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Isolation, Extraction and Industrial Application of Catalasefrom Various Samples

Jhanvi D. Bhatt*, Rushita Gandhi, Arpita Damor, Manisha Baranda, Shah Rupal K.

Department of Microbiology and Biotechnology, School of Sciences, Gujarat University, Ahmedabad,Gujarat.
E-mail Id-Jhanvi.bhatt21@gmail.com

*Corresponding Author

Abstract

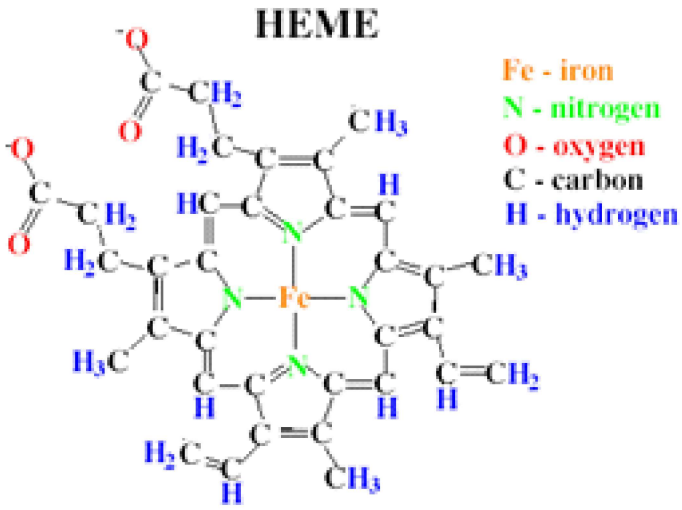
Catalase is an oxidoreductase enzyme that contains tetrameric bifunctional groups which catalyze conversion of toxic hydrogen peroxide into gaseous oxygen and water. Because of this ability, it holds an important plant in different fields such as dairy industry in cheese ripening process, textile industry for removal of bleaching agents, in cosmetic industry as well as food industry as antioxidant and also in paper industry for treatment of pulp. The enzyme has highest turnover of all the enzymes known, that is one molecule of catalase can convert 40,000 molecules of H_2O_2 into water and molecular oxygen. In the present study, isolation of bacteria producing catalase was carried out from various samples, extraction of the enzyme was done, the enzyme activity was checked, production was carried out in a specific medium and the practical applications of this enzyme from the isolates were shown.

Keywords :Bleaching,Repairmechanism,Identification, Shimazdu u.v. spectrophotometer.

Introduction

Catalase is an ubiquitous antioxidant enzyme that degrades H_2O_2 into H_2O and O_2. Various organisms constitute to this enzyme to defend themselves from toxic effects of $H_2O_2$and against oxidative stress. It is a significant component of the cell defense mechanism against oxidative stress, as it scavenges hydrogen peroxide to oxygen and water. All aerobic microorganisms have evolved complex inducible repair mechanisms in the form of this enzyme, to alleviate the damaging effects of active oxygen." Catalase" term was coined by Oscar Loew in 1900 .The enzyme also finds application in textile and food industries were H_2O_2 removal is a neccesity.Catalase are of three types.Catalase contain heme group in their structure and are called as heme catalases.Catalase Peroxidase contain both peroxidase and catalase. Mn catalase contains Mn in place of Fe in its catalytic center,so they are called pseudo catalase.Catalase has been employed in various analytical

and diagnostic methods in the form of biosensors and biomarkers in addition to its other applications in textile , paper, food and pharmaceutical industries.The references used for this research are described below at the end of this article.



Materials and Methods

Water samplescollection :-Effluent or Marine water collected aseptically with optimized parameters such. as temperature and ph.[Samples were collected from R.O. Water treatment Plant,Narol {pH -7.26 and temperature – 29.4 ·C } ;Marine sample from Kavi Kamboi,Bharuch,Gujarat,Sewage effluent sample from Naroda,GIDC]

Isolation of catalase producing bacteria

Spread plating by using serial dilution blanks of $10^{-6}$of Normal saline , 0.1 ml of culture in the plates from10^{-2}, 10^{-4} and 10^{-6}. Spread plating of serially diluted sample was done on petriplates containing nutrient agar, petriplates were incubated at 37°C for 24 After 24 the petriplates were examined to check the growth of bacteria.

Screening

After isolation the screening was performed by bubble test in which on a clean slide dry slide single colony was placed and a drop of H_2O_2 were placed. If the colonies produced bubbles then it is catalase positive and colonies which does not produce bubble are catalase negative.

Identification

After identifying catalase positive bacterial colonies, each catalase positive colony was further streaked on freshly prepared nutrient medium and then incubated at 37°C for 24 and the identification of positive organisms was done through Hi media biochemical kit.

