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Isolation of amylase producing bacteria from the gut of *Labeo rohita*

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Abstract- In the past few years, advancement has clearly been observed in the field of medicine and life sciences in which microbiology has played a crucial role in the elevation of therapeutic industries, food industry and many more. In the present investigation, bacteria was isolated from the gut of *Labeo rohita* by spread plate technique using NA media and characterised as *Bacillus* sp. The amylase producing property of bacteria in vitro was confirmed by using starch agar media. Amylase is a proteinaceous enzyme that helps in the breakdown of starch, amylose and amylopectin that are nothing but the naturally occurring polymers of alpha-glucose. Isolated bacteria were rod shaped and Gram positive having a considerable starch hydrolysing capacity. The isolated bacteria can be used to produce amylase enzyme which can be utilized in digestive supplements, food, textile, paper and detergent industries.

Key words: Amylase enzyme, Gut bacteria, *Labeo rohita*, Alpha-glucose

INTRODUCTION

Fish body specially the gut remains in symbiotic relationship with beneficial bacteria.[1] These bacteria are termed as probiotics. The beneficial bacteria help its host to digest food by producing digestive enzymes and keep a check on harmful microbes by competitive exclusion.[2]

Digestive tract of fish gets bacteria from its surrounding water and food,[3] but not all bacteria that enter inside can establish themselves in gut. Bacteria that adapt itself for the harsh gut environment survive and others are killed by the digestive enzymes. However, the maximum quantity of the bacteria is found in the intestinal portion and least in the stomach region.[4] High nutrient availability in the intestine makes it a favourable site for bacterial growth.[1]

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Amylase is an enzyme that helps in the breakdown of complex carbohydrate into simple sugar by hydrolysing glycosidic bonds. In humans, it is produced by salivary gland and exocrine part of pancreas.[5] There are numbers of bacteria that have been found having amylase producing capacity like *Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens* that are used for commercial purpose.[6] Amylase enzyme is widely used in digestive syrups, food, textiles, papers, fermentation and detergent industries.[7]

In the present investigation, an attempt has been made to isolate the amylase producing bacteria from the gut of *Labeo rohita* and study the effect of temperature on its growth.

MATERIALS & METHODS

Collection of fish

Healthy *Labeo rohita* fishes were collected from the Fish farmer's training centre, Shalimar, Dhurwa, Ranchi,

Jharkhand. They were then starved for 24 hours in order to clean their intestinal tract.[8]

Collection of bacteria

Body surface of fish was washed with ethanol.[9] The ventral body surface was opened with scissors and gut was removed. 1g of intestinal portion was weighed and homogenised with distilled water.[8] Homogenate was centrifuged and cell free supernatant (CFS) was collected. CFS was serially diluted up to 10^{-6} dilution.[10] 10^{-6} dilution was plated by using spread plate technique on nutrient agar media[11] and incubated at 37° for 24 hours. The random individual bacterial colony was picked and purified by using streak plate technique.[12] Purified bacterial colony was stored in slants at 4°.

Characterization of bacteria

Isolated bacteria were carefully identified using the guidelines available in the Bergey's manual of systemic bacteriology.[13] Gram staining was also performed for identification.

Screening of amylase production

Amylase production was tested by using starch agar media.[14] Isolated bacteria was streaked on starch agar media and incubated at 37° for 48 hours. Then the plate was washed with iodine solution and result was observed.

Effect of temperature on bacterial growth

Temperature is considered as an important factor for bacterial growth. In the present investigation 2-3 colonies of isolated bacteria were inoculated in 50 ml of nutrient broth and incubated at temperature ranging from 25°, 35°, 40° and 50° for 24 hours. Then 1ml of sample was plated from 10^{-6} dilution from each temperature on NA media and incubated overnight at 37°. The bacterial population was then counted and colony forming unit (CFU) per ml of sample was calculated.

