International Journal of Scientific Research and Management (IJSRM)
||Volume||5||Issue||11||Pages||7449-7455||2017||
Website: www.ijsrm.in ISSN (e): 2321-3418
Index Copernicus value (2015): 57.47 DOI: 10.18535/ijsrm/v5i11.14

Biodegradation of aflatoxin by peroxidase enzyme produced by local isolate of *Pseudomonas* sp.

Ahmed Majeed Abd Zaid

College of science
University of Al-Qadisiyah

Abstract

Twenty isolates of bacterial isolates were screening for peroxidase activity. The isolate 13 was performance higher peroxidase activity among all isolates with activity (23 U/ml). The selected isolate was characterized and identified according to morphological and biochemical tests as Pseudomonas aeruginosa. The optimum pH for peroxidase activity was 6 with activity (42) U/ml. The result showed an increase in the activity at 40°C and peroxidase activity reached to (71) U/ml. The peroxidase from Pseudomonas sp able to degrade aflatoxin B1 after 24h and the maximum degradation (87%) was attained after 72 h at enzyme concentration 71 U/ml**.**

Key words: *Pseudomonas sp., Peroxidase, Biochemical tests, pH, Temperature, Aflatoxin.*

Introduction

Aflatoxins are secondary metabolite produced by the moulds *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxin-producing moulds are widely distributed in nature (Arim, 1995). The main biological effects of aflatoxins are carcinogenicity, immunosuppression, mutagenicity, and teratogenicity. Aflatoxins cause liver damage, decrease milk yield, decrease egg production and overall performance, and suppresses immunity in animals consuming low dietary concentrations (Ramosa *et al*., 1996). Aflatoxin B1(AFB1) is considered the most potent, having hepatotoxic, hepatocarcinogenic, mutagenic, and teratogenic effects in many animal species, and classified as a Group I carcinogen in humans (Tedesco *et al.*, 2004).

Several studies on physical and chemical strategies for the reduction of aflatoxins have been reported (Kabak *et. al*. 2006). Nevertheless, none of these strategies completely fulfils the necessary efficacy, safety and cost requirements (Zhao *et al.* 2011). These disadvantages encouraged recent emphasis on the biological degradation of aflatoxins. Biological detoxification of AFB_1 by fungal and bacterial isolates or their secondary metabolites has been reported, such as *Armillariella tabescens, Pleurotus ostreatus* (Motomura *et al*. 2003).

Specific enzymes that are capable of degrading aflatoxins have been purified from microbial systems. The detoxification by specific enzymes avoids the drawback of using the microorganism, which may, in addition

to its degradation activity, change flavor or impair the nutritional value and acceptability of the product (Wu *et al*., 2009).

Nuclear Magnetic Resonance (^{1}H-NMR) and High Resolution Electrospray Mass Spectrometry (HR-ESI-MS) analysis suggested that AFB1 is first oxidized to AFB1-8, 9-epoxide by peroxidase and then hydrolyzed to AFB1-8, 9-dihydrodiol (Wang *et al.*, 2011).

This study aimed to examine the ability of crude peroxidase from *Pseudomonas* sp. for degradation of aflatoxin B1 (AFB1).

Material and methods

Isolates and chemicals:

Twenty isolates. were obtained from Baghdad university department of biology. The isolates were characterized and identified on the basis of morphological and biochemical tests.

All reagent chemicals were purchased from Hi – Media and Sigma-Aldrich, India.

Production media

The production media that was used for peroxidase production was composed of (g/l): peptones 10, NaCl 5, yeast extract 2, beef extract 1 and agar 3.

Screening of bacterial isolates for peroxidase production

This procedure was down according to Kalyani *et al.*(2011) A loop-full of bacterial culture of *Pseudomonas* sp was inoculated into the production medium and incubated for 48 h at 37°C. The cells were collected by centrifugation at 4,000 rpm for 20 min and suspended in citrate-phosphate buffer (0.1 M, pH 5). The cell suspension was gently homogenized and disrupted by sonication. The homogenate was centrifuged under cold conditions at 10,000 rpm for 20 min, the supernatant obtained was collected and used as a crude source of peroxidase. Then the ability of each isolate for peroxidase production were tested by measure the activity of peroxidase of each isolate.

