CHARACTERIZATION, IDENTIFICATION AND OPTIMIZATION OF CHITINOLYTIC RARE ACTINOMYCES ISOLATED FROM SINAI SOIL, EGYPT

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ABSTRACT

Actinomycetes are highly economic and biotechnologically useful prokaryotes able to produce wide range of bioactive secondary metabolites such as antibiotics and enzymes. The present study, aimed to isolate and screen actinomycetes from various soil samples collected from different regions of Saini, Egypt for chitinase production. In instantaneous isolation and screening of actinomycetes from Sixty-one soil samples, only eight isolates were found to have the capability to produce chitinase on colloidal media. Actinomycete strain A13 exhibit highest chitinase production while other strain shown poor production as compared to an index of relative enzyme activity. The most potent chitinolytic actinomycete isolate No. A13 was selected for further studies concerning their identification. Morphological, physiological, and phylogenetic analysis (16S rRNA). In addition to biochemical studies and culture characteristics as well as the chemical analysis of the cell wall, were carried out for the isolate under study. Based on the phenotypic and genotypic accumulated characteristics of the most potent chitinolytic actinomycetes isolate and consulting the recommended International Key’s of Bergey’s Manual for identification of actinomycetes, it was found that this isolate matched with Amycolatopsis orientalis by 97% with some differences and was given the name Amycolatopsis orientalis A13 sp. nova. The results revealed that the chitinase produced by Amycolatopsis orientalis A13 sp. nova can be optimized to produce the highest yield of chitinase and used as biocontrol agent.

Keywords: rare, Actinomycetes, Chitinolytic, Saini soil, chitinase, optimization
Introduction

Actinomycetes are filamentous Gram-positive bacteria, characterized by a complex life cycle belonging to the phylum Actinobacteria, which represents one of the largest taxonomic units among the 18 major lineages currently recognized within the Domain Bacteria. *Actinobacteria* are widely distributed in both terrestrial and aquatic ecosystems, mainly in soil, where they play an essential role in recycling refractory biomaterials by decomposing complex mixtures of polymers in dead plants, animals, and fungal materials. They are also important in soil biodegradation and humus formation as they recycle the nutrients associated with recalcitrant polymers, such as chitin, keratin, and lignocelluloses (Mukesh et al., 2014). For more than a decade, actinomycetes, *Aspergillus fumigatus*, and *Penicillium* sp. have been recognized as the dominant culturable micro-organisms in composting bioaerosols (Wéry, 2014). Enzymes created by Actinomycetes are rates as potential biocatalysts to bring of many specific reactions. Enzymes originated from Actinomycetes source are generally noticed as safe for the environments and they are functional at a wide range of temperature, pH or other extreme conditions (Mukhtar et al., 2017).

A few Actinomycetes genera have the ability to produce some types of chitinases when grown on chitin substrate, some actinomycetes genera (*Aeromicrobium*, *Microbacterium*, *Nocardioides*, and *Solirubrobacter*) were detected only on chitin-media (Jacquiod et al., 2013).

Medium with chitin as the sole nitrogen and carbon source could be selective for isolation of Actinomycetes (Lingappa & Lockwood, 1961; Hsu & Lockwood, 1975; Lacombe-Harvey et al., 2018). When grown on solid medium containing the colloidal chitin as a sole Carbon source, clearing zone surrounding colonies reveals that their growth depends, at least the evidence on their capability to solubilize chitin (Lacombe-Harvey et al., 2018; Yildirim-Aksoy et al., 2019).

Chitin agar is still used for selective isolation of chitinolytic actinomycetes as well as actinomycetes interacting with plants (Golinska et al., 2015). Several chitinolytic actinomycetes strains have been found to protect plants against plant diseases or to promote their growth. Some of these chitinolytic strains can even assume an endophytic means after their pass in plant tissues through cross root development areas, other natural openings or wounds (Santi et al., 2013). Chitinolytic actinomycetes strains used as commercial fungicides (Doumbou et al., 2001; Rey & Dumas, 2017). Although biocontrol has been shown to contribute to plant protection (Doumbou et al., 2001), chitinases produced by the actinomycetes are also thought to participate in antagonistic interactions with pathogenic fungi (El-Tarabily et al., 2000; Rey & Dumas, 2017).

