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Isolation and Screening of Pectinolytic Streptomyces Sp. From Soil Samples of Egypt.

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ABSTRACT

Pectin is a major part of all plants. Peptidases hydrolyze peptic substances. The purpose of the present study was to isolate and screen pectinolytic Streptomyces sp. from different soil samples of Egypt. Total 526 Streptomyces sp. were isolated. Primary screening of pectinolytic Streptomyces sp. was made by pectin agar plates. Two hundred thirty three isolates were had pectinolytic activity, providing clear zones, represented (44%). These positive isolates divided into two groups fair and good one. Fair group that contain clear zone diameter < 10mm, represented 42.9% and good one that contain clear zone diameter ≥10 mm represented 57.1%. By secondary screening of the good isolates using pectin broth medium, five isolates (2, 5, 8, 72 and 103) give the best results (≥15 U/mg). By screening the five isolates using different media (M1, M2, M3 and M4), isolates (2, 5 and 103) provide the best results on M2 pectin nitrate broth medium, while 8 and 72 give the best results with M1 pectin broth medium. By screening using different agro-industrial waste products as lemon peel, orange peel, rice husk and wheat bran, isolate 5 give maximum activity (21.5 U/mg) with lemon peel nitrate medium.

Keywords: Peptidases, Pectinolytic Streptomyces sp., Different media, Different agro-industrial wastes.

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INTRODUCTION

Biotechnological products like enzymes are most significant products for human needs in large scale part of environmental, industrial and food technology especially the one that obtained from microbial origin. Peptidases are cluster of enzymes that help in breakdown of peptic parts which are mostly within plant tissues. The pectin, an indispensable component present in between middle lamella and cell wall of plant cells composed of as many as seventeen various monosaccharides and at least seven various polysaccharides forming a most complicated bio-macromolecule in nature [1]. Peptidases are naturally produced by different organisms such as fungi, bacteria, yeasts, nematodes, other harmful microorganisms, plants and insects. A quarter sales of global food enzymes rely upon microbial peptidases. Even though fungal peptidases are being industrially exploited, peptidase creation from actinomycetes has also been reported earlier [1, 2, 3, 4,5,6,7 and8].

Actinomycetes play a major role in the vegetable residue degradation. Actinomycetes produce extracellular enzymes like cellulose, xylonite and peptidase as they are effective degraders of plant debris. It is a specific class of gram-positive bacteria (Actinomycetales) of prokaryote. In 16SrDNA tree, actinomycetes form a definite phylogenetic series and have been a major technological importance in the past years, with the discovery of numerous range of metabolites produced by its various genera [9].The diversity in actinobacterial populace in sediment and samples of water from the submarine environment of Tamil Nadu and diverse genera viz. Streptomycin, Actinopolyspora, Actinomadura, Nocardiosis, Micromonospora and Actinomycete have been observed in earlier studies [10]. They have ability to synthesize chemically diverse and commercially important bioactive substances like pigments, antibiotics and enzymes, etc [11].

Streptomycin sp. is significant one in soil ecology and present worldwide in soil. They provide the characteristic earthy smell to soils [12]. They are considered as one of all significant bacteria, due to their capacity to develop the soil properties as well as producing several extracellular enzymes as secondary products [13, 14]. Pectin degrading enzymes reported from actinomycetes are mainly pectate lyases [15].

Pectate lyase from the actinomycete Thermomonospora fiscal was purified and characterized [16]. Pectate lyase has lately been detected from Streptomycin viridochromogenes [17]. Pectinolytic enzymes from actinomycetes have been used for the degumming of ramie pan fibers [18]. Peptidase was also produced and purified from Streptomycin sp. QG-11-3[19]. Also peptidase was produced and purified from Streptomycin lyrics by [1].

The use of wastes as low cost residues, inexpensive, available and safe substrates in enzyme production is especially interesting for countries where agro-industrial residues are abundant. The Disposal of fruit peels is becoming the threatened problem over fruit industries. So, fruit peels will be used as a potential substrate for the production of peptidase. Among them, citrus, apple and orange are most widely used because it contains large amount of pectin. For using in industry, peptidases can be made out of pectin-containing wastes [20], such as apple pomade and grape skin area [21, 22], tamarind kernel [23] and citrus peel [24]. All of these materials contain variable levels of pectin: lemon peel (around 35%, [25] and apple pomade (up to15%; [22], whereas grape skin and tamarind kernel have minimum pectin content 4%; [21] and <2%; [26], respectively.