RESULTS

Morphologically the isolated bacteria were found having large circular colonies of pale-yellow colour with little raised elevation. The isolate was Gram positive and identified as *Bacillus* sp. [Fig. 1]

The amylase producing capacity of bacteria was performed on Starch agar media which was followed by washing with iodine solution. A clear zone was observed around the bacterial growth [Fig.2]. The clear zone represented that the amylase enzyme was synthesised which was responsible for the breakdown of starch into free glucose. The isolated bacteria were grown at different temperatures ranging from 25° to 50° in which it was observed that the maximum bacterial growth was at 35° which was 43×10^{6} CFU/ml. This means that the optimum temperature for bacterial growth is 35°. [Fig. 3]

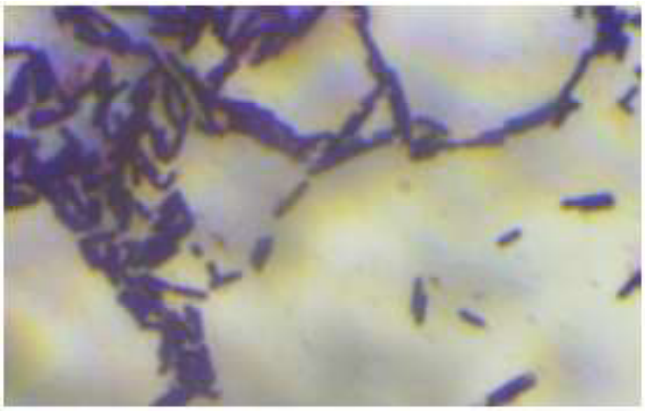

Fig. 1- Gram positive Bacillus bacteria

Fig. 2- Qualitative analysis of amylase enzyme production using Starch Agar Media. Plate showed clear zone around bacterial growth

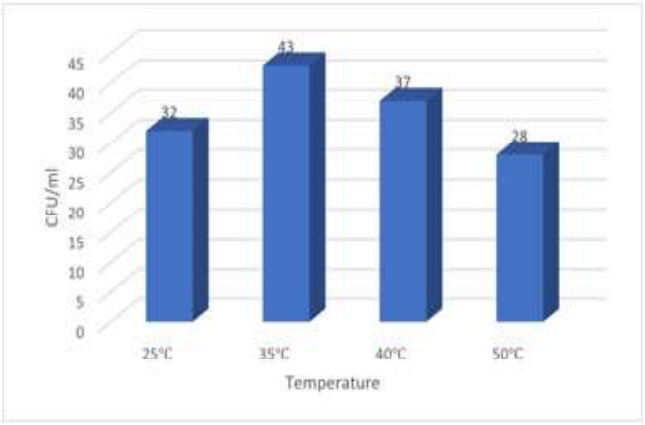

Fig. 3- Effect of Temperature on bacterial growth

DISCUSSION

Gut associated bacteria exhibits a wholesome effect on animals.[15] These bacteria are known as probiotics. In most of the studies in freshwater fish the beneficial gut bacteria were found to be of *Bacillus* sp. like *Bacillus subtilis, Bacillus cereus, Bacillus licheniformis,*[16-18] which shows resemblance with our present study in which isolated bacteria was identified as Gram positive, *Bacillus* sp. having starch degrading property.

Kar *et al.* (2008)[19] suggested that the amylase synthesis is mostly observed in bacteria living in herbivorous animals. Starch is considered as macro molecule made up of long chain of alpha glucose.[20] Amylase producing capacity of isolated bacteria helps in the degradation of starch leading to the formation of free glucose molecules. This was also reported by Ghosh *et al.* (2002)[21]. The release of monomer is due to breakdown of α-1,4- glycosidic bond and α-1,6-glycosidic bond. Glycolysis is the primary machinery for energy production within the cells.[22] As glucose is a simplest energy source that is why many bacterial cells prefer to pick up glucose [23] from the mixture of complex energy sources by the process catabolite repression.[24]

The amylase production was confirmed by the formation of clear zone around the bacterial growth after washing with iodine solution. This is due to the absence of starch at the clear zone due to amylase activity. Apart from isolation and characterization of bacteria having amylase producing capacity, the present study was also focused on observing the effect of temperature on bacterial growth. It was found that optimum temperature of 35^{o} showed maximum growth of bacterial colonies. It was also found that the deviation from the optimum temperature for example 40^{o} or 25^{o} leads to the declination in bacterial count. This shows resemblance with the result of Balaji *et al.* (2012)[9]. Though temperature affects the rate of metabolism and growth of organism, however, Rahman *et al.* (2005)[25] found that the enzyme secretion by bacteria is also dependent on temperature.

From the application point of view, starch hydrolysing property of amylase enzyme makes it very popular among industries that deal with food, paper and textile. Amylase is a major enzyme for carbohydrate digestion in human body. Thus it is also widely used in digestive syrups like Dizicum and Allpeptine. In beer producing industries, amylase is widely used for fermentation process known as brewing. Considering the economic importance of amylase enzyme, the isolated bacteria can be used to produce amylase enzyme in a very fast and cost-efficient manner, which not only provides an alternate way of amylase production but also boosts the economy.

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