

Isolation, characterization and optimization of amylolytic *Serratia liquefaciens* from municipal sludge

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Abstract

Amylases are one of the most important industrial enzymes with wide variety of applications ranging from conversion of starch to sugar syrups, food, feed fermentation, textile, detergent and paper industries. In present investigation study, amylase producing bacterial strain was isolated from municipal sludge sample and was identified as *Serratia liquefaciens* on the basis of morphological, biochemical and cultural characteristics. Amylase activity was optimized at different parameters such as pH, temperature, carbon and nitrogen sources. The results reveal that optimum pH and temperature for maximum amylase activity was 7 and 37°C respectively whereas the highest activity was observed when fructose as carbon and tryptone as nitrogen source.

INTRODUCTION

The enzymes can serve as an important molecular device for bioconversion of hazardous waste into bio-friendly compost which can be used to fertilize the soil, without affecting the ambiance. When the most remarkable characteristics of enzyme that is catalytic power and specificity taken into account; amylase is one of the important biocatalyst (Samanta *et al.*, 2013).

Amylases are enzymes that hydrolyze starch molecules to generate progressively smaller polymers composed of glucose units (Windish and Mhatre, 1965). Today, a large number of microbial amylases have almost completely replaced the chemical hydrolysis of starch. The main advantage of using microorganisms for the production of amylase is the ability to bulk produces the enzyme and the easy manipulation of microbes to achieve enzymes with desired characteristics. Moreover, the

stability of microbial amylases is higher than those of plant and animal (Tanyildizi *et al.*, 2005).

Wastewater is generated by residential, institutional, commercial and industrial establishments and includes household liquid wastes from baths, toilets, kitchens and sinks that are disposed off via sewers (Sonune and Garode, 2015). The main organic contaminants in municipal wastewater are polysaccharides, proteins and lipids. The bacteria present in such wastewater have potential to produce the enzymes which are required for hydrolysis of these organic materials and use this material as a food or nutrients. Such microbes may be used for bioremediation of municipal wastewater as well as for enzyme production (amylases, proteases, lipases etc.). The aim of the present study was to isolate and identify the amylase producing bacterial isolate from municipal sludge and to optimize amylase activity for various parameters.

MATERIALS AND METHOD

Sample collection and isolation of bacteria: The sludge sample was collected from main drainage from Nanded city by standard procedure given in APHA and was subjected for serial dilution. The last dilution of sample was spread on nutrient agar plates by spread plate method. The plates were kept for incubation at 37°C for overnight for 24h. The bacterial isolates from plates were purified and subcultured on nutrient agar slant and maintained at 4°C in refrigerator for further study.

Screening of amylase producing bacteria: All purified bacterial isolates were streaked on the starch agar plate and incubated at 37°C for 48 hours. After incubation iodine solution was flooded with dropper for 30 seconds on the starch agar plate. The clear zone of hydrolysis around the colony indicates positive result.

Identification of bacterial isolate: The bacterial isolate which showed maximum zone of hydrolysis was examined for morphology characters (Gram staining, spore staining, motility) and biochemical tests such as Indole production, methyl red, Vogues Proskauer's, citrate utilization, triple sugar iron, nitrate reduction, urease, catalase, oxidase, sugar fermentation such as glucose, lactose, mannitol, sucrose and trehalose (Holt, 1994).

Enzyme production and assay: For enzyme production, nutrient broth was inoculated with a single isolated colony and incubated for 24 h at 37°C with continuous shaking on a rotary shaker at 150 rpm. From this, 1 ml (1%) of inoculum with optical density 0.5 at 600 nm was transferred to 100 ml sterile nutrient starch broth in 250 ml Erlenmeyer flask. The incubation was carried out on a rotary shaker at 150 rpm, 37°C for 48 hr. The bacterial cells were removed by centrifugation at 4000 rpm for 10 minutes at 10°C in cooling centrifuge and the supernatants were used as crude enzyme for determination of enzyme activity. The estimation of amylase activity was carried out according to Miller (1959) method.

