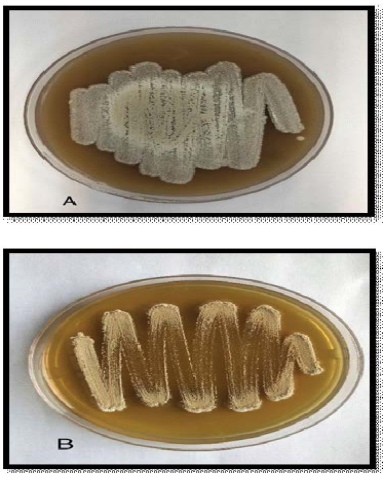

Figure 1: FMB18 isolate on: (A) starch casein nitrate agar, and (B) Pectin agar medium.

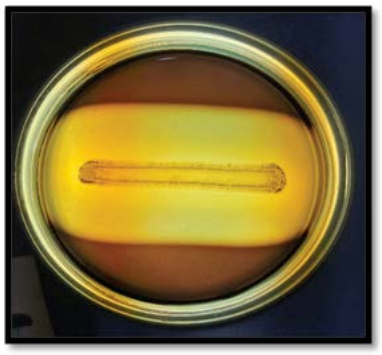

Figure 2: Zone of pectin hydrolysis on pectin agar medium of isolate FMA18 after 5 days' incubation.

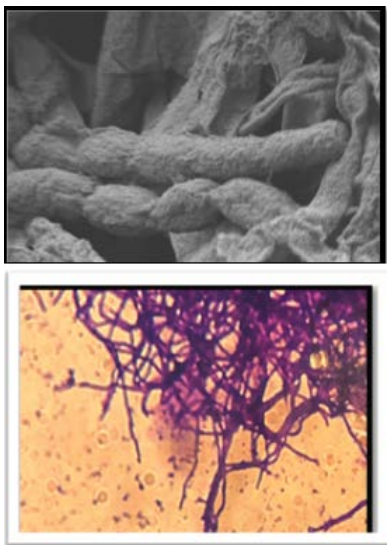

Figure 3: *Streptomyces aurantiogriseus* (A) FMA18 under scaning electron and, (B) light microscope after Gram stain

The selected bacterium grew on different media suggested by international Streptomyces projects (ISP). The growth and color of the aerial and substrate mycelia in addition to the reverse color were determined. The selected bacterium grew well on all tested media. The growth was good or very good. The colonies on all media were white

Table 1: Characteristics of *Streptomyces aurantiogriseus* FMA18.

Results of Isolate FMA18	Test parameter
Gram positive	Gram stain
Rectiflexible	Spore chain
Well developed	Aerial and substrate mycelia
Positive	Resistance to penicillin
Positive	Keratinase production
Positive	Hydrolsis of starch
Positive	Hydrolsis of Protein
Negative	Hydrolsis of Gelatin
Negative	Citrate test
Positive	Fermentation of Lactose

Table 2: Cultural characteristics of the selected isolate FMA18 on different ISP media.

Soluble pigment	Substrate mycelia color	Aerial mycelia color	Colony Properties	Growth	Type of agar media
Negative	Cream	Cream	White, spreading, powdery	Good	Tryptone-Yeast Extract agar (ISP1)
Negative	Yellow	White	Yellow edges spreading	Good	Yeast Extract - Malt Extract (ISP2)
Light Brown	Pale brown	White	White, spreading, powdery	Good	Oatmeal (ISP3)
Negative	Brown	White	Cream, Non-Spreading, Granular	Very good	Inorganic Salt Starch Agar (ISP4)
Negative	Yellow	White	White, spreading, powdery	Very good	Glycerol Asparagines Agar Plate (ISP5)
Pale yellow	Yellow	White	White, spreading, powdery	Very good	Tyrosin Agar (ISP7)

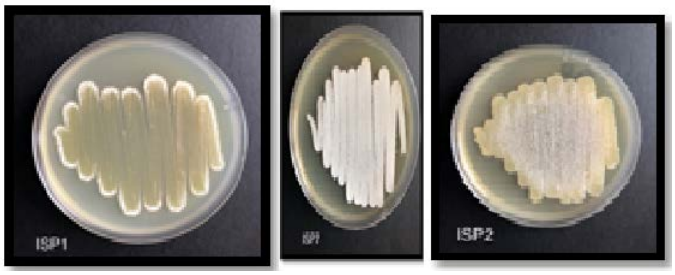

Figure 4: Growth of *Streptomyces aurantiogriseus* FMA18 in different media.

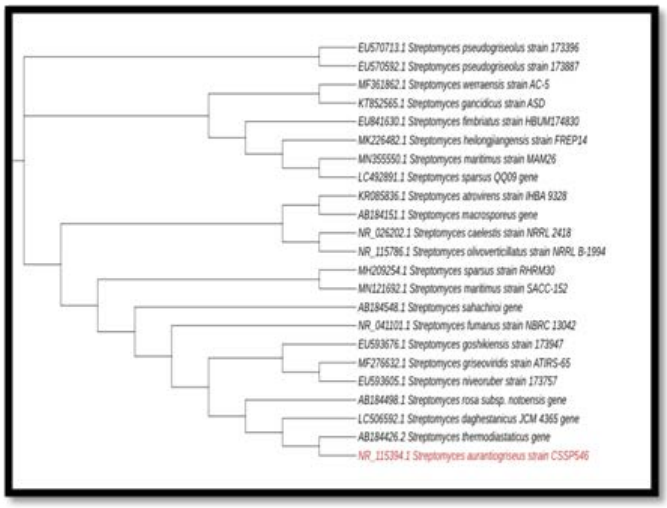

Figure 5: Phylogenetic tree constructed on the basis of 16S rRNA gene sequences of Streptomyces aurantiogriseus strain SD with other *Streptomyces* sp. obtained from GenBank database. Their names and respective accession numbers are shown on the tree.

with powdery appearance. The aerial mycelia on all tested media was white while the color of the substrate mycelia was ranged from yellow, pale brown to brown. The color or the produced pigment was pale yellow or pale brown and in some cases absent (Table 2 and Figure 4).

DNA was extracted from the selected bacterium and molecular identification was carried out using 16 sr RNA. It was belonging to genus Streptomyces and identified as *Streptomyces aurantiogriseus*. Phylogenetic tree constructed on the basis of 16S rRNA gene sequences of Streptomyces species obtained from GenBank database are shown in Figure 5.

Conclusion

The Actinomycetes are active degraders of pectic substance present in nature. The Actinomycetes utilize agricultural wastes for the production of pectinase enzyme therefore, it is potential to make enzyme in cost-effective and eco-friendly manner. The enzyme production and application in industries using Streptomyces sp. are very scanty. Henceforth, Actinomycetes can be employed to produce pectinase as it has numerous benefits and optimistic alternative than other species in future.

Acknowledgment

The authors are grateful to King Abdulaziz University, Department of Biology, College of Science for providing laboratory facilities.

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