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OPTIMIZATION AND REPRESSION OF AMYLASE PRODUCTION FROM NOCARDIOPSIS SP. MIT 6 ISOLATED FROM MITHAPUR, COASTAL REGION OF GUJARAT

[1]Ashish B. Kalasava, [2]Kruti G. Dangar, [3]Gira P. Mankad and [4]Satya P. Singh
UGC-CAS Department of Biosciences, Saurashtra University,
Rajkot, Gujarat (India).

ABSTRACT

Salt tolerant and high G+C content actinomycete Nocardiopsis sp. Mit 6 (KJ556535.1) was isolated from Mithapur, the coastal region of Gujarat, India. The isolate was screened for the extracellular amylase production and growth pattern. Maximum production of extracellular amylase was carried out by "One factor variable at time" method. The influence of various factors such as Starch 1-3%, Salt 5-15% and pH 4, 5, 6 was studied on the amylase production. Nocardiopsis sp. Mit 6 could grow and repressed amylase production with the presences of monosaccharide and disaccharide carbon sources. Optimization and repression of the amylase production is explored for biotechnological potential.

KEYWORDS: *Nocardiopsis sp., Amylase, Haloalkaliphilic actinomycetes*

INTRODUCTION :

Now a day's, exploring the different habitats with culture independent approaches such as metagenomics, proteomics and genomics are widely applicable [1,2]. However, tapping the potential of cultivable microbes are reliable approach to determine the residing microorganisms in habitat [3,4]. Actinomycetes belong to bacteria domain and *Actinobacteria* family. It shows the Gram-positive, characteristics with high GC containing, and fungus like branched hyphae observe in microscopy examination [5,6,7]. The diverse habitats such as coastal region, marine sediment, salt lakes, saline soil, sea water, brines and many more are the recognized habitats for the isolation of the halophlies/salt tolerant actinomycetes [8,3,9]. *Nesterenkonia, Nocardiopsis, Salinispora and Streptomonospora,* and family *Actinopolyspora, Saccharomonospora, Yuhushiella, Prauserella, accharopolyspora and Amycolatopsis* genera and species [10] naturally residing in diverse saline niches. From the several halophlices actinomycetes the various molecules are reported such as antibiotics, heat shock proteins [11,12,13]. *Halomonas meridiana* [14], *Halothermothrix orenii* [15] and *Nocardiopsis species* [16] were reported for production of amylases from the saline habitats. Biotechnological compatible protease were recently reported from the specie of *Nocardiopsis xinjiangensis* strain OM-6 [17]. While the study based on the *Nocardiopsis sp.* Mit 6 endeavored to investigate an assortment of parameters such as salt and pH involving growth and amylase production. The enzymes may show better variation in their characteristics and hence captured the biotechnological application prospects.

2. MATERIALS AND METHODS

2.1. Detection of extracellular amylase

The production of the extracellular amylase was screened on the starch agar plates [18].

2.2. Amylase production in liquid culture

2.2.1. Inoculums preparation and Enzyme assay

The inoculum preparation and Enzyme assay was performed by using starch as substrate as described earlier [18]. Amylase was estimated by DNSA method. One unit of amylase activity (U) was described as the enzyme liberating of maltose per min under the assay condition

2.3. Effect of NaCl and Starch on the growth and amylase production

The effect of salt and starch on the growth and amylase production was studied by varying the 5-15% at a constant pH 8 in 1% starch medium and 1-5% at pH 8 at 10% (w/v) NaCl at 30º C under shaking at 120 rev/min respectively with 10% inoculums. The growth and enzyme activity were quantified for 10 days at a time intervals of 24 hrs at 28º C.

2.5. Repressive effect of various medium components on *Nocardiopsis sp.* Mit 6 for amylase production

Repression of enzyme secretion by glucose and maltose was studied by incorporating maltose and glucose (1% w/v) into the optimized medium individually and in combination. α -amylase activity was expressed as percentage relative activity

2.5.1. Repression of amylase production by Glucose in *Nocardiopsis sp.* Mit 6

Glucose was evaluated for their effect on amylase production by addition 1% (w/v) glucose in the optimized medium of *Nocardiopsis sp.* Mit 6**.** The medium contained (g/100ml); NaCl 10; pH 8, yeast extract 0.5, peptone 0.5, starch 1 with 10% inoculums. The enzyme activity was quantified for 4th days at time intervals of 24 hrs at 28º C under shaking condition (120 rpm).

