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RESEARCH ARTICLE

Isolation &Identification of Catalase Producing *Bacillus spp:* A Comparative Study

Babiker Mohamed Babiker[1] Mohamed Abd Elmahamoud Ahmed[2] and HananMoawia Ibrahim[3]

1. Alnahda University, Faculty of Pharmacy, Sudan
2. Elrebat University, Faculty of Pharmacy, Sudan
3. Central Lab. Ministry of Higher Education & Scientific Research, Sudan

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Corresponding Author

HananMoawia Ibrahim.

Abstract

Catalase, an oxidoreductase enzyme, works as a detoxification system inside living cells against reactive oxygen species formed as a by-product of different metabolic reactions. This study was carried on 50 samples collected from different locations in Khartoum State for catalase production. Twenty nine out of fifty sample (58%) showed a typical characteristics of Bacillus species, fifteen out of twenty nine (51%) showed catalase positive reaction, Five out of fifteen (33%) gave highest catalase activity using hydrogen peroxide method. The strains with higest activity were identified as B. subtilis, B. pasteurii, B. coagulans, B. sphaericus and B. alvei according to chemical and morphological characteristic. The activity of the enzyme measuring spectrophotometrically according to the decrease in the absorbance of hydrogen peroxide. B. subtilis gave highest catalase activity of 135.2 µM/ml in 48hr. incubation, which could be a promising isolate for catalase production for commercial scale.

Introduction:-

Catalase is a haem-containing enzyme belonging to the oxido-reductase family. It is a significant component of the cell defense mechanism against oxidative stress, as it scavenges hydrogen peroxide to oxygen and water (Foyer and Noctor, 2000). All aerobic microorganisms have evolved complex inducible repair mechanisms, in the form of this enzyme, to alleviate the damaging effects of active oxygen (McCord and Fridovich, 1988). Catalase also plays a role in maintaining redox homeostasis of the cell as a part of the antioxidant response system. Catalase has also 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. New applications for catalases are constantly emerging thanks to their high turnover rate, distinct evolutionary origin, relatively simple and well-defined reaction mechanisms. (Mullineaux et al., 2006). The enzyme is found in a wide range of aerobic and anaerobic organisms. Catalase is used in a wide range of industrial processes and they are important enzymes in the global enzyme market. The industrial enzyme market is now growing to reach from US$ 2.2 billion to US $3.4 billion by 2015. This escalation is due to the emergence of improved production technologies and new applications of engineered enzymes. The demand for industrial enzymes in matured economies such as the US, Western Europe, Japan and Canada has been during recent times, while developing economies of Asia-Pacific, Eastern Europe, Africa and Middle East regions have emerged as the fastest growing markets for industrial enzymes (Baljinder and Munish, 2014). Catalase has also 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. New applications for catalases are constantly emerging thanks to their high turnover rate, distinct evolutionary origin, relatively simple and well-defined reaction mechanisms. Catalases are used in a wide range of industrial and medical processes and they are important enzymes in the global enzyme market. The use of catalase for construction of electro-chemical biosensors has been investigated for a range of applications (Vatsyayan et al., 2010). It is use in many applications such as in medical as inhibition of tumor metastasis, and treatment of catalasemia (catalase deficiency) which linked with diabetes mellitus, and biomedical and clinical diagnosis also in food industry such as

peroxide removal during pasteurization of milk, and detection of calcium in milk and water samples, and prevention of lipid oxidation in uncooked meat and many other pharmaceutical and environmental applications. Hence, the presence of hydrogen peroxide in industrial wastewater constitutes serious disposal problems and requires special attention. An eco-friendly alternative to the aforementioned problem is the development of enzyme based (catalase) bioremedial system which catalysis the degradation of hydrogen peroxide into water and oxygen. The radicalmediated modification of DNA, proteins, lipids and small cellular molecules is associated with a number of pathological processes, including atheroscle- rosis, arthritis, diabetes, cataractogenesis, muscular dystrophy, pulmonary dysfunction, inflammatory disorders, ischemia-reperfusion tissue damage and neurological disorders such as Alzheimer's disease (Frlich and Riederer, 1995). Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in reproductive reactions. Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second (Chelikani et al., 2004). A quick and straight forward biosensor based method for detection of mastitis infection in milk was developed to detect increased activity of catalase in infected milk samples (Futo et al., 2012). Due to unique properties such as region and stereo specificity, biocatalysts play a key role in processes for manufacturing of enantiomeric products with minimum formation of wasteful by-products. Furthermore, microbial enzymes have largely replaced the traditional plant and animal enzymes due to their high activity, rapid multiplication, easy handling and easy genetic manipulations (Puri et al., 2012). The information on the studies of catalase for amphibia has been scarce until now. The present study aims to isolate and characterize of microorganism that gave higher catalase production and to compare between these strains in their catalase production.