MATERIALS AND METHODS

Isolation of Streptomycin sp. from soil samples

Twenty five cultivated agriculture soil samples were collected from different areas of Egypt. Pectinolytic Streptomycin sp. were isolated from collected soil samples by serial dilution method and spread on starch nitrate agar plates. Serial dilution was done by taking one gram of soils were transferred into 100 ml of sterilized distilled water, shaking for two hours. The resulting soil suspensions were serially diluted and plated onto starch nitrate agar [27]. Following pouring plate technique, the inoculated plates were incubated at 28 °C for 7-14 days. Firm cartilaginous rough chalky colonies of Streptomycin sp. were selected and purified. Starch nitrate agar medium contains Starch, 20g; KNO₃ 2.0g; K₂HPO₄ (anhydrous), 1.0g; MgSO₄.7H₂O, 0.5g; NaCl, 0.5g; CaCO₃3.0g; FeSO₄.7H₂O, 0.01g; Distilled water 1000ml.

Preliminary screening of peptidase activity on solid plates

The cultures were screened for their ability to grow and utilize polygalacturonase using pectin as a sole carbon source in a defined synthetic basal medium. Fresh cultures of the Streptomyces isolates were screened for utilization of pectin using a solid medium containing: KH₂PO₄ (anhydrous), 4.0g, Na₂HPO₄ (anhydrous), 6.0g, Pectin (from apples), 5.0g, (NH₄)₂SO₄, 2.0g, Yeast extract, 1.0 g, MgSO₄ · 7H₂O, 0.0001g, CaCl₂ 0.001g, agar, 10.0g, distilled water, 1000ml. Hydrolysis zones were detected after 4 and 6 days by flooding plates with an aqueous solution of certified solution (hexadecyl-trimethyl-ammonium-bromide) (1% w/v) [28].

Secondary screening of Polygalactouranase by active isolates by submerged fermentation medium

Heavy spore suspensions (1ml) of the active Streptomycin strains used as inoculums for 50ml broth medium in 250 ml conical flasks, which were incubated on a rotary shaker (NEW BRUNSWICK SCIENTIFIC, EDISON, N. J., USA) (180 r.p.m) at 28 °C for five days. After incubation the supernatants were separated by filtration, the filtrates were used as crude enzymes sources. 250 ml conical flasks contains 50 ml of the following medium: KH₂PO₄ (anhydrous), 4.0 g, Na₂ HPO₄ (anhydrous), 6.0g, Pectin (from apples), 5.0 g, (NH₄)₂SO₄, 2.0 g, Yeast extract, 1.0g, MgSO₄ · 7H₂O, 0.0001g, CaCl₂ 0.001g, distilled water, 1000ml using pectin as a sole carbon source [28].

Polygalacturonase assay:

polygalacturonase was determined according to [29] using 1.0 % (w/v) polygalactourinic acid (Sigma) in 0.2M phosphate buffer pH 7.0 as substrate for enzyme reaction. The assay reaction mixture contain 0.5 ml substrate + 0.5 ml diluted enzyme solution in the buffer was incubated at 40 °C for 10 min, the reaction was stopped by addition of 1ml of dinitrosalicylic acid (DNS) reagent solution followed by keeping in boiling water for 3 mins, then put in cold ice. The amount of reducing sugars liberated was determined by the DNS method [30] using D-galactourinic acid (Sigma) as a standard.

One unit (1U) of enzyme activity is equal to the 1 μ Mol of reducing sugars released per ml per min, measured in terms of D-galacturonic acid, produced as a result of enzyme substrate reaction.

Protein determination

The protein concentration was determined in the enzyme solution according to [31]. Bovine serum albumin was used as a standard.

Screening of the most active Streptomycin isolates for polygalacturonase using different media

The most active isolates that showed clear zone (equal or more than ten mm) were screened for polygalacturonase production in submerged medium using the following different media.