2.5.2. Repression of amylase production by Maltose on *Nocardiopsis sp.* Mit 6

Maltose was evaluated for their effect on amylase production by addition 1% (w/v) Maltose in the optimized medium of *Nocardiopsis sp.* Mit 6. The medium contained (g/100ml); NaCl 10; pH 8, yeast extract 0.5, peptone 0.5, starch 1 with 10% inoculums. The enzyme activity was quantified for 4th days at time intervals of 24 hrs at 28º C under shaking condition (120 rpm).

2.5.3. Repression of amylase production by Glucose + Maltose on *Nocardiopsis sp.* Mit 6

The glucose and maltose were evaluated for their effect on amylase production by the addition of 1% (w/v) of these sugars in the optimized medium of *Nocardiopsis sp.* The enzyme activity was quantified for 4th days at time intervals of 24 hrs at 28º C under shaking condition (120 rpm).

2.6. Amylase characterization

The temperature and pH profile

The crude amylase activity was screened with various parameters such as temperature 25º C to 90º C and in 20 mM concentrations of different buffers such as Sodium phosphate buffer (pH 8), Tris–HCl

buffer (pH 9) and Acetate buffer (pH 5) at with 1% starch. The substrate as starch in the range of 1-3% was analyzed on the amylase activity.

3. RESULT

3.1. Screening for the extracellular amylase production Detection of amylase by plate assay

After adding Gram's iodine on starch agar media, the appearance of clear zone surrounding the colonies indicated the production of extracellular amylase against blue background. Colony Diameter 2.3 cm and Ratio 0.9 cm

3.2. Effect of NaCl and Starch on the growth and amylase production

For the maximum amylase production with influence of varying NaCl concentrations: 5%, 10% and 15% and starch 1-3% were studied in the liquid medium containing 1% starch for 10 days at the time intervals of 24 hrs at 28º C after inoculating 10% inoculums under shaking condition (120 rpm). *Nocardiopsis sp.* Mit 6 had optimum amylase production 180.16 U/mL at 5% salt on 7th day of growth *Nocardiopsis sp.* Mit 6 had amylase production 275.4 U/mL at 10% salt on 4th day of growth. *Nocardiopsis sp.* Mit 6 had amylase production of 148.14 U/mL at 15% salt on 5th day of growth. *Nocardiopsis sp.* Mit 6 had optimum amylase production of 273.16 U/mL at 1% starch on 4th day of growth. *Nocardiopsis sp.* Mit 6 had optimum amylase production of 143.46 U/mL at 2% starch on 7th day of growth *Nocardiopsis sp.* Mit 6 had optimum amylase production of 168.12 U/mL at 3% starch on 8th day of growth Table 1 displayed the maximum amylases activity with optimum condition.

Table 1. Maximum amylases activity with optimum condition.

SALT	5% (7th day)	10% (4th day)	15% (5th day)
	180.16 U/mL	275.4 U/mL	148.14 U/mL
STARCH	1% (4th day)	2% (7th day)	3% (8th day)
	273.16 U/mL	143.46 U/mL	168.12 U/mL

3.3. The characterization amylase

3.3.1. Effect of pH on amylase activity

The optimum pH for crude amylase of *Nocardiopsis sp.* Mit 6 was pH 8 (401.5 U/mL) (Fig. 1).

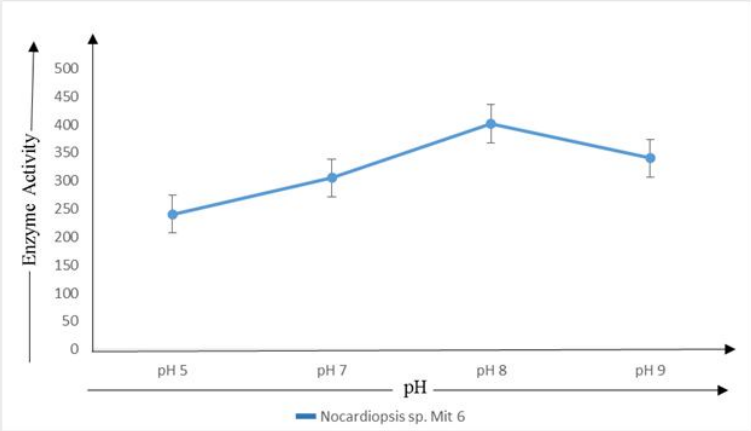

Fig. 1. pH optima of partially purified amylase from *Nocardiopsis sp.* Mit 6

3.3.2. Effect of temperature on the amylase activity

The optimum temperature for the activity of the crude amylase of *Nocardiopsis sp.* Mit 6 was 37 ºC (440.2 U/mL) (Fig. 2).