Materials and Methods:-

This study (Lab. work) was done at the Microbiology Department / Central Laboratory during May to August 2015.

Soil sample:-

Fifty soil samples from different locations in Khartoum State were collected into sterilized plastic bags and then transferred to labbeled screw bottles. Samples were taken from 15-20 cm depth after removing approximately 3cm of earth surface.

Isolation and identification of microorganism:-

Isolation of microorganism was performed by dilution plate technique (You and Park, 2004). In this technique, 1gm of each soil sample was taken in 9 ml of sterilized distilled water in pre-sterilized test tubes. Serial aqueous dilution were prepared by transferring one ml of each soil suspension into 9 ml of sterilized distill water in sterilized test tubes. One ml (10^{-1} – 10^{-7}) of each soil suspension were applied separately into sterilized Petri-dishes with nutrient agar (which has been prepared by taken 28 g of agar base and poured it into 1 L of water, sterilized using autoclave, poured it into petri-dishes and left to cooled and incubated for 24 hr. at 37˚C and checked it for contamination), after gently rotating, the plates they incubated at 37˚C for 24 hr. Colonies, with *Bacillus* morphological characteristic that appeared in the incubated plates, were repeatedly sub-cultured for further studies. Identification of *Bacillus* species were done according to Bergey's Manual of Determinative Bacteriology (Don et al., 2005).

Enzyme Extraction:-

An isolated colony from a freshly grown slant was transferred into a 100 ml conical flask containing 50 ml of broth media (g^{-1} of distilled water) peptone 8, yeast extract 4, NaCl 2, pH of 7.2 and incubated at 30°C in a shaking incubator at 150 rpm followed by centrifugation at 10,000×*g* for 15 min at 4ºC. The clear supernatant (extracellular crude extract) was used for enzyme assay. (Loewen and Switala, 1987).

Enzyme assay:-

Catalase activity was measured spectro-photometrically by monitoring the decrease in absorbance at 240 nm caused by the decomposition of hydrogen peroxide. The reaction mixture composed of 2.4 ml of 50 Mm phosphate buffer pH of 7.0, then 0.5 ml hydrogen peroxide was added and 100 µL of the extracellular crude extract.

Catalase activity (µmole/ml) =

$$\frac{\text{Decrease in absorbance of hydrogen peroxide at 240 nm}}{\text{Molar extinction coefficient of hydrogen peroxide at 240 nm}}$$

(Anderson et al., 1995), (Paar et al., 2001).
One unit of catalase activity is defined as the amount of activity required to convert 1µ mole of hydrogen peroxide to water and oxygen per minute at 25C.

Result and discussion:-

Screening, isolation and identification of the microorganism:-

In this study fifty presumptive isolates were recovered from different area in Khartoum State, each isolate was given a number. Twenty-nine out of fifty (58%) was determined as Bacillus spp., fifteen out of the twenty nine (53%) Bacillus isolate was found to be catalase positive Fig (1). Five Bacillus spp. isolate that showed strong catalase positive was further identified and characterized according to the manual (Table 1). It revealed that the isolates were differ in their reactions, hydrolysis , their growth in NaCl and 55˚C, indicating that they were different species. Nagy et al. 1997 extract and purified catalase from Mycobacterium tuberculosis. While Blaut, 1998 extracte catalase from Rhodobacter sphaeroides. Brown- Peterson et al., 1995. Purify and characterize a mesohalic catalase from the halophilic bacterium Halobacteriumhalobium.