- 1- Hankin medium, represented M1, [28] containing (g/l): KH₂PO₄ (anhydrous), 4.0 g, Na₂HPO₄ (anhydrous), 6.0g, Pectin (from apples), 5g, (NH₄)₂SO₄, 2.0g, Yeast extract, 1.0g, MgSO₄ · 7H₂O, 0.0001g, CaCl₂ 0.001g, distilled water, 1000 ml. using pectin as a sole carbon source.
- 2- Pectin nitrate medium, represented M2, [27], containing (g/l): Pectin, 20g; KNO₃ 2.0g; K₂HPO₄ (anhydrous), 1.0g; MgSO₄ · 7H₂O, 0.5g; NaCl, 0.5g; CaCO₃ 3.0g; FeSO₄ · 7H₂O, 0.01g; Distilled water 1000 ml.
- 3- Horikoshi medium, represented M3, [32], containing (g /l): glucose 5.0, peptone 5.0, yeast extract 5.0, KH₂PO₄ 1.0, MgSO₄ · 7H₂O 0.1, pH 8.0 was used as a basal medium.
- 4- Modified Horikoshi medium, represented M4, containing (g /l): pectin 5.0, glucose 5.0, peptone 5.0, yeast extract 5.0, KH₂PO₄ 1.0, MgSO₄ · 7H₂O 0.1, pH 8.0 was used as a basal medium.

Screening by using different industrial and agricultural wastes

Screening the most active Streptomycin isolates by using the best medium for polygalacturonase production for each isolate by using different industrial and agriculture wastes like lemon peel, orange peel,

rice husk and wheat bran. Orange bagasse (Ob), lemon peels (Lp), Wheat bran (Wb) and rice husk (Rs) were obtained from local market in Egypt. Orange bagasse (Ob) and lemon peels (Lp) cut into small pieces (~2 mm), washed with tap water several times in order to remove all water soluble compound then dried and grinded.

RESULTS AND DISCUSSION

Isolation of Streptomyces

The current study, isolation and screening process of peptidase producing Streptomycin was carried out from the agricultural soil gathered from different places of Egypt. Streptomyces are usually round, convex shaped colonies, moreover their rooting growth into the medium. Based on these characteristics, 526 streptomyces colonies were randomly chosen and isolated from different soil sources of Egypt on the basal starch nitrate medium. Starch nitrate agar medium was found to be very effective for the isolation of the actinomycetes in confirmation with the reports [1, 3, 4, 5, 33 and 34]. Results showed that El Sharkia showed the largest number of the isolates, (200 isolates) which represented 38%, followed by El Qalioubia, (120 isolates), which represented 23%, El Esmailia, (41 isolates), which represented 8%, Aswan, (50 isolates), which represented 9%, Luxor, 45 isolates, represented 9% and As suit, (70 isolates), which represented 13% respectively, as shown in figure 1.

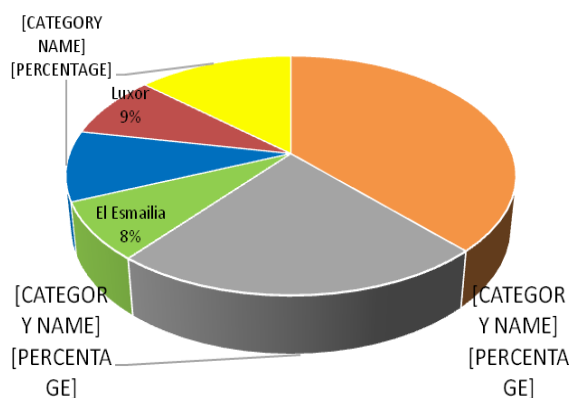


Figure 1: Total number of streptomyces isolated from different soil sources of Egypt.