Optimization for amylase production: The effect of different parameters on the amylase production by the isolate was optimized for various conditions such as pH, temperature, carbon and nitrogen sources. The effect of pH for amylase production was determined by culturing the bacterial isolate in the production media with different pH such as 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. The enzyme assay was carried out after 48 hours of incubation. The effect of temperature on amylase production was studied by the incubating the culture media at various temperatures such as 25, 30, 37, 45 and 55°C. The enzyme assay was carried out after 48 hours of incubation.

The composition of media greatly affects the growth and production of extracellular amylase production in bacteria (Srivastava and Baruah, 1986). In present study, the effect of different carbon sources such as glucose, lactose, fructose and sucrose were studied by replacing the original carbon source of the medium with equivalent carbon amount (2%) of each of the tested carbon sources. Similarly, different organic (yeast extract, tryptone and beef extract) and inorganic (potassium nitrate) nitrogen sources were studied by replacing the original nitrogen source of the medium with equivalent nitrogen amount (0.6%) of each of the tested nitrogen sources.

RESULTS AND DISCUSSION

In present study, total eight amylase producing bacterial isolates were screened from sludge sample. Among them, culture No. 8 showed highest zone of clearance on starch agar. Hence, this isolate was used for further study. On the basis of morphological, cultural and biochemical characteristics, the isolate was identified as *Serratia liquefaciens*. Similar finding was also reported by Garode and Sonune, 2015. There were previous reports on isolation of enzyme producing bacteria from wastewater (Garode and Sonune, 2013; Garode and Sonune, 2014).



Fig. 1: Zone of hydrolysis after 72 hr incubation by culture No.8 isolate

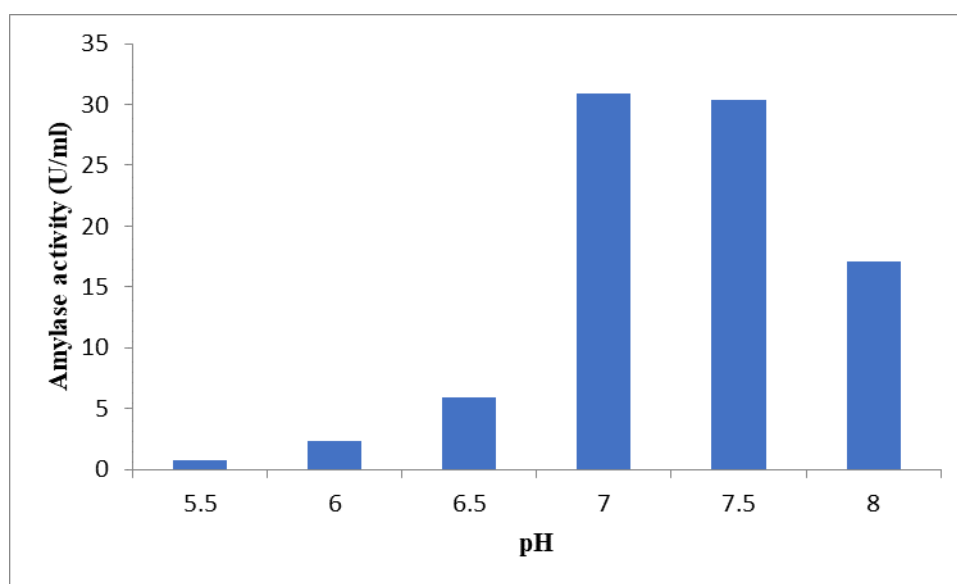


Fig. 2: Effect of pH on amylase activity (U/ml)

Medium optimization for maximum enzyme production is a most important step for its commercial usage. In present study (Fig. 2), it was observed that as pH increased from 5.5 to 7, the activity was also increased. The maximum amylase activity was found to be 30.91 U/ml at pH 7 while further increased in pH the amylase activity was significantly decreased. It may be due to denaturation of protein above optimum pH. Similarly, Vijayalakshmi *et al.*, (2012) reported highest amylase activity at pH 7 for *B. subtilis*.