The present study aimed to isolate, identification, and characterization of the most prominent chitinolytic actinomycete bacteria from soil sample (desert soil of El Tur, Sinai) and optimization of production parameters for maximum chitinase production.
Material & Methods

Isolation of Actinomycetes from soil sample:

Soil sampling

Sixty-one soil samples were collected from different locations from Egyptian Desert regions Sinai (El Tur, Nwaba, Ras Sidr and Saint Catherin). Every sample was a mixture of soil collected from 3 to 5 holes whose depth was around 10 to 20 cm (Yassine, 2013). The surface layer of soil removed, and the central portion was collected in sterile plastic bags. Samples mixed to ensure uniformity and passed through a 3 mm sieve to remove stone and root fragment.

Processing of soil samples

Each sample was divided into two parts. One part was used as air dryer for one week at room temperature to be used as dry sample and the second part was taken as the preliminary treatment of soil samples by 1:10 w/w CaCO₃ with A high relative humidity was maintained in the jar by water saturated filter paper and incubated it for several days at 28°C. Such procedure proved to be rather efficient in regard to increasing the number of the isolates including representatives of rare genera as compared with the routine methods and this helps in reducing the relative counts of bacteria and molds (Alferova & Terekhova, 1988).

Actinomycetes isolation

For isolation of Actinomycetes colonies, the dilution method described by (Oskay et al., 2004) was used. Suspensions of soil samples were prepared by shaking 5 gm of the soil in 50 ml sterile distilled water, allowed to stand for 10 min. Serial dilutions were prepared from 10⁻¹ to 10⁻¹⁰ using sterile distilled water. Under aseptic conditions, 0.1 ml of each dilution was spread by a glass spreader on Starch Nitrate agar and Yeast-Malt Extract (ISP2) agar plate (Shirling and Gottlieb, 1996) and incubated for 7 days at 28 -30°C.

The developed colonies of Actinomycetes which are characterized by their sharp round edges on culture medium are picked up by a sterile needle and then re-inoculated on the isolation medium, sub-cultured on slants of the same medium and stored for studies.

Primary Screening for enzyme activity of Actinomycetes isolates

Preparation of colloidal chitin

Colloidal chitin was prepared by the method of (Hsu & Lockwood, 1975) with some our modifications as follows. The practical grade chitin powder purchased from (QualiKems, India) was used to prepare the colloidal chitin. Chitin powder (5 g) was
dissolved in 100 ml of ice concentrated hydrochloric acid and continuously stirring at 4 °C for 2 h. Chitin was precipitated as a colloidal suspension by adding it slowly to 500 ml of ice 95% ethanol with continuously stirring at 4 °C for 1 h and then incubate at 27°C overnight. The suspension was centrifuged at 4°C for 15 min at 3000 rpm and the pH of precipitate after dissolving in distilled water was adjusted to 7.0 by addition of 5 N NaOH and the colloidal suspension was washed with distilled water for desalting. After desalting, the suspension was centrifuged at 3000 rpm for 15 min at 2°C and the precipitate was collected for further use as colloidal chitin.

Screening for production of chitinase activity

Qualitative assay

Chitinolytic ability of the actinomycetes isolated from soil samples were determined using the effects of different media supplemented with colloidal chitin (0.1 %). Chitinase production were tested using three culture media, basal medium (Vyas & Deshpande, 1989) colloidal chitin medium (Deng et al., 2007) and ISP2 with replacing glucose with Colloidal chitin (ISP2CC). Each plate containing media was inoculated with a loop of growing margins of six- day-old isolates on a Yeast-Malt Extract (YEME) plate and incubated for 7 days until zone of chitin clearing were seen around and beneath the colonies. Clear zone diameter was measured in (mm) at the average of three-dimension measure and used as an indicator of chitinase activity. An index of relative enzyme activity (RA) for each isolate was calculated by dividing the total area of activity (the area of the clearing zone less the area of the colony) by area of the colony of 1 or greater (Bradner et al., 1999; Duncan et al., 2006).