Preliminary screening of peptidase activity on solid plates

The Streptomycin isolates were screened for their ability to grow and produce peptidase using solid petri dishes. The results showed that, the number of isolates which non produced peptidase was 293 isolates represented 56%. Two hundred thirty three isolates produced peptidase activity represented 44% as shown in figure 2 and 3. The selected isolates were checked out for their ability to hydrolyze pectin in the main screening step. The strains were spotted on to pectin-agar plates and four days after inoculation, when the plates were flooded with certified solution (1%); clear zones appeared around effective colonies, indicating the usage of pectin. White coloring appeared in the left over area of the dish, this is because of reaction of certified with the remained pectin present. The efficiency of certified in finding pectinolytic activity is well established [1, 5 and 8]

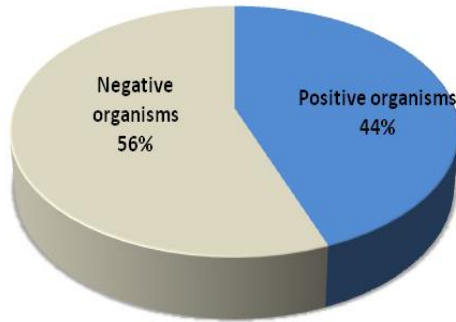


Figure 2: Survey of the isolated streptomycetes for peptidase production.

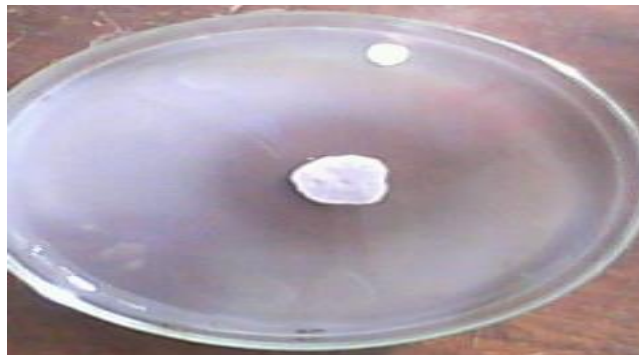


Figure 3: Photograph showing pectin clear zones of Streptomycin sp. pectin clear zone, in millimeter diameter.

In primary screening, 293 isolates did not give zone of clearance, represented negative group (56%) and 233 Streptomycin isolates (44%) showed zone of clearance as shown in figure 2. The positive isolates were classified as good and fair producers, selected on the basis of pectin clear zone in millimeter diameter for their peptidase activity. One hundred thirty three isolates were found to be good producers that have clear zone more than 10 mm in diameter represents 57.1%; other fair one contains 100 isolates that have clear zone diameter of less than 10 mm in diameter represents 42.9% as shown in figure 4.

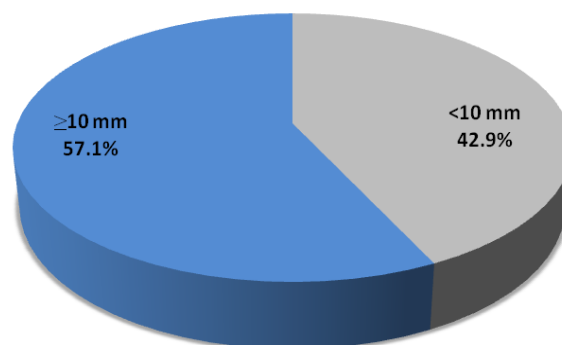


Figure 4: The pectinolytic Streptomycin sp. divided into two clusters, good and fair producers

Survey of the good group of streptomycetes isolates for polygalactouranase production in submerged medium

Since the present investigation was focused on good polygalacturonase producers, 133 isolates which showed dish clearance more than ten millimeter represented (57. 1%), were exposed to secondary screening

process by submerged fermentation by using pectin synthetic medium [28]. The results showed that, among the 133 isolates, five isolates were more than 15U/mg specific activity, represents (4%), 27 isolates range from 10 to 15 U/mg, represents (20%), and 72 isolates range from 5 to 10 U/mg, represents (54%) and 29 isolates were up to 5 U/mg, represents (22%), as shown in figure 5.

