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Qualitative analysis of catalase production by the gut bacteria of *Labeo rohita*

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Abstract- Enzyme producing bacteria has become popular among the researchers due to its remarkable participation in the field of textile, food and beverage. Apart from producing beneficial enzymes, bacteria are easy to handle. Persistent and cost-efficient production is possible as well as epigenetic modification can also be performed conveniently. Hydrogen peroxide is a highly reactive chemical compound produced by nearly all organisms that live in the presence of oxygen. This chemical is a Reactive Oxygen Species (ROS) produced during numerous metabolic processes of living cell and can perpetrate oxidative damage to cellular components. To cope up with its harmful effect catalase enzyme plays a crucial role. It converts hydrogen peroxide into water and oxygen and, thus, protects the cells from oxidative damage. In the present investigation an attempt has been made to isolate the catalase producing bacteria from the gut of fish *Labeo rohita*. It is a freshwater herbivore fish commonly found in Indian freshwater ponds, rivers and streams. Bacteria was isolated on NA media and purified by streaking. The bacteria were identified as Gram-positive *Bacillus* species. Catalase producing ability of bacteria was identified in vitro by using the chemical hydrogen peroxide and a positive test result was observed. The optimum temperature for bacterial growth was also identified which was about 40°C. In fabric industry, catalase is used for the removal of excess hydrogen peroxide from fabric. This enzyme is mostly used along with other enzymes in food processing industries. Catalase is extensively used for food preservation. It is also used for elimination of oxygen from wine before bottling. Contemplating the beneficial effects of catalase, the isolated bacteria from the gut of *Labeo rohita* can be used for mass production of this enzyme in a cost-efficient manner.

Key words: Hydrogen peroxide, Catalase, *Labeo rohita,* Reactive oxygen species (ROS), Oxidative damage

INTRODUCTION

Existence of living organisms is a great example of coordination between physiological as well as molecular components. With the advancement of science and technology a meticulous microscopic world came into existence that is microbiology, which is the scientific study of microorganisms. Bacteria, which is one of the class of microorganisms are considered to be cosmopolitan in distribution.[1,2] Not only in the environment, but they are also reported to be found inside the body of living organisms[3] in which some bacteria are harmful but some are found suitable or beneficial.[4]

Gut bacteria of fish have dragged the attention of many researchers and thus various researches have been done in this field. The micro flora of the digestive tract represents a pivotal and diversified enzymatic potential. Ray *et al.* (2012)[5] reported that some fish species accomplish their need of intestinal enzyme from the bacteria inhabiting the gut. Apart from helping in digestion of food, these bacteria also help in the protection of its host body from other pathogens by competitive exclusion.[6]

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Hydrogen peroxide (H_2O_2) is a highly reactive chemical compound widely found in living cells and has been reported to cause oxidative damage to the cell in many ways.[7] This hydrogen peroxide (H_2O_2) can be neutralized by an enzyme called catalase. This catalase enzyme is reported to be produced by some bacteria which are termed as catalase positive. The catalase enzyme which is produced by the bacteria has various industrial significances.[8] In the present investigation an attempt has been made to extract catalase positive bacteria from the gut of freshwater fish *Labeo rohita.*

MATERIALS & METHODS

Healthy *Labeo rohita* fish was bought from the fish farmers training centre, Shalimar, Dhurwa, Ranchi and brought to the laboratory for further investigation. Fish was starved for 24 hrs in order to clear its digestive tract.[9]

Collection of bacteria

Using ethanol, outer surface of fish was washed properly. The ventral portion of fish body surface was opened and gut was removed. Removed gut was washed thrice with distilled water. About 1g of intestine was homogenized in 9ml of distilled water and this was followed by centrifugation. 1ml of cell free supernatant (CFS) was diluted serially using serial dilution method[10] upto 6^{th} dilution. 1ml of 6^{th} dilution was inoculated on NA media plates and incubated at 37°C for 24 hours. Using streak plate technique, a randomly picked bacterial colony was purified.[11]

Identification and characterization of bacteria

Using Bergey's manual of determinative bacteriology[12], the isolated bacteria was identified carefully and further preceded for Gram staining.

Screening of catalyst production

A loop full of isolated bacteria was placed on a clean glass slide. Then 1-2 drops of hydrogen peroxide (H_2O_2) were poured on the bacteria and result was observed.

Effect of temperature on bacteria

Temperature has a significant role in the bacterial growth and metabolism. In the present investigation a loop full of isolated bacteria was inoculated in 50 ml of nutrient broth and incubated at different temperatures in separate test tubes for 24 hours. The temperatures taken were 25°C, 35°C, 40°C, 50°C and 60°C.

After the incubation, each sample was serially diluted upto 6^{th} dilution. 1ml of sample from each test tube, that was kept at different temperatures was taken and spread over NA media plate and incubated for 24 hours. Bacterial colonies were then counted and colony forming unit (CFU) per ml of sample was calculated.

RESULTS

Isolated bacteria from the gut of *Labeo rohita* were analysed. The morphological analysis showed cream coloured, large, circular bacterial colonies. [Fig. 1] The edge of the colonies was also found to be smooth. The isolated bacteria were Gram positive and a *Bacillus* species.