Fig (1) Catalase +ve

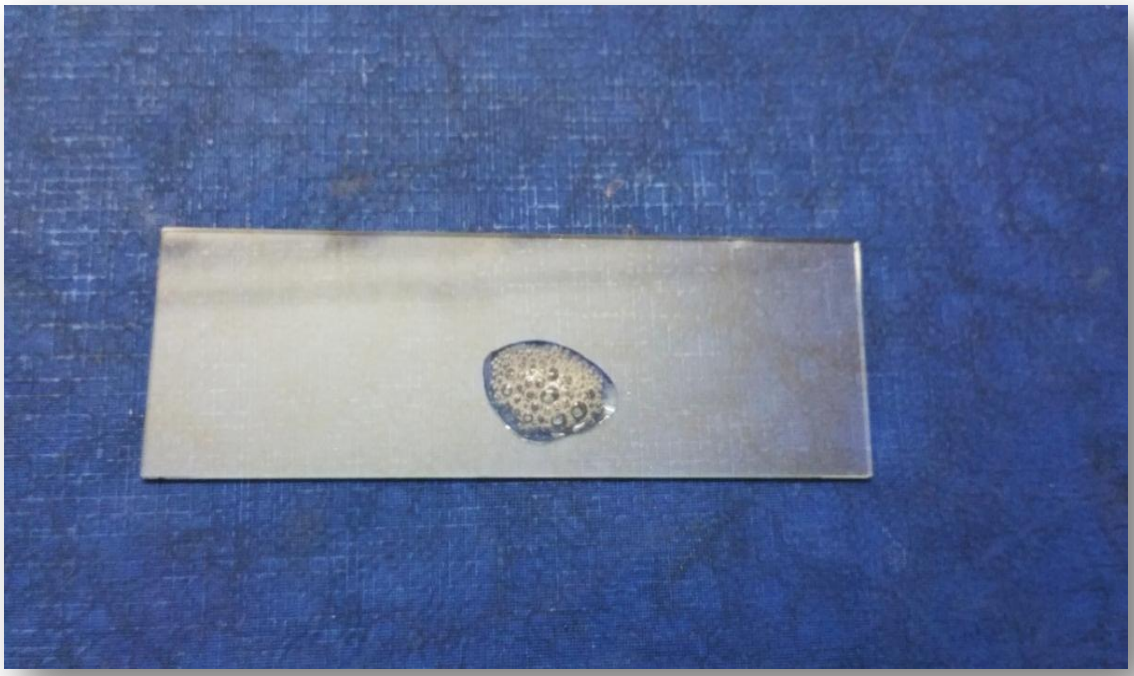

Table (1): Biochemical characteristics of the *Bacillus* isolates:-

Sample Number	Starch hydrolysis	V.P	citrate Utilization	Manitol test	Nitrate reduction	Growth at 6.5% NaCl	Growth at 55˚C
1	+	+	+	-	-	+	-
3	+	+	+	-	-	-	-
9	+	+	-	-	-	-	-
14	+	+	+	-	-	+	-
16	+	+	+	-	-	+	+
18	-	+	-	-	+	-	-
20	+	+	+	-	-	+	+
27	-	-	-	-	-	-	-
30	+	+	+	-	-	+	-
32	+	+	-	-	-	-	-
35	+	-	+	-	-	-	-
37	-	+	-	-	+	-	-
42	+	+	+	-	-	-	-
44	+	+	+	-	-	+	+
48	+	-	+	-	-	-	-

(+) Positive. (-) Negative.