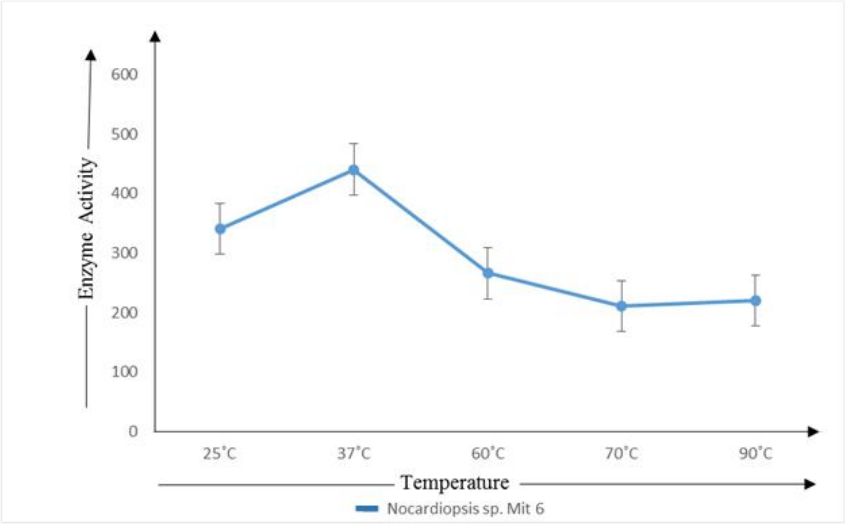

Fig. 2. Temperature optima of crude Amylase from *Nocardiopsis sp.* Mit 6

3.3.3. Substrate specificity

The effect of substrate concentrations on the activity of the crude amylase was carried out at varying starch concentration in the range of 1-3%. The optimum starch concentration for the crude amylase activity of *Nocardiopsis sp.* Mit 6 was 1% (Fig. 3).

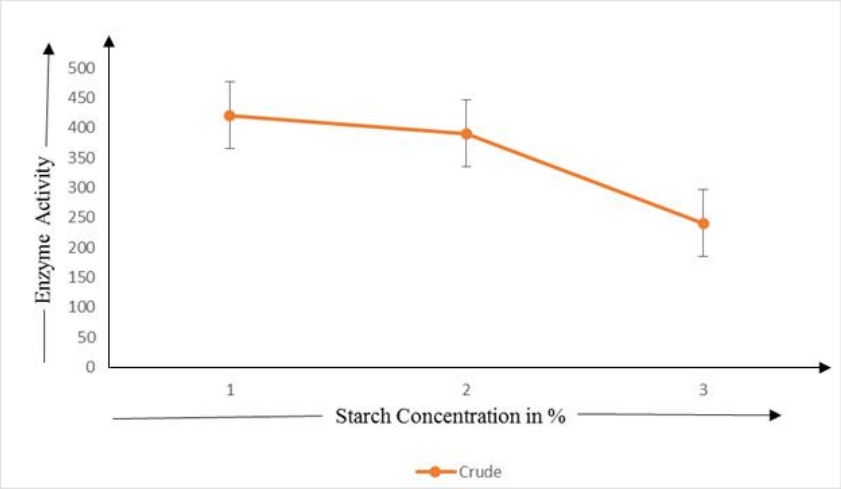

Fig. 3. Substrate Optima of crude amylase from *Nocardiopsis sp.* Mit 6

3.4. Repressive effect of various medium components on Mit 6 for amylase production

3.4.1. Repression of amylase production by Glucose in *Nocardiopsis sp.* Mit 6

The repression medium contained 1% starch with 1% glucose as carbon sources. Standard medium contained only 1% starch as carbon source. For *Nocardiopsis sp.* Mit 6, in repression medium, the amylase activity was 454.68 U/ml, while in standard medium, amylase activity was 520.28 U/ml, on 4th day of growth (Fig. 4).