Quantitative determination of chitinase

Individual 250-ml Erlenmeyer flask containing 50 ml of ISP2 without glucose broth amended with 10 mg/ml of colloidal chitin were prepared. Flask containing broth media as above were inoculated with a culture disk (5 mm diameter) taken from the growing margins of six- day-old isolates on a Yeast-Malt Extract plate of actinomycete isolate and incubate for 7 days (Balouiri et al., 2016). After incubation, the suspension from each flask were ice-centrifuged for 30 min at 13000 g. The supernatant was collected in sterile tubes and was used as a source of the crude enzymes.

Chitinase Enzyme assay

Chitinase activity was determined according to the method described by (Tweddell et al., 1994) and modified by (Reissig et al., 1955; Singh et al., 1999). The reaction mixture consisting of 1 mL of colloidal chitin (1 %) in 50 mM sodium phosphate buffer, pH 6.8 and 1 mL of enzyme solution. After incubation at 50 °C for 1 hour, the reaction was stopped by boiling, centrifuged and chitinase activity was calculated by measuring the release of N-acetyl -D-glucose amine (NAG). Reducing sugar concentration was determined by optical
density at 570 nm using a scanning spectrophotometer (UV). N-acetylglucosamine used as the calibration standard. The concentration of NAG in the supernatant is determined by the procedure of Reissig et al. (1955). Specific activity (U= 1 unit of chitinase) was defined as the amount of the enzyme that released 1µmol of NAG mg⁻¹ protein h⁻¹. The concentration of protein was estimated by Lowry’s method (Lowry et al., 1951) using bovine serum albumin as standard.

Optimization of enzyme production

The effect of different operational parameters on chitinase production was optimized further. About 1% culture was inoculated into the previously mentioned medium and evaluated one by one keeping other factors constant. For the determination of optimum temperature, the chitinase production medium was prepared in different flasks and the chitinase production was assayed after incubating the medium at different temperatures (20, 25, 28, 30, 35, 40 and 45 °C). The optimum pH for the chitinase production was investigated by varying the initial pH of the culture medium from 5 to 9 and at optimized temperature. For the determination of optimum incubation time for chitinase production, the isolate was allowed to grow for various incubation periods viz: 4, 5, 6, 7, 8, 10 and 11 days at 30 °C. Effect of different colloidal chitin concentrations on the chitinase productivity were tested. For the determination of optimum nitrogen source our organic nitrogen sources (arginine and tryptone), two inorganic nitrogen sources (ammonium nitrate and ammonium sulphate) were added to production media. Where carbon source optimized by ISP2 medium lacking its carbon sources was supplemented with different types of sugars at equi-molecular amounts of carbon sources. The carbon sources were represented by, D-glucose, D-fructose, soluble starch, sucrose, tween80, and glycerol, centrifuged at 10,000 rpm for 15 min and the supernatant was subjected to chitinase assay.

Methods used for classification of the most potent chitinase-producing actinomycete isolate No. A 13

1. Conventional taxonomy

(a) Studies concerning the morphological characteristics

Determination of the spore-bearing hyphae and spore chains morphology were done by cover slip culture technique (Kawato & Shinobu, 1959) or by direct microscopically examination to the surface of the culture on the growth plates. Electron microscope study was done for spore chain morphology and spore surface.

(b) Studies concerning the cultural characteristics

Diaminopimelic acid (DAP) in the whole cell was analyzed according to (Becker et al., 1964) and (Yamaguchi, 1965). The color of sporulating aerial mycelium, substrate mycelium and soluble pigments in media were recorded in accordance with the guidelines
established by the International Streptomyces Project (Shirling & Gottlieb, 1966) ISP methods. Colors were assessed on the scale developed by (Kelly & Judd, 1976).