Table (2): Qualitative catalase activity from identified organism:-

Strain no.	Identification	Catalase test
1	*B. subtilis*	+++
14		
30		
3	*B. coagulans*	++
42		
9	*B. alvei*	++
32		
16	*B. licheniformis*	+
20		
44		
18	*B. pasteurii*	+++
37		
27	*B. sphaericus*	++
35	*B. megaterium*	+
48		

+ Mid + + moderate + + + vigorous

Table (2) shows that the identification and characterization of the 15 *Bacillus* species, it revealed that they were 7 types of *Bacillus*, that was: *B. subtilis, B. coagulans, B.licheniformis, B. sphaericus, B. pasteurii, B.megatorium* and *B. alvei*. The highest catalase positive selected were (*B. subtilis, B. pasteurii, B. coagulans, B. sphaericus* and *B. alvei*) respectively for catalase production

Extraction and activity of catalase:

The enzyme was extracted by centrifugation of the fermented broth at 10,000×*g* for 15 min at 4ºC. The clear supernatant (extracellular crude extract) was used for enzyme assay. Table (3) shows that extracellular catalase activity using concentration µmol/ml was measured using molar extinction coefficient for hydrogen peroxide at 240 nm as suggestion by Paar, et al., (2001), (molar extinction coefficient for hydrogen peroxide at 240 nm is 36 $M^{-1}cm^{-1}$). Noticibly the result obtained by *B. subtilis* gave highest extracellular catalase activity compared with the other *Bacillus* at all time incubation 0-72 hr. Extracellular catalase from *B. subtilis* gave enzyme activity of 135.2 µM/ml,

B. pasteurii, of 127.4 µM/ml, *B. sphaericus* of 116.8 µM/ml, *B. coagulans* of 93 µM/ml and finally *B. alvei* of 56.1 µM/ml at stationary phase of 48 hr incubation. Min-Jung, *et al.*, 2004 extract a crude catalase from bull frog liver with total activity of 2.81 U. While Blaut, (1998) isolate and characterize a catalase from the nonsulfur phototrophic bacterium *Rhodobacter sphaeroides.* Brown-Peterson et al., 1995 stated that when subjected to the stress of growth in a relatively low-salt environment (1.25 M NaCl), the halophilic bacterium *Halobacterium halobium* induces a catalase of 0.105 U/ml.

Table (3): Catalase activity using concentration µmoles/ml

Strain	0h	24h	48h	72h
B. subtilis	5.6 µM/ml	122.8 µM/ml	135.2 µM/ml	129.3 µM/ml
B. coagulans	2.8 µM/ml	83.7 µM/ml	93 µM/ml	88.8 µM/ml
B. alvei	2.8 µM/ml	47.4 µM/ml	56.1 µM/ml	53.9 µM/ml
B. pasteurii	2.8 µM/ml	112.6 µM/ml	127.4 µM/ml	117.8 µM/ml
B. sphaericus	5.6 µM/ml	107.3 µM/ml	116.8 µM/ml	111.2 µM/ml

Table (4) shows that extracellular catalase activity was measured spectrophotometrically by monitoring the decrease in absorbance at 240 nm caused by the decomposition of hydrogen peroxide. *B. subtilis* showed highest extracellular enzyme activity of 0.134 at 48 hr incubation, and decrease at 72hr. (Increase as a decreasing rate). Compare with study done by Alex et al., 1999, That catalase was extracted from *Escherichia coli* has been confirmed to be located intracellular, also our result agree with study done by Naclerio et al., 1995 that showed strong catalase activity was secreted by *Bacillus subtilis* cells during stationary growth phase in rich medium

Table (4) Catalase activity using U.V Absorbance at 240 nm

Strain	0 hr	24 hr	48 hr	72 hr
B. subtilis	4.8	0.579	0.134	0.347
B. coagulans	4.9	1.988	1.652	1.802
B. alvei	4.9	3.293	2.982	3.061
B. pasteurii	4.9	0.948	0.415	0.760
B. sphaericus	4.8	1.137	0.794	0.998

Conclusions:-

Fifty soil samples brought from different locations in Khartoum state. 30% of the isolates gave positive catalase test and identified as (*B. subtilis, B. pasteurii, B. sphaericus, B. coagulans, and B. alvei*) they were considered as strong source of catalase. Interestingly maximum catalase activity was observed after 48hr of incubation of the five selected isolates in batch fermentation. Comparing within the five species studied, *B. subtilis* was found to be a potent producer of catalase enzyme of 135.2 µM/ml within 48hr of inoculation. These isolate could produce active catalase for commercial usage and for industrial applications.

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