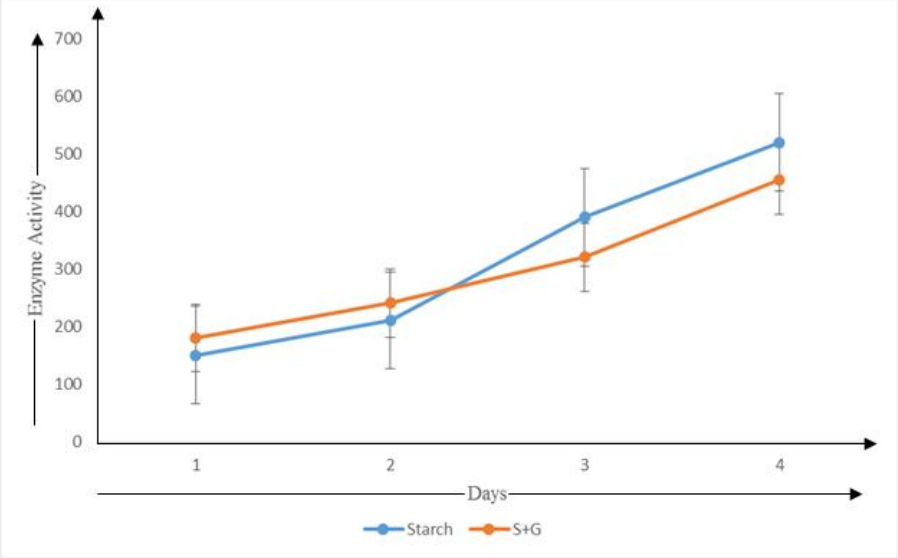

Fig. 4. Repression of amylase production by glucose on *Nocardiopsis sp.* Mit 6

3.4.2. Repression of amylase production by Maltose on *Nocardiopsis sp.* Mit 6

The repression medium contained 1% starch along with 1% maltose as carbon sources. Standard medium contained only 1% starch as carbon source. For *Nocardiopsis sp.* Mit 6, in repression

medium amylase activity was 437.68 U/ml, while in standard medium, the amylase activity was 520.28 U/ml, on the 4th day of growth (Fig. 5).

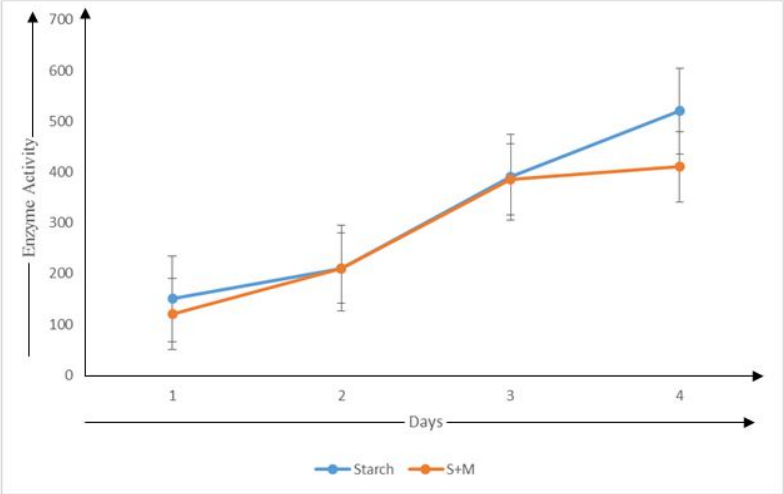

Fig. 5. Repression of amylase production by maltose on *Nocardiopsis sp.* Mit 6

3.4.3. Repression of amylase production by Glucose + Maltose on *Nocardiopsis sp.* Mit 6

The repression medium contained 1% starch along with 1% Glucose and 1% Maltose as carbon sources. Standard medium contained only 1% starch as carbon source. For *Nocardiopsis sp.* Mit 6, in repression medium, the amylase activity was 462.06 U/ml, while in standard medium, amylase activity was 520.28 U/ml, on the 4th day of growth (Fig. 6).

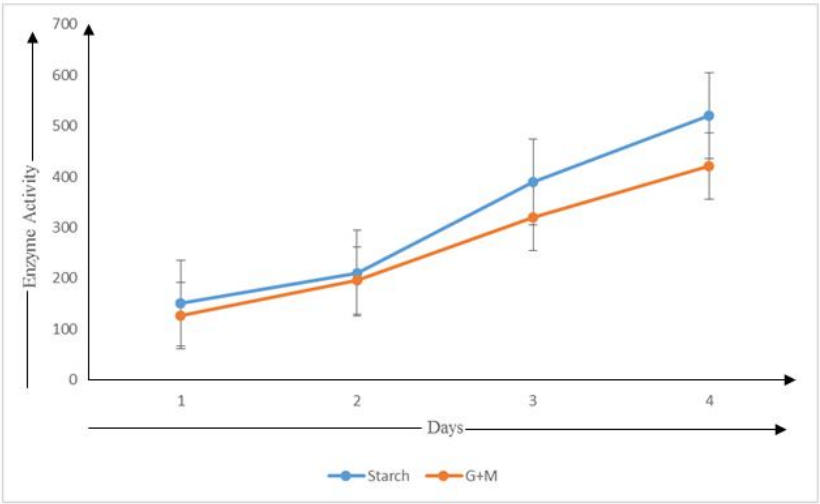

Fig. 6. Repression of amylase production by Glucose + Maltose on *Nocardiopsis sp.* Mit 6