Cultural and morphological characteristics :-

Identification of catalase positive microorganisms was done based on shape, size, and structure of various inclusions namely spore, phosphate granules, and capsule by methods given below :-

1) Gram staining
2) Spore staining (Bathalomew and Mittwers method)
3) Capsule staining (Hiss method)
4) Metachrome staining (loffler's staining method)

Enzyme extraction:-

From freshly grown active slant an isolated colony was transferred into 100ml conical flask containing 50 ml of broth media and incubated at 30°C in a shaking incubator at 150rpm followed by centrifugation at 10,000 ×g for 15 min at 4°C . The supernatant (extracellular crude extract) was used for enzyme assay .

Enzyme assay :-

Catalase activity was measured spectro-photometrically by monitoring the decrease in absorbance at 240nm through Shimadzu U.V. spectrophotometer caused by decomposition of hydrogen peroxide. The reaction mixture composed of 2.4 ml of 50Mm phosphate buffer ph of 7.0 then 0.5ml hydrogen peroxide was added and 100 **µl** of the extracellular crude extract.

$$\text{Catalase activity (µmole/ml)} = \frac{\text{Decrease in absorbance of hydrogen peroxide at 240nm}}{\text{Molar extinction coefficient of hydrogen peroxide at 240 nm}}$$

Results

The results of isolated colonies observed under microscope for the study of colony characteristics are described in Table 1.

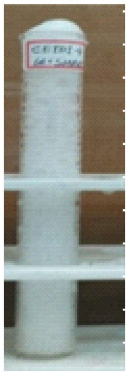
Table 1:-Colony Characteristics of Catalase Positive Organisms

SR NO.	SAMPLE NAME	SIZE	SHAPE	MARGIN	ELEVATION	TEXTURE	OPACITY	PIGMENT	CATALASE REACTION	DILUTION
1	JRE3	Large	Round	Uneven	Flat	Rough	Transparent	nil	positive	10^{-6}
2	JAR1	Small	Round	Uneven	Flat	Rough	Translucent	nil	positive	10-^{6}
3	AM	Large	Round	even	Flat	Smooth	Transparent	nil	positive	10^{-4}
4	CEPTI4	Medium	Round	even	Flat	Rough	Opaque	nil	positive	10^{-4}

Table 2 Morphological Characteristics of the Isolated Positive Catalase Colonies

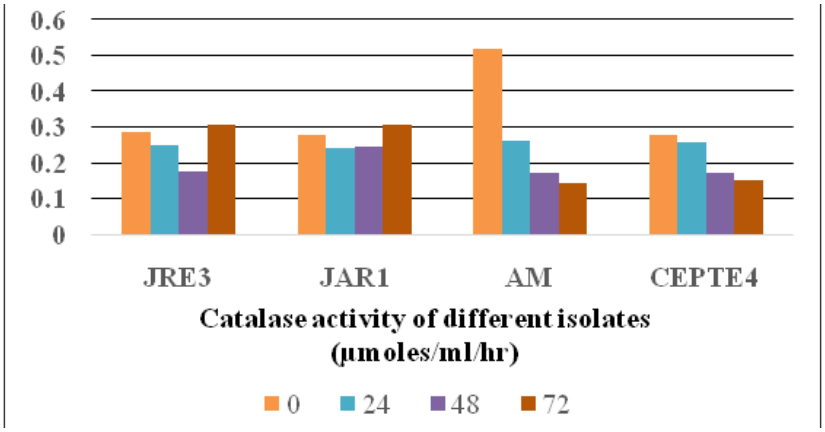
SR NO	CULTURE	GRAM STAINING	KOH TEST	SPORE STAINING	CAPSULE STAINING	METACHROME STAINING
1	JRE3	Gram positive rods,motile	Negative	Positive	Positive	Positive
2	JAR1	Gram positive rods,motile	Negative	Positive	Positive	Positive
3	AM	Gram positive rods,motile	Negative	Positive	Positive	Positive
4	CEPTI4	Gram negative rods,motile	Positive	Positive	Positive	Positive

- **The screening of Catalase positive isolates through slide test and the tube test is shown in Figure 1 and Figure 2 respectively.**



- **The results of enzyme assay of positive isolates based on the standard protocol are representedgraphically in Graph 1**

GRAPH 1:- CATALASE ACTIVITY THROUGH ENZYME ASSAY



Discussion

On the basis of above observations,the colony characteristics as noted on Nutrient agar plate

under 10x magnification of the microscope are noted. Further, it is concluded that the isolates obtained are motile organisms possessing spore, capsule and phosphate granules.Also theyshow optimum catalase activity up to 48 hours which is proved from the results of enzyme assay as shown in the graph. From the qualitative analysis it's depicted that the isolates have high potential of breaking H_2O_2 and thus they can be explored for various industrial applications where breaking of hydrogen peroxide is an utmost necessity.

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