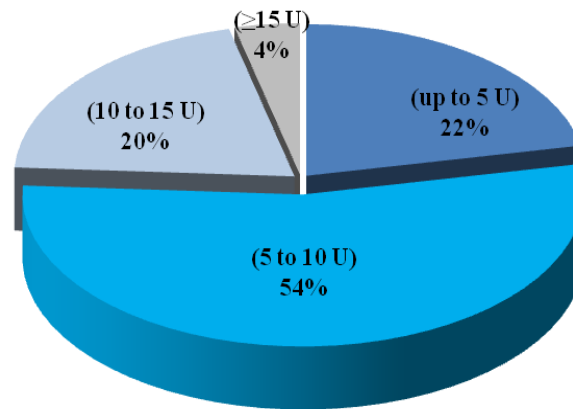


Figure 5: Survey of the good group of Streptomyces isolates for polygalacturonase production.

Screening of the most active Streptomyces sp. for polygalacturonase using different media

Screening of the most active five Streptomyces isolates (no. 2, 5, 8, 72 and 103) that have maximum polygalacturonase activity (≥15 U/mg) by using different media (M1, M2, M3 and M4).

The results showed that isolate no. 2, 5 and 103 provide the best results on pectin nitrate medium (M2) with pectin as only carbon source, while isolate no. 8 and 72 provide the best results on Hankin medium (M1) with pectin as only carbon source as shown in figure 6. Out of the four growth media processed, two have been reported for actinomycetes (Horikoshi medium and pectin nitrate). Decrease or increase of enzyme level production by using other media may be due to amount of carbon and nitrogen not well suited for production [1]. This was comparatively greater than peptidase from Streptomyces sp. J9 [4], 1122 U/L and 923 U/L by using apple and orange pomade respectively, polygalacturonase from Streptomyces lyrics was 18.3 U/mL [1] and enzyme activity and enzyme activity from Streptococcus sp. SB5 was 12.75 ± 0.12 U/mg protein [35].

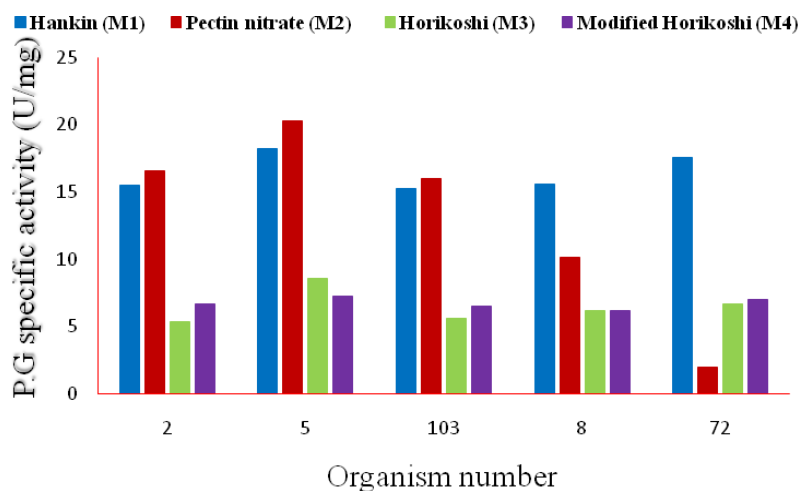


Figure 6: Screening of the most active five Streptomyces Isolates for polygalacturonase using different media.

Screening of the most active five isolates by using different industrial and agricultural wastes by using best medium for each isolate

The use of wastes as low cost residues, inexpensive, available very safe substrates in chemical production is especially interesting for countries where agro-industrial residues are abundant. This type of in turn helps to alleviate global warm in and environmental pollution. In this study we record, successful usage of agro-industrial waste products for polygalacturonase production.