Peroxidase activity assay

Crude Peroxidase activity was determined spectrophotometrically at a specific wavelength according to Kalyani *et al.*(2011), the increase in absorbance due to the oxidation of a selected substrate. The increase in absorbance was followed at 420 nm in order to monitor the oxidation of guaiacol. Assays were conducted at temperature (30 °C), The reaction mixture contained 1 mM of guaiacol, 10 mM H2O2 and potassium phosphate buffer (pH 6.5). One unit of enzyme activity was defined as the amount of enzyme required to oxidize the 1 µmol of substrate.. Enzymatic activity was determined by measuring the increase in absorbance at 420 nm for guaiacol with a spectrophotometer (UV-9200 , UK).

Determination of optimum pH

The effect of pH on peroxidase activity was determined within a pH range of 1–9 by using guaiacol as a substrate; 0.1 M citrate-phosphate buffer (pH 3.0−5.6) , 0.1 M potassium phosphate buffer (pH 5.8 – 8) and 0.1 M Tris-HCl buffer (pH 9.0) .

Determination of optimum temperature

Optimum temperature of the crude peroxidase was examined over the temperature range of 20–80°C with guaiacol as the substrate at its optimal pH value.

Biodegradation of aflatoxin by crude peroxidase enzyme

The reaction mixture (3 ml) contained phosphate buffer (0.1 M, pH 6), H2O2 (10 mM), aflatoxin B1 (1.33 µg/ml) and peroxidase (71 U/ml). Then the tubes incubated at different times (24hr, 48hr and 72hr) at 30°C in shaking incubator 150rpm.The absorption spectrum of aflatoxin B1(360 nm) was measured using spectrophotometer (UV-9200 , UK). Aflatoxin B1degradation was measured by monitoring the decrease in absorbance at its respective wavelength and expressed in terms of percentage.

Results and discussion

Screening of bacterial isolates for peroxidase production

Screening of bacterial isolates by measuring peroxidase activity. It was found that the isolate 13 produced the highest levels of peroxidase with activity (23 U/ml) fig. (1), so it was selected for further studies.

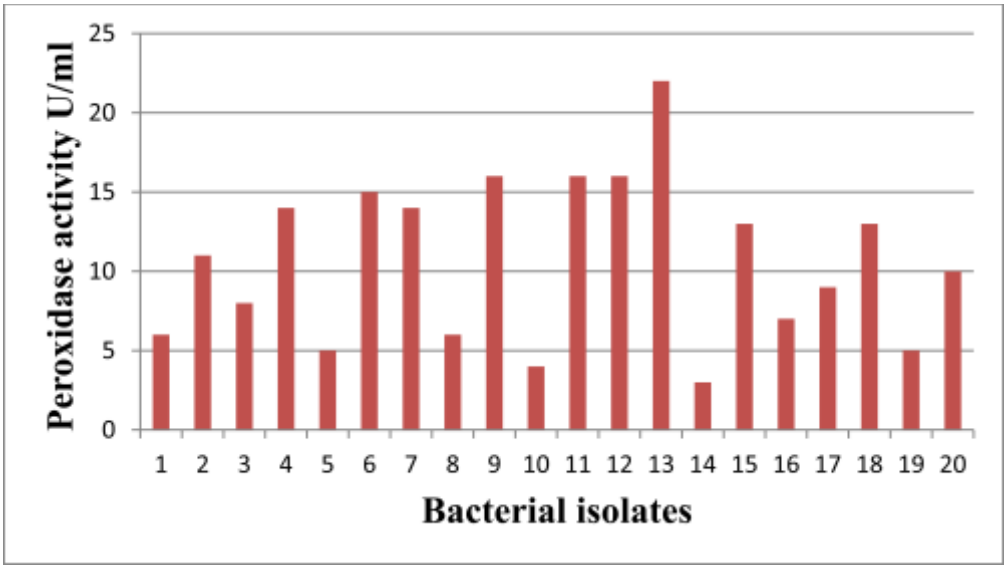

Figure (1): screening of bacterial isolates for peroxidase production

Identification of bacterial isolates

According to the morphological and biochemical tests, the selected isolate with higher peroxidase activity were identified as *Pseudomonas aeruginosa* table (1) and these result are compared with (Dortet *et al.* 2012; Zierdt and Schmidt 1964 ; Bruins *et al.*2004).

