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FULL LENGTH ARTICLE

Amylase activity of aquatic actinomycetes isolated from the sediments of mangrove forests in south of Iran

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Abstract In this study amylase producing actinomycetes were isolated from the sediments of mangrove forests in the south of Iran and the rate of amylase activity was measured. The samples of sediments were collected from one hundred different places in mangrove forests of the south of Iran. Collected samples were diluted then they were purified on the starch (casein agar) culture and Woodruff. After that they were examined in terms of amylase production on agar–starch culture. The activity of the produced amylase by the isolated aquatic actinomycetes was measured by dinitrosalicylic acid (DNS) method. The results showed that aquatic actinomycetes were isolated from 86 per 100 places in spring (86%) and from 61 per 100 places in summer (61%). The highest rates of producing enzyme were related to isolated samples in spring (62.97 U/ml). Biochemical and Bergey's book tests showed that the most isolated aquatic actinomycetes belonged to *Streptomyces* genus. As regards this, it is economical and easy to isolate the aquatic actinomycetes which produce amylase that is used in different industries in Iran from the sediments of mangrove forests of the south of Iran. So the isolated strains in this study can be suitable candidates for amylase production after genetic manipulation.

Introduction

Actinomycetes are Gram positive and filamentous bacteria which are found freely or saprophytically in different habitats such as soil, warm water, marine sediment. They include important genera such as *Micromonospora*, *Nocardia* and *Streptomyces* whose large genomes enable them to produce some types of secondary metabolites, antibiotics, industrial enzymes, antitumor materials, nutritional metabolites and pesticides (Anderson and Wellington, 2001; Salami, 2004). Recent studies have shown that the aquatic actinomycetes can be a good and important source to discover new biological products such as industrial enzymes for example; amylase is produced too much by the aquatic actinomycetes (Lam, 2006; Ramesh and Mathivanan, 2009). Amylase is the enzyme which hydrolyzes starch. It is used to convert starch to glucose and in paper, textile, alcohol (from starch), nutritional and detergents industries (Upadek and Kottwitz, 1997; Van der Laan and May, 1995). Nowadays it is applied in medicine, biotechnology

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and chemistry, too (Kiran et al., 2004). The most important bacterial genera that produce amylase are *Bacillus*, *Streptomyces*, *Micrococcus*, *Pseudomonas*, *Arthrobacter*, *Escherichia*, *Proteus* and *Serratia* (Van der Maarel et al., 2002; Shafiei et al., 2011). One of the important fungi producing amylase is *Paecilomyces* which produces an amylase resistant to heat (Van der Laan and May, 1995; Michelin et al., 2010). Amylases are produced by some genes. This gene in *Bacillus* has 1948 bases and coded a protein with 483 amino acids with molecular weight of 5520 Da (Nakajima et al., 1986a,b). Quantity of amylase that is used in different industries in Iran is about one hundred ton in a year and all of them are imported from other countries (Moghbeli and Noshiri, 2009). Until now, no extensive studies have been conducted about the aquatic actinomycetes and their ability to produce industrial enzymes in Iran. Iran has various seasons so it is possible to isolate different strains of aquatic actinomycetes (Dehnad et al., 2009). The mangrove forests ecosystem grows mainly in the south of Iran in tropical and semitropical coast (Amigues et al., 2002). Because of continuing impact of tides these areas are appropriate for growing different microorganisms (Goodfellow and Williams, 1983). In attention to the area of mangrove in the south of Iran and convenient isolation of aquatic actinomycetes which can produce amylase that is important for different industries. The aim of this project was the isolation of amylase producing aquatic actinomycetes from mangrove forests in the south of Iran during the time period of spring and summer and measured the rate of amylase production.

Materials and methods

Sampling

Samples were collected from depths of 1–10 cm from 100 different places in mangrove forests, Nayband gulf, 320 km in the southeast of Bushehr in spring and summer year 2014. Then they were placed in sterile plastic containers (Selvin et al., 2009).

The sediment sample cultured

One Gram of transferred sediments to the lab was suspended separately in 9 ml of sterile saline solution (0.9%) physiological serum and after ten minutes when heavy particles precipitated the supernatant was diluted to 1/10,000 with sterile physiological serum. In the next step 100 μL of diluted solution with the ratio1/100, 1/1000 and 1/10,000 was cultured in the starch–casein–agar (soluble starch 10 g, vitamin free casein 0.3 g, KNO_3 2 g, NaCl 2 g, K_2HPO_4 2 g, $MgSO_4{\cdot}7H_2O$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO4{\cdot}7H_2O$ 0.01 g, agar 20 g, D.W 1000 ml, pH 7.0 ± 0.2) culture (Ramesh et al., 2006).

Isolation and purification of actinomycetes colonies

The agar plates were investigated in terms of colony appearance, colony color, and conidia type (spore configuration) after incubation. Then each selected colony was cultured separately and linearly in starch casein–agar and woodruff to purify the isolates (Ramesh and Mathivanan, 2009) and selected isolates were further evaluated using biochemical tests and Gram staining.

Screening the amylase production of the isolates

Isolated aquatic actinomycetes was grown on starch–agar (6 g/L bacteriological peptone, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.5 g KCl, 1 g starch, 10 g agar, 1000 ml D.W). Then it was assessed by the povidone–iodine solution halo of the analyzed starch after heating at 28 ± 2 °C in an oven for 24 h and the colonies with bigger halos were selected for the next step (Nakajima et al., 1986b).

Amylase activity

The amylase activity was measured by dinitrosalicylic acid (DNS) method (Harley and Prescott, 2002). For this purpose equivalent concentration to half McFarland was prepared from new cultured aquatic actinomycetes in the special culture medium for amylase production that was concluded: 6 g/L bacteriological peptone, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.5 g KCl and 1 g starch. The concentration of bacterial suspensions was prepared as half McFarland in order to decrease the possibility of error in this step. Fermentation was implemented at 28 °C in 150 rpm for 24 h. 1.5 ml of the culture broth was centrifuged in 4500 rpm at 4 °C for 10 min after fermentation (Amoozegar et al., 2003) and one milliliter of the supernatant was transferred to a new sterile test tube. Enzymatic reaction was initiated by adding one milliliter of 1% (w/v) starch solution at 40 °C for 30 min. The reaction was stopped by adding one milliliter DNS and absorbance was measured in 540 nm (Smit et al., 1996; Mosbach, 1976). Exact of 0.5 ml of the solution containing enzyme was put under boiling temperature to make inactive the enzyme in order to prepare the blank solution and then all the steps of enzyme measurement (DNS method) were performed according to the sample. The above mentioned tests were carried out in triplicate for all samples.

The standard curve of the amylase activity was drawn by diluting glucose, DNS method and reading the absorption in 540 nm in order to find the rate of the amylase activity of the isolated samples (Godfrey and West, 1996). That is why different glucose concentrations were used (Table 1) and by virtue of DNS method the wavelengths were examined in 540 nm and standard curve of amylase enzyme activity was drawn. The tests were conducted three times to draw the glucose standard curve.

Identification of the isolate

The genus of the isolated aquatic actinomycetes which had the ability to produce amylase was identified according to the

Table 1 Glucose absorbance in 540 nm.

Concentration of glucose (g/L)	Absorbance in 540 nm
5	0.215
20	0.476
35	0.682
50	0.883
65	1.04
80	1.32
95	1.39
110	1.68