(c) Studies concerning the physiological properties

Was carried out according (Tresner & Danga, 1958; Pridham & Gottlieb, 1948; Shinobu, 1958; Pridham and Gottlieb 1948).

2. Molecular and phylogenetic identification:

DNA isolation and amplification of 16S rRNA gene:

A single, pure colony of the isolate was taken from the plate grown culture and was suspended in 10μl TE buffer [10mM Tris (pH 8.0), 1m MEDTA]. The samples were mixed briefly using cyclo mixer, and 5.0μl of this suspension was applied to FTA® Classic Card (Whatman International Ltd.) and allowed to dry for at least 1h at room temperature. Further processing of the sample was done according to the manufacturer’s instructions.

The 16S rRNA genes were amplified using forward (5’- AGA GTT TGA TCC TGG CTC AG -3’) and reverse (5’- GGT TAC CTT GTT ACG ACT T-3’) primers (Xcelris Labs Ltd., India) known to be conserved among all known bacteria (Ulrike et al. 1989). PCR was performed in an automated thermal cycler (PTC-200, M J Research Inc.) with an initial 92°C denaturation for 2min 10sec; followed by 35 cycles of 92°C for 1min 10sec, 48°C for 30sec, 72°C for 2 min 10sec and a final extension at 72°C for 6min 10sec (Massol-Deya et al., 1995). The PCR products were resolved by electrophoresis on 1.5% agarose gel (Bio-Rad, Hercules, CA) and visualized using gel documentation system (UVI pro, UVI tec). DNA isolation and purification, 16S rRNA gene amplification and sequencing was carried out at Genetic Analyzer Unit; Sigma Scientific Services Co., El-Dokki, Egypt.

Results and Discussion

Isolation of actinomycetes from soil samples.

A total of 18 morphologically different actinomycetes were isolated from Sixty-one soil samples collected from different locations from Egyptian Desert regions Sinai (El Tur, Nwaba, Ras Sidr and Saint Catherin).

Qualitative determination of chitinolytic activity

Only Eight isolates have the capability to produced chitinase on colloidal chitin (CC) media and ISP 2 supplemented by colloidal as Carbon source (ISP2CC) showed by clear zone formed around them, these eight isolates were 7A1, 7A2, 11, A2, 32, A13,23M and 22. No growth was observed on CC medium except isolates No.11,23M. When these isolates compared according to calculating of An index of relative enzyme activity (RA) for each
isolate, A13 was ranked as the most potent chitinolytic actinomycete producer (Table 1, Fig. 1) and was chosen for further studies.

| Isolation | Mean of colony diameter (mm) | Mean of clear zone diameter (mm) | RA |
|-----------|-------------------------------|----------------------------------|-----|
| A13       | 10.67                         | 28.33                            | 1.656 |
| 7A2       | 4.50                          | 6.00                             | 0.333 |
| 22        | 9.50                          | 10.50                            | 0.105 |
| 11        | 5.00                          | 9.43                             | 0.887 |
| 7A1       | 12.67                         | 18.33                            | 0.447 |
| A2        | 10.50                         | 14.50                            | 0.381 |
| 32        | 9.67                          | 13.67                            | 0.414 |
| 23M       | 6.17                          | 11.33                            | 0.838 |

RA = An index of relative enzyme activity

Table 1: An index of relative enzyme activity (RA) for isolates on ISP2CC media.

**Figure 1:** Evaluation of Chitinolytic activity assay

**Quantitative determination of chitinase**

The most potent isolate for chitinolytic activity (A13) grew well on Colloidal Chitin broth medium (CC) and ISP2 supplemented with colloidal chitin as sole source of Carbon (ISP2CC). The chitinolytic activity were recorded for CC Broth media as triple replicate with chitinase activity as following 132.94, 165.32, 153.25 U/ml and protein measure 6.05, 7.30, 6.89 U/mg while for ISP2CC recording chitinase activity as 262.80, 286.47, 293.47U/ml and protein measuring 5.361, 5.880, 10.571U/mg, so ISP2CC medium was selected for further procedures.