Table (1): Microscopic examination and biochemical tests of bacterial isolates

Gram Staining	**Negative**
Shape	**Rods**
Motility	**Motile (Unipolar)**
Capsule	**Non-Capsulated**
Spore	**Non-Sporing**
Catalase	**Positive (+ve)**
Oxidase	**Positive (+ve)**
MR	**Negative (-ve)**
VP	**Negative (-ve)**
Indole	**Negative (-ve)**
Citrate	**Positive (+ve)**
Urease	**Negative (-ve)**
H2S	**Negative (-ve)**
Gelatin Hydrolysis	**Positive (+ve)**
Coagulase	**Negative (-ve)**
Pigment	**Positive (+ve)**
Fermentation of	
Arabinose	**–**
DNase	**–**
Fructose	**–**
Glucose	**Negative (-ve)**
Lactose	**Negative (-ve)**
Maltose	**Negative (-ve)**
Mannitol	**Positive (+ve)**
Mannose	**–**
Ribose	**–**
Sucrose	**Negative (-ve)**

Determination of optimum pH

The optimum pH for peroxidase activity was 6 with activity (42) U/ml, with a decrease in peroxidase activity at the pH value moved towards alkaline range (8.0-90), it was also noticed a decrease in the activity of the enzyme at acidic values (3.0-5.0). However, peroxidase activity was highest at natural pH (6-7) fig. (2). This bacterial peroxidase, which has been reported previously, show maximum activity at pH values close to 3.0 (Dawkar et al. 2009; Ghodake et al. 2009).

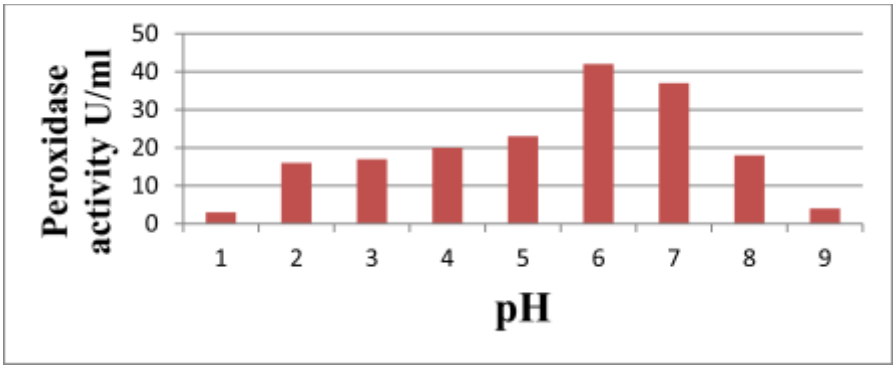

Figure (2) : Effect of pH on peroxidase production

Determination of optimum temperature

The result showed an increase in the activity at 40°C and peroxidase activity reached to (71) U/ml then the activity was declined with increasing temperature up to 40°C, and minimum activity observed at 80°C was (11) U/ml fig. (3). This supports earlier observations that lignolytic enzymes show maximum activity at 40°C (Hossain and Anantharaman 2006).