4. DISCUSSION

Nocardiopsis alba OK 5 and *Streptomyces clavuligerus* Mit 1 were earlier isolated and reported for protease production while *Nocardiopsis sp.* Mit 6 was reported for amylase production from the coastal region of Gujarat [19,20].

In this study, *Nocardiopsis sp.* Mit 6 grew on salt medium and produces amylase. Amylase production was inducible. Maximum amylase production was carried out with combination of salt, pH and starch based on one factor variable at time. The high concentration of salt 10%, alkaline pH 8 and

starch 1% promoted maximum production of amylase activity (275.4 U/mL) and growth of *Nocardiopsis sp*. Mit 6.

Nocardiopsis alba OK 5 and *Streptomyces clavuligerus* Mit 1, *Nocardiopsis sp.* Mit 6 maximum production of enzyme was shown during the late phase of growth [19,20]. The result reveled that salt tolerant actinomycetes required at least one week for maximum production.

The *Nocardiopsis xinjiangensis* [21] and *Nocardiopsis halotolerance sp. nov.* [22] were isolated from the saline habitats and grew at 0-10% w/v NaCl and pH 7-10. *Nocardiopsis sp.* Mit 6 was able to optimally grow at 10% salt and pH 8. The enzymes from the saline habitats have great significance applications in food, leather and pharmaceuticals industries.

Nocardiopsis sp. 7326 and *Nocardiopsis aegyptia sp.* grown at 35° C and 25° C pH 8 and pH 5 respectively. Both the strain also reported with amylase production [23]. Similarly, *Nocardiopsis sp.* Mit 6 strain in the present study, produced maximum amylase at 28° C under the alkaline conditions.

The effect of different carbon sources on amylase production was shown on *Nocardiopsis sp.* for maltose, lactose, and glucose. The results revealed that the addition of a glucose and maltose induced both growth as well as α-amylase production. The positive effect of inositol and starch or maltose on the amylase production by *Bacillus stearothermophilus* and *Thermoactinomyces vulgaris* has been reported. The repression of the enzyme secretion studied for monosaccharides and disaccharides suggested that different concentrations of maltose and glucose into the basal medium play an important role.

Bacillus subtilis JS-2004 strain was studied for α-amylase production in waste potato starch liquid medium. Optimum enzyme production was reported with 72 U/mL activities after 48 hrs at pH 7.0 and 50° C calcium and yeast extract acted as an inducer for growth and α-amylase production, but a strong repression effect was observed with the presence of 1.0% glucose [24]. The *Bacillus subtilis* isolate secreted the α-amylase at 37° C with pH 7.0. The glucose acted as a repressor in the culture medium and enzymes activity was decreased [25]. In our study, enzyme activity was repressed with 1.0% with glucose and maltose.

Further repression and purification studies are based on the potential of *Nocardiopsis sp*. Mit 6 for amylase production. Amylase production was repressed gradually with the addition of maltose ad glucose as carbon sources along with a combination of (Glucose + Maltose) in starch contained medium growth was quite stable and enzyme production was repressed. The results are quite interesting in the view of the fact that actinomycetes from saline habitats are explored only in limited sense and many features of their enzymes are quite suitable for wider applications.

5. CONCLUSION

Mit 6 belonged to *Nocardiopsis sp*. with different base pairs. The isolates were screened for extracellular amylases in the range of 5-15% NaCl and pH 5-10. Carbon sources, salts effects were investigated for amylase production. To evaluate the effect of monosaccharide and disaccharide on α-amylase production was chosen as additional carbon sources, and the results indicated that it may be a good stimulator for α-amylase production in *Nocardiopsis sp*. Mit 6. Glucose and maltose repression of amylase biosynthesis as part of their adaptation to growth on recalcitrant, heterogeneous mixed polysaccharides. The results discussed above assess the recent outlook of research are quite interesting in the biotechnology view of actinomycetes from saline habitats.

6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

7. ACKNOWLEDGEMENTS

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