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# THE USE OF ANTIFUNGAL ACTIVITY OF *Lactobacillus plantarum* DSMZ20191 IN BIOPRESERVATION OF DIETS USED FOR FEEDING OF THE NILE TILAPIA(*Oreochroms niloticus*) FINGERLINGS

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## ABSTRACT

This study was designed to examine the antifungal activity of *Lactobacillus Plantarum* DSMZ 20191 on a variety of molds which normally contaminated the fish diet and my cause it`s deterioration and also affect it's shelf life. We used different concentration of the antifungal metabolites (1,2,3,4 and 5 ml/100g of the fish diets) to demonstrate it`s role in inhibition of fungal growth during storage of fish diets. According to our study *Lb. plantarum* DSMZ 20191exhibit high activity against *Aspergillus sp.* which contaminated animal fish diet especially at concentration of 5%, while in plant diet the antifungal activity against *Aspergillus sp., Fusarium sp. and Penicillum sp.*was lower. Finally the activity against *Aspergillus sp.* and *Fusarium sp.*in mixed fish diet was moderate.In conclusion LAB can be used in biopreservation due to their antifungal activity against a variety of molds which contaminate different food and feed and can cause serious deterioration and this can eliminate the use of chemical preservatives.

**Keywords:** Lactic acid bacteria, *Lb. plantarum* and Biopreservation

## INTRODUCTION

Food and feed spoiling moulds and yeasts cause great economic losses worldwide. Furthermore, the presence of moulds with the concomitant production of allergenic spores and possibly mycotoxins makes them serious potential health hazards (Pitt and Hocking, 1999). Aflatoxins, part of a large group called mycotoxins, are toxic substances produced as a result of mold growing on grain, feedstuff and other foods. Mycotoxigenic fungi such as *Fusarium* and *Penicillium* are serious hazard for human health (Dalie *et al.*, 2009). Filamentous moulds and yeasts are common spoilage organisms of food products as stored crops, bread and feed such as hay and silage (Bullerman, 1977). During the last years there has been a growing interest in biopreservation to prevent spoilage and extend the shelf life of foods (Stiles, 1996). The reduction of mould and yeast growth in food and feed production and storage is thus of primary importance and there is great interest in developing efficient and safe strategies for this purpose. The application of biopreservation has received much attention in recent years. Lactic acid bacteria (LAB) are known to produce different antimicrobial compounds and are important in the biopreservation of food and feed, Lindgren and Dobrogosz (1990) and (Messens and de Vuyst, 2002). LAB are of special interest as biopreservation organisms since they have a long history of use in food and are 'generally regarded as safe' organisms. Their preserving effect mainly relates to the production of organic acids, i.e. lactic and acetic acid (Stiles, 1996), but bacteriocins, produced by some strains, are also of importance (Dodd and Gasson,1994). The majority of the large numbers of

reports on antimicrobial activity of LAB have focused on antibacterial effects (Dodd and Gasson, 1994) while reports on antifungal effects are few. Lavermicocca *et al.*, (2000) reported on the production of the antifungal compounds phenyllactic acid and 4-hydroxyphenyllactic acid by a sourdough *Lactobacillus plantarum* strain. In addition, bacteriocin-like substances and other low molecular mass compounds produced by LAB have been reported as antifungal (Niku-paavola *et al.*, 1999) and (Okker*et al.*, 1999). *Lactobacillus coryniformis* strain Si3 can produce a proteinaceous antifungal compound (Magnusson and Schnurer, 2001).Strom *et al.*, (2002) also identified antifungal cyclic dipeptides from a silage *L. plantarum* strain.The application of LAB with the simultaneous control of factors that affect the fungal growth can help to minimize food spoilage, (Belal *et al.*, 2013). The aim of this study is to demonstrate the role of lactic acid bacteria in inhibition of fungal growth due to the production of many antifungal metabolites which can