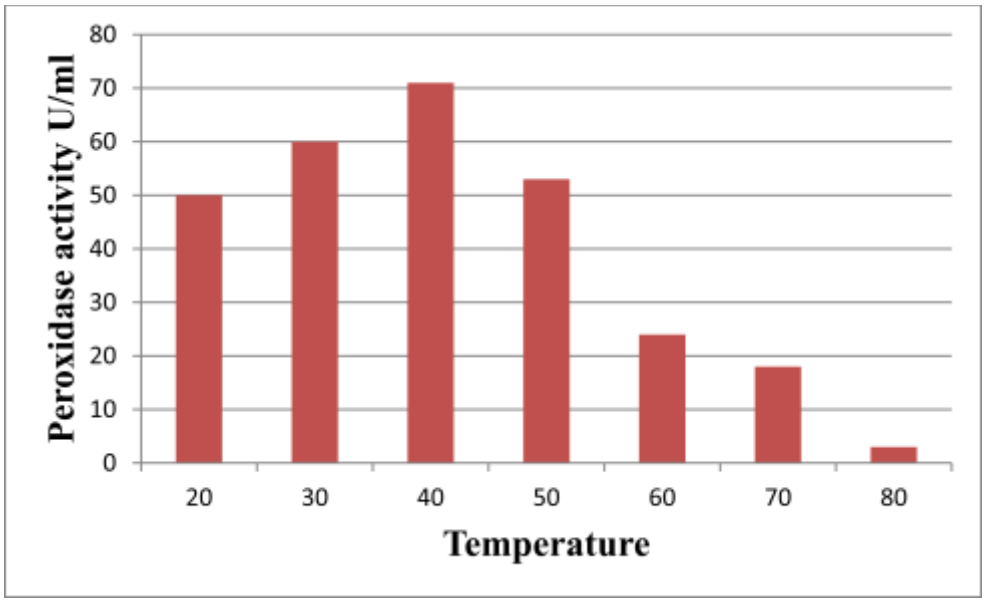

Figure (3) : Effect of temperature on peroxidase production

Biodegradation of aflatoxin by crude peroxidase enzyme

The degradation potentiality of peroxidase to aflatoxin B1 showed that after 24 h the level of AFB1 was reduced to 56% in the presence of 71 U/ml enzyme while the maximum degradation (87%) was attained after 72 h at enzyme concentration 71 U/ml (fig 4). MnP from *Phanerochaete sordida* Yk-624 removed approximately 70% of AFB1 after 24 h and reach to complete detoxification by multitreatment with MnP (Wang *et al.*, 2011). Culture supernatant of *Myxococcus fulvus* was able to degrade AFB1, AFG1 and AFM1 effectively in solution. Significant reduction of AFB1 (71.89%), AFG1 (68.13%) and AFM1 (63.82%) were observed after 48 h treatment with the culture supernatant from strain ANSM068 (Zhao *et al.*, 2010). These findings are in agreement with those obtained by Alberts *et al.* (2006) who reported that AFB1 was effectively degraded by extracellular extracts from *Rhodococcus erythropolis* liquid cultures, with only 33.2% residual AFB1 after 72 h. These results also went parallel with those obtained by Lillehoj *et al.* (1971) who reported that AFM1 (8 μg mL^{-1}) is completely removed from the liquid medium of *Flavobacterium aurantiacum* by incubation with 5×10^{10} resting cells per milliliter for 4 h.

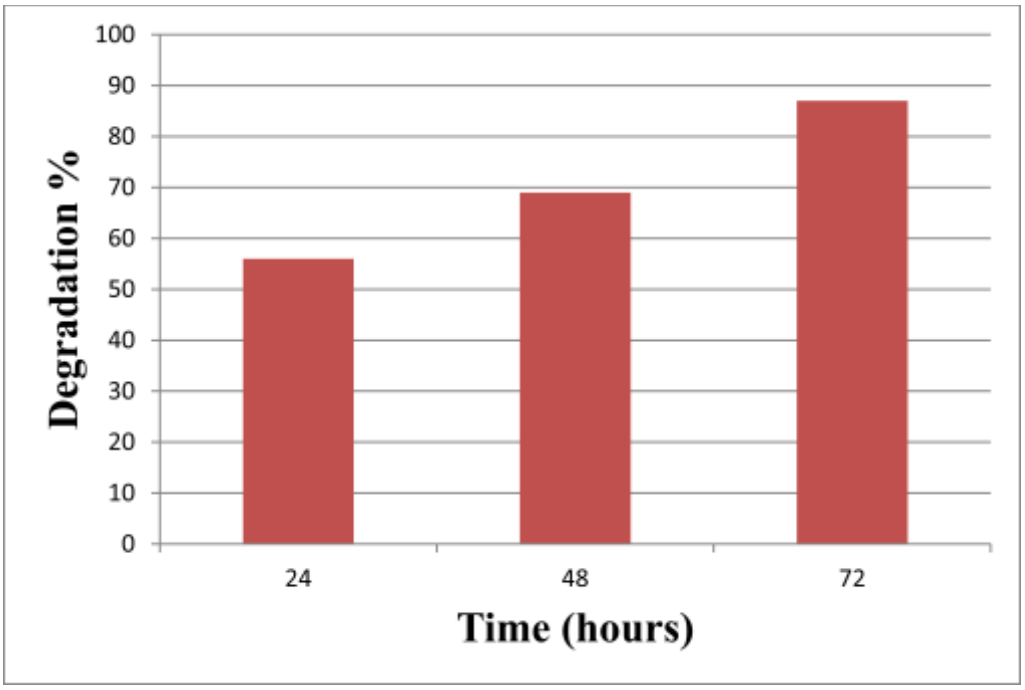

Figure (4) : degradation of aflatoxin B1 by peroxidase

Conclusion

In conclusion, production of peroxidase by *Pseudomonas* sp. was confirmed and the enzyme activity was affected by pH and temperature change. The ability of peroxidase to degradation of aflatoxin B1 makes it as an efficient tool for the detoxification of aflatoxin from the environment.