**Taxonomic studies of the most potent chitinase-producing actinomycete isolate No. A13**

The morphological, physiological, cultural, and biochemical characteristics for isolate No. A13 were carried out (Tables 2, 3 and Figures 3, 4).
### Types of media

| Types of media                        | Growth | Aerial mycelium | Substrate mycelium | Diffusible pigments |
|--------------------------------------|--------|-----------------|--------------------|---------------------|
| Starch-nitrate agar                  | +<sup>p</sup> | white           | yellow             | Yellowish brown     |
| Inorganic-trace salt- starch agar (ISP 4) | +<sup>p</sup> | white           | Pale yellowish     | Yellowish brown     |
| Glycerol asparagine agar (ISP 5)    | +      | white           | cream              | -                   |
| Yeast extract- malt extract agar (ISP 2) | +      | white           | Pale yellowish     | +/- purple          |
| Oatmeal agar (ISP 3)                | +      | white           | Pale yellowish     | -                   |
| Melanin pigment media 1-Tryptone yeast extract (ISP 1) | +<sup>p</sup> | white | yellow | - |
| 2- Peptone yeast extract iron agar (ISP 6) | +      | white           | Pale yellowish     | -                   |
| 3- Tyrosine agar (ISP 7)             | +      | white           | cream              | Purple              |

+ <sup>p</sup> grow - absent +/- produced after long period incubation  

**Table 2**: Cultural and physiological characteristics of the actinomycetes isolate No. A13.

| Characters                  | Isolate A13 |
|-----------------------------|-------------|
| **Morphological characters:** |            |
| Spore mass                  | Brilliant gray |
| Spore chain                 | fragmenting branched aerial hyphae |
| Spore surface               | Smooth      |
| Diffusible pigment          | +           |
| **Physiological characters:** |            |
| DAP                         | meso-DAP    |
| Pattern Sugar               | Arabinose and galactose |
| Melanin pigment             | -           |
| Growth at 45 °C             | -           |
| **Resistance to**           |            |
| Rifampicin (10 μg/ml)       | R           |
| Penicillin G (20 μg/ml)     | R           |
| Gentamycin (5 μg/ml)        | R           |
| Tobramycin (50 μg/ml)       | R           |
| Neomycin (8 μg/ml)          | R           |
| Streptomycin (20 μg/ml)     | R           |
| **Growth at (% w/v):**      |            |
| NaCl (7.0)                  | -           |
Utilization of:

| Utilization          |   |
|----------------------|---|
| Sucrose              | + |
| meso-Inositol        | + |
| Mannitol             | + |
| Glucose              | + |
| Arabinose            | + |
| Fructose             | + |
| Glycerol             | + |
| Maltose              | + |
| Mannose              | + |
| Xylose               | + |
| Adonitol             | + |
| Cellobiose           | + |
| Tween 80             | + |

(+)= positive, (-)= negative (R)= Resistant

**Table 3:** Morphological and biochemical characteristics of actinomycetes isolate No. A13

**Molecular Identification of the most potent chitinase-producing actinomycete isolate A13**

The 16S r-RNA gene sequencing of strain A13 resulted in 97% of similarity with *Amycolatopsis orientalis*, therefore the strain A13 was designated as *Amycolatopsis orientalis* strain A13 which was submitted in the NCBI GenBank and accession number MN704757.1 was obtained. The phylogenetic tree of strain A13 was constructed by calculating bootstrap values by neighbor tree joining method which has been presented in Figure 2.

![Phylogenetic Tree](image)

**Figure 2:** Phylogenetic Tree Representing Position of Strain A13 With Neighbor Joining Method.
From eighteen isolates showed chitinolytic activity, isolate No. A13 showed the strongest activity. The identification of isolate No. A13 was done according to Bergey’s Manual (Bergey & Holt, 1994; Williams, 1989), in which the basis for the definition depends on the morphological characteristics and the pigments produced in addition to the physiological and genetic studies (amplification and sequencing of the 16S rRNA).