Catalase activity of the isolated bacteria was tested using hydrogen peroxide (H_2O_2). A positive test result was identified due to the occurrence of brisk effervescence when bacteria came in contact with hydrogen peroxide H_2O_2. [Fig. 2]

Survival of the isolated bacteria was also tested at different temperatures. The selected range of temperature was between 25°C to 60°C. After incubation of bacteria at different temperatures, a maximum bacterial growth was observed at the temperature of 40°C, which means that 40°C is the optimum temperature for the isolated bacteria. On the other hand, deflection of temperature from 40°C showed fall in bacterial growth. [Fig. 3]

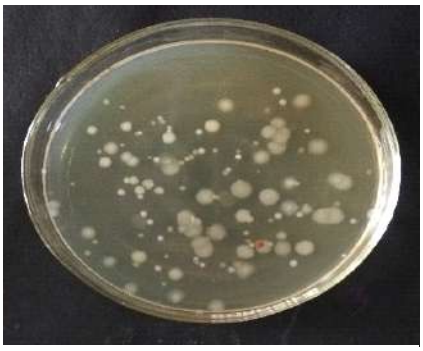

Fig. 1- Bacterial growth on NA media

Fig. 2- Positive catalase test result, Showing brisk effervescence when H_2O_2 was poured on loop full of bacteria

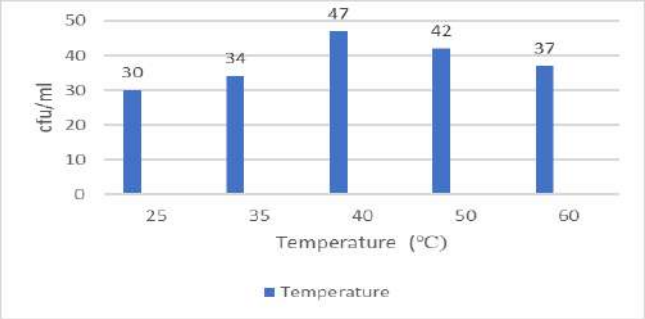

Fig. 3- Effect of temperature on bacterial growth

DISCUSSION

Enzymological and growth studies have revealed the enzymes producing ability of many bacteria. These enzymes are responsible for digestion and providing immunity to its host. Recent studies have also put a light on the capability of bacteria for utilizing Reactive Oxygen Species (ROS), like hydrogen peroxide (H_2O_2), and for its signalling and survival.[13] Reactive Oxygen Species (ROS) are a group of molecules that are produced in the cell through metabolism of oxygen. Endogenous Reactive Oxygen Species (ROS) such as hydrogen peroxide (H_2O_2) have been recognized as a destructive molecule.[14]

Watt *et al.* (2004)[15] have documented that hydrogen peroxide (H_2O_2) causes toxic effect in many ways, some of which are lipid peroxidation and corrosive damage. However, in 1970s, a small collection of studies reported that exogenously added hydrogen peroxide (H_2O_2) could mimic the signalling activity of some hormones.[16] Gough and Cotter (2011)[14] have also reported that hydrogen peroxide (H_2O_2) can modulate enzyme activities by altering its working mechanism which is known as oxidative modulation.

Catalase is an enzyme that is also found in biological systems. It actually catalyses the hydrogen peroxide (H_2O_2) decomposition which ultimately converts it into non-toxic compounds i.e. water and oxygen. It is a well known enzyme for protecting cell from oxidative damage.[17]

$$2H_2O_2 \xrightarrow{\text{Catalase enzyme}} 2H_2O + \underset{\text{Air bubbles}}{O_2\uparrow\uparrow}$$

Whitten bury (1964)[18], stated that bacteria like *Bacillus*, *Leuconostoc*, *Streptococci* and *Pediococci* have the capability of producing catalase enzyme. This shows resemblance with the result obtained in present investigation in which the isolated bacteria was found to be a *Bacillus* species which is also catalase positive.

So far, the reaction velocity is concerned; it has extremely high turnover number which means one molecule of catalase can convert millions of hydrogen peroxide (H_2O_2) molecule into water and oxygen per second.[19,20] This shows resemblance with the present experiment in which brisk effervescence was observed which is the indication of high reaction rate of hydrogen peroxide (H_2O_2) and catalase.

Catalase enzyme has a great role in protecting the cells and other biological components from damage. However, Zamocky *et al.* (2008)[21] opined that only selective organisms have catalase producing ability. In some studies, it has also been reported that catalase activity is acid resistant, which means catalase activity will not get affected by stomach acid if the bacteria is fed as probiotic.[18,22]

On the other hand, Kirchman *et al.* (2005)[23] opined that temperature plays a significant role in the growth and metabolism of bacteria. Membré *et al.* (2005)[24] added that effect of temperature can vary from species to species of microbes. On examining the effect of temperature on the isolated bacteria, it was observed that 40°C was the optimum temperature for its growth. So far, the commercial aspect is concerned, catalase enzyme has a significant role in textile industries as an agent for removing excess hydrogen peroxide from fabric. Catalase is also enormously used for food preservation. It is also in use for elimination of oxygen from wine before bottling.[25,26]

Considering the multiple benefits of this catalase enzyme and due to the easy handling of bacteria, the isolated bacteria can be used for mass production of enzyme catalase in cost efficient manner.

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