References

1. Wang J, Ogata M, Hirai H, Kawagishi H (2011). Detoxification of aflatoxin B1 by manganese peroxidase from the white-rot fungus *Phanerochaete sordida*YK-624. FEMS Microbiol Lett. 314:164–169.
2. Zhao LH, Guan S, Gao X, Ma QG, Lei YP, Bai XM, Ji C (2010). Preparation, purification and characteristics of an aflatoxin degradation enzyme from *Myxococcus fulvus* ANSM068. J Appl Microbiol. 110:47–155.
3. Alberts JF, Engelbrecht Y, Steyn PS, Holzapfel WH, Van Zyl WH (2006). Biological degradation of aflatoxin B1 by Rhodococcus erythropolis cultures. Int J Food Microbiol. 109:121–126.
4. Lillehoj EB, Stubblefield RD, Shannon GM, Shotwell OL (1971). Aflatoxin M1 removal from aqueous solutions by *Flavobacterium aurantiacum*. Mycopathol Mycol Appl. 45:259–266.
5. Hossain SM, Anantharaman N (2006) Activity enhancement of ligninolytic enzymes of Trametes versicolor with bagassepowder. Afr J Biotechnol 5:189–194.
6. Dawkar VV, Jadhav UU, Telke AA, Govindwar SP (2009) Peroxidase from Bacillus sp. VUS and its role in the decolorization of textile dyes. Biotechnol Bioprocess Eng 14:361–368.

7. Ghodake GS, Kalme SD, Jadhav JP, Govindwar SP (2009) Purification and partial characterization of lignin peroxides from Acinetobacter calcoaceticus NCIM 2890 and its application in decolourization of textile dyes. Appl Biochem Biotechnol 152:6–14.
8. Kalyani, DC, Phugare, SS, Shedbalkar, UU and Jadhav, JP (2011) Purification and Characterization of a Bacterial Peroxidase from the Isolated Strain Pseudomonas sp. SUK1 and Its Application for Textile Dye Decolorization. Annals of Microbiology, 61, 483-491.
9. Dortet, L, Poirel, L, & Nordmann, P (2012). Rapid identification of carbapenemase types in Enterobacteriaceae and Pseudomonas spp. by using a biochemical test. *Antimicrobial agents and chemotherapy*, *56*(12), 6437-6440.
10. Zierdt, CH, & Schmidt, PJ (1964). Dissociation in Pseudomonas aeruginosa. *Journal of bacteriology*, *87*(5), 1003-1010.
11. Bruins, MJ, Bloembergen, P, Ruijs, GJ., & Wolfhagen, MJ (2004). Identification and susceptibility testing of Enterobacteriaceae and Pseudomonas aeruginosa by direct inoculation from positive BACTEC blood culture bottles into Vitek 2. *Journal of clinical microbiology*, *42*(1), 7-11.
12. Kabak B, Dobson AD, Var I (2006). Strategies to prevent mycotoxin contamination of food and animal feed: A review. Crit. Rev. Food Sci. Nutr. 46:593–619.
13. Motomura M, Toyomasu T, Mizuno K, Shinozawa T (2003). Purification and characterization of an aflatoxin degradation enzyme from *Pleurotus ostreatus*. Microbiol. Res. 158:237–242.
14. Arim, RH (1995). Present status of the Aflatoxin situation in the Philippines. Food additives and contaminations 12(3): 291-296.
15. Ramosa, AJ, Hernandez , E, Plfa-Delfina, JM and Merino, M (1996). Intestinal absorption of zearalenone and in vitro study of non-nutritive sorbent materials. International Journal of Pharmaceutics 128: 129-137.
16. Tedesco, D, Steidler, S, Galletti, S, Tameni, M, Sonzogni,O. and Ravarotto L (2004). Efficacy of silymarin-phospholipid complex in reducing the toxicity of Aflatoxin B1 in broiler chicks. Poultry Science 83:1839–1843.
17. Wu, Q, Jezkova, A, Yuan, Z, Pavlikova, L, Dohnal, V and Kuca, K (2009). Biological degradation of aflatoxins. Drug Metabolism Reviews 41(1): 1–7.