Therefore, it was found that isolate No. A13 matched with Amycolatopsis orientalis by 97% with some differences and was given the name Amycolatopsis orientalis A13 sp. nova. (Nanjo et al., 1990) was first who purify the chitinase from Nocardia orientalis (present name: Amycolatopsis orientalis), (Murata et al., 2005) also reported chitinase enzyme from the culture filtrate of Amycolatopsis orientalis.
Optimization of chitinase production by *Amycolatopsis orientalis* A13 sp. nova.

**Effect of different incubation temperatures on the chitinase production**

For the detection of the suitable incubation temperature for maximizing chitinase activity, it could grow on growth medium adjusted at pH (7) and incubated at different temperatures covering the range from 20-45 °C for 6.0 days. Data represented graphically in Figure (5) revealed that, the optimum temperature capable of promoting chitinase productivity by *Amycolatopsis orientalis* A13 sp. nova was at 30 °C. Similar observation were also reported by *(Kim et al., 2003)* with *Streptomyces* sp. M-20, other reports also maximum enzyme production from *Bacillus* sp. R2 at 30°C *(Cheba et al., 2018)*. In general, most of the soil borne *Streptomyces* sp. showed the optimum temperature for maximum chitinase production lies between 30 to 40°C *(Gomes et al., 2001; Shanmugaiah et al., 2008; Subramaniam et al., 2012)*.

![Figure 5: Effect of different incubation temperatures on the chitinase production by *Amycolatopsis orientalis* A13 sp. nova.](image)

**Effect of the initial pH values on the chitinase production**

Effect of different initial pH values on the enzyme specific activity produced by *Amycolatopsis orientalis* A13 sp. nova was studied. For this purpose, growth medium was adjusted at different initial pH values covering the range of (5-8) and incubated at 30 °C for six days. It could be concluded from the results represented graphically in Figure (6) that; the optimum initial pH value capable of promoting activity of Enzyme biosynthesis by *Amycolatopsis orientalis* A13 sp. nova was found to be at the value of 6.0. Below and above this pH value the enzyme(s) yield decreased gradually. Similarly, *(Jahangiri et al., 2019)* reported the enzymatic activity of purified chitinases from *Aeromonas* sp. *PTCC 1691* was optimal between pH 6 and 8, with a maximum at 6.0 *(Donderski &*
Trzebiatowska, 2000) showed the maximum activity of chitinases produced by planktonic bacteria at pH 6.0 and at 30°C.

**Figure 6**: Effect of the initial pH values on the chitinase production by *Amycolatopsis orientalis* A13 sp. nova.

**Effect of different incubation periods on production of chitinase**

This experiment was conducted to detect the suitable incubation period needed to produce the highest yield of chitinase by *Amycolatopsis orientalis* A13 sp. nova. The chitinase productivity was detected at time intervals of 4, 5, 6, 7, 8, 10 and 11 days on ISP2CC medium, in submerged conditions. Data illustrated graphically in Figure (7) revealed the relation between specific activity of Enzyme and time of incubation that gave rise to a higher chitinase synthesis after 4 days of incubation and decreased thereafter while show slight increase after 11-day due production of pigmentation that interfere with assay. Similar observation was also reported by (Miranda-Chavez, 1978) with *Streptomyces albus* and *S. griseus.*(Karunya et al., 2011) reported the highest chitinase production at 4 days from *Bacillus subtilis.*

**Figure 7**: Effect of different incubation periods on production of chitinase by *Amycolatopsis orientalis* A13 sp. nova.
Effect of different colloidal chitin concentrations on the chitinase production

The effect of different colloidal chitin concentrations on the chitinase productivity by *Amycolatopsis orientalis A13 sp. nova* was tested by add different concentrations of colloidal chitin to ISP2CC media viz: 3, 5, 10, 15, and 20 mg/ml were applied as represented graphically in Figure (8). Maximum activity of the chitinase was recorded in the presence of colloidal chitin at 5 mg/ml. Similar observation were reported that the optimal concentration ranged between 0.5 and 1 % by (Cheba & Zaghloul, 2017) with *Bacillus sp.*

![Figure 8: Colloidal optimization](image)

**Figure 8:** Effect of different colloidal chitin concentrations on the chitinase production by *Amycolatopsis orientalis A13 sp. nova*.