Temperature is one of the most important factors that controls the growth and production of metabolites by microorganisms. The present results

(Fig. 3) showed that, the highest activity was observed at 37°C (66.81 U/ml) while further increased in temperature showed reduction in amylase activity. Aiba *et al.* (1983) reported that high temperature may inactivate the expression of gene responsible for the starch degrading enzyme. Dash *et al.*, (2015) reported highest amylase activity at 37°C for *B. subtilis*. The effect of different carbon sources suggest that α -amylase was an inducible enzyme and gets induced in the presence of carbon sources (Fig. 4). In present investigation, four sugars (glucose, fructose, lactose and sucrose) were used for study.

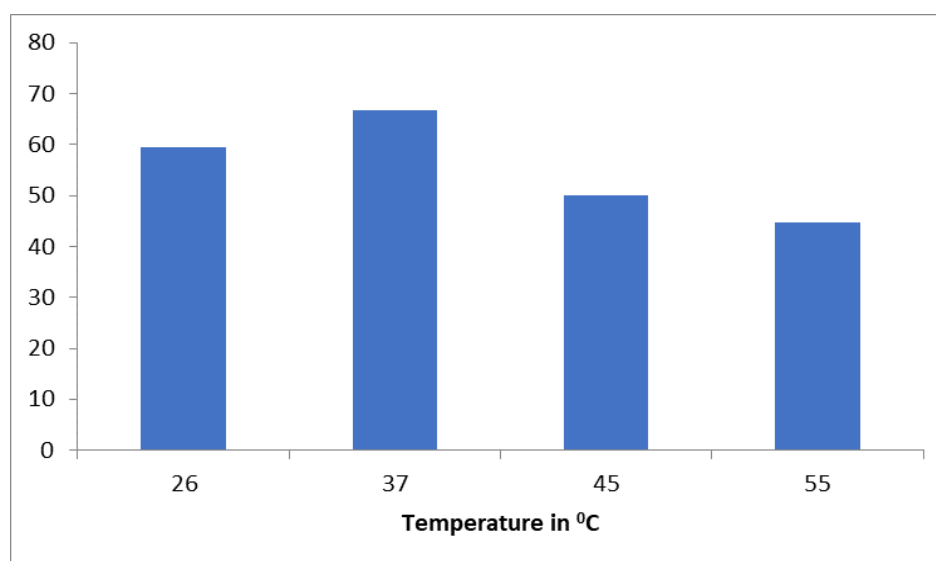


Fig. 3: Effect of temperature on amylase activity (U/ml)

The results showed (Fig. 4) that fructose showed highest amylase activity (46.72 U/ml) followed by glucose (45.87 U/ml) and lactose (44.48 U/ml) whereas lowest activity was observed in case of sucrose (44.19 U/ml). Dharani Aiyer, (2004) reported high yield of amylase in media containing fructose as sole carbon source whereas Salva and Moraes (1995) reported highest activity by *B. subtilis* when glucose as carbon source. Similarly, three organic nitrogen sources (yeast extract, tryptone and beef extract) and one inorganic nitrogen source (KNO_3) were used for study (Fig. 5). Among the organic nitrogen sources, tryptone (42.91 U/ml) proved to be the most suitable followed by yeast extract 24.92 U/ml and beef extract 22.52 U/ml as compared to the inorganic N_2 sources i.e KNO_3 (18.28 U/ml). It has been

previously found that organic nitrogen sources usually have stimulating effects (Hewitt and Solomons, 1996) and our findings are similar to them.