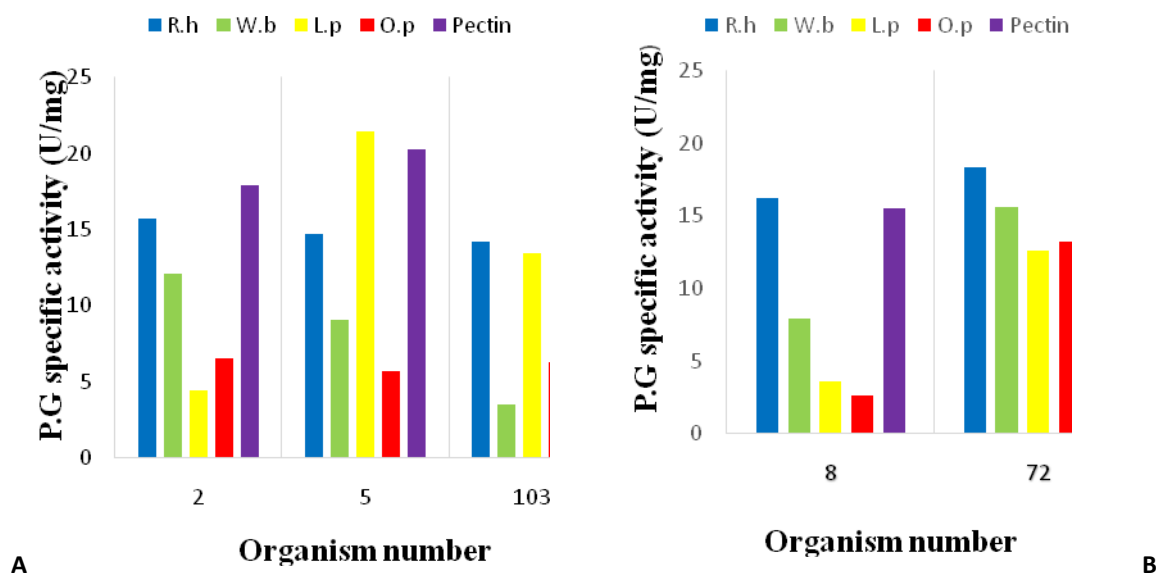


Figure 7: Screening for polygalacturonase using different agro-industrial wastes, (A) screening of isolates no. 2, 5 and 103 using M2 medium and (B) screening of isolates no. 8 and 72 using M1 medium.

From the results we find that M2 is the best medium for polygalacturonase by isolate no. 2, 5 and 103 using pectin as the sole carbon source as shown in figure 6. By screening using agro-industrial wastes like orange peel, lemon peel, rice husk, wheat bran instead of pectin in M2 medium for isolate (no. 2, 5 and 103), the isolate no. 5 give maximum enzyme activity by using lemon peel as the sole carbon source (21.5 U/mg) as compared to pectin (20.29 U/mg), while using rice husk, wheat bran and orange peel give lower enzyme activity (14.7, 9.1 and 5.7 U/mg respectively as compared to pectin (20.29 U/mg). In case of isolates no. 2 and 103 by using different agro-industrial wastes instead of pectin in M2 medium, enzyme activity lower by using all used agro-industrial wastes (lemon peel, orange peel rice husk and wheat bran) as compared to pectin as shown in figure 7(A).

In case of isolates (no. 8 and 72), we found that, M1 is the best medium for enzyme activity by using pectin as the sole carbon source as shown in figure 6. By screening of these isolates using agro-industrial wastes like orange peel, lemon peel, rice husk, wheat bran instead of pectin by using M1 medium, both isolates no. 8 and 72 give higher enzyme activity using rice husk 16.2 U/mg and 18.4 U/mg respectively as compared to pectin in medium 15.57 U/mg and 17.55 U/mg respectively, while using lemon peel, orange peel and wheat bran give lower enzyme activity as compared to pectin in medium as shown in figure 7(B).

Complex carbon sources such as agro-industrial wastes and pectin in fermentation medium have been reported as substrates that induce the peptidase activity in *Streptomyces* sp. This really reported by [19 and 36]. *Lentinus edodes*, also called Chinese mushroom, was successfully grown on apple pomace and other lignocelluloses wastes. Saadoun, et al., 2013 also use agriculture waste for peptidase production [4]. Hours et al., 1988 reported that, production of peptidase by *Aspergillus* foresides from apple pomace fermentation [37]. Many researchers reported peptidase production using fruit processing industrial waste materials [38, 39 and 40].

CONCLUSION

The agriculture soil was seemed to be good source for isolation of peptidase producing Streptomycin sp. The results obtained in our study strongly support the suitability of Streptomycin sp as good prospect for polygalacturonase production. We can produce enough amount of peptidase at a cheaper rate using agriculture wastes which will become a boon for our industries that are utilizing these enzymes for several processes. Further studies were made for taxonomical, identification of peptidase producing Streptomycin sp and maximization for peptidase production.

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