Effect of supplying different nitrogen sources on the chitinase production:

The effect of different nitrogen sources on the chitinase productivity by *Amycolatopsis orientalis A13 sp. nova* was tested by add other nitrogen sources to ISP2CC medium where, four different nitrogen sources with different conc. viz: organic form such as arginine, tryptone and inorganic form as NH$_4$NO$_3$, and (NH$_4$)$_2$SO$_4$ were applied as represented graphically in Figure (9). Maximum activity of the chitinase was recorded in the presence of ammonium nitrate 0.06 g/ml. (Gangwar et al., 2016; Shalaby et al., 2019) reported The maximum chitinase activity was obtained from medium supported by Ammonium nitrate as nitrogen source.
Figure 9: Effect of supplying different nitrogen sources on the chitinase production by Amycolatopsis orientalis A13 sp. nova.

Effect of supplying different carbon sources on the chitinase production:

For the detection of the suitable carbon source for maximizing the chitinase productivity by Amycolatopsis orientalis A13 sp. nova, it could grow on growth medium containing different carbon sources viz: D-glucose, D-fructose, soluble starch, sucrose, glycerol, and tween 80. Data represented graphically in Figure (10) revealed that, the highest chitinase activity could be obtained in the presence of commercial D-fructose followed by D-glucose. (Shivalee et al., 2018) showed formulated production medium supplemented with fructose greatly influenced the chitinase production by Streptomyces pratensis strain KLSL55.

Figure 10: Effect of supplying different carbon sources on the chitinase production by Amycolatopsis orientalis A13 sp. nova.

Conclusion

Several actinomycetes isolates were isolated from different Sinai areas can produce chitinase enzyme. This study implies that the presence of highest chitinolytic activity of A.
orientalis A13 sp. nova and the Results of this experiment could be considered in improving the strategy to use as a biocontrol agent.

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خصائص وتعريف والظروف المثلى لإنتاج الكيتيزاز لنوع من الأكتينوميسيتات النادرة

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الملخص العربي

تهدف هذه الدراسة إلى عزل بعض الأكتينوميسيتات النادرة والمحملة للكيتيزاز من عينات تربة من البيئة الصحراوية

وجملة الدراسة الحالية تم عزل ١١ عزلة أكتينوميسيتات من عينات التربة وأظهرت النتائج أن ٨ عزلات فقط لها القدرة على تحليل الكيتيز الذكي تحيطي على الكيتيز كمصادر اساسي للكربون واعتبرت عزلة واحدة ومن ثم تم اختيار هذه العزلة لأجراء التجارب مخل الدراسة.

تم تعريف العزلة باستخدام الطرق المورفولوجية والفسيولوجية والاختبارات البيوتيممائية وتحليل النتائج الجيني ١٦٨ RNA ونسبة ٩٧% مع وجود بعض Amicolatopsis orientalis A13 sp. nova اختلافات مما يرجح كونه نوع جديد وقد سمى الجينات.

ودراسة الظروف المثلى لإنتاج إنزيم الكيتيزاز المحمل للكيتيزاز بواسطة تلك العزلة تبين ما يلي:

درجات حرارة مثلي عند ٣٠-٣٧ درجة مئوية ودرجة الأسد البيضوري ٣٧-٥٠ درجة أم تحسن شد وتركيز الكيتيز الغروي ملء الكل من الوسط الغذائي كما أضح أيضا ان نترات الأمونيا والفرنكوز أفضل مصدر للكيتيزاز والكربون على التوالي.

الكلمات المفتاحية: الأكتينوميسيتات , نادرة الوجود , تحل للكيتيزاز , تربة سيناء , إنزيم الكيتيزاز , الظروف المثلى