CONCLUSION: In present study, total eight bacterial isolates were screened for amylase production from municipal sludge sample. Out of them, one isolate showed highest zone of clearance on starch agar plate was identified as *Serratia liquefaciens*. *Serratia liquefaciens* was optimized for some parameters such as pH, temperature, carbon source and nitrogen source for amylase production. The present results showed that the maximum activity of amylase was found to be at pH 7, 37°C temperature, fructose and tryptone as carbon and nitrogen source respectively.

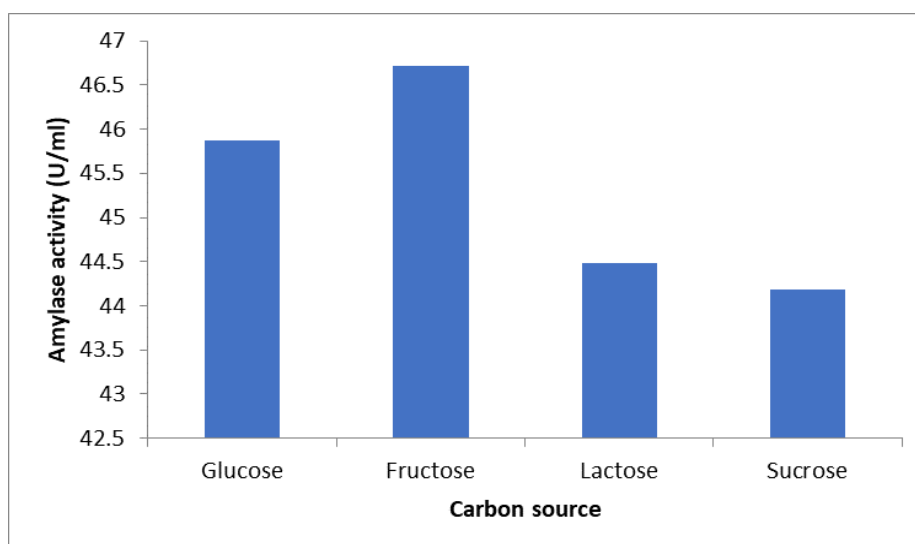


Fig. 4: Effect of carbon sources on amylase activity (U/ml)

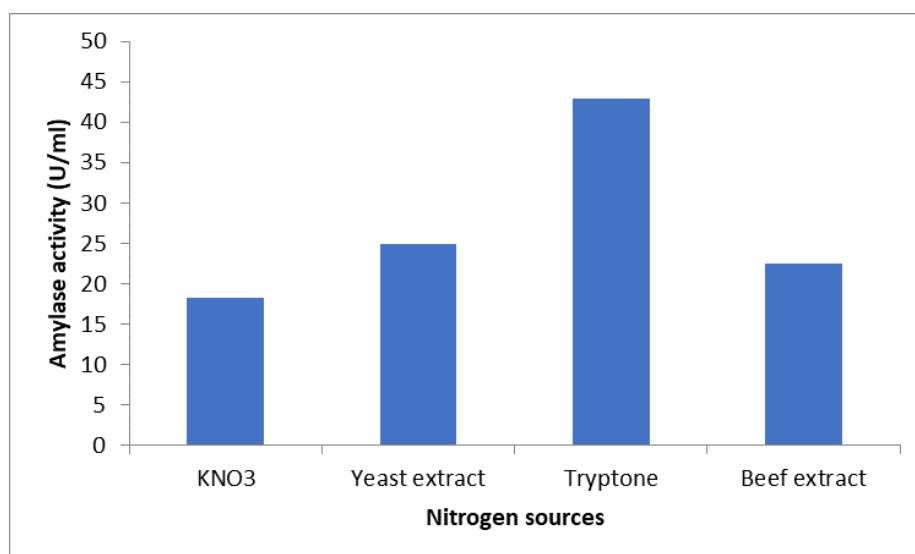


Fig. 5: Effect of nitrogen sources on amylase activity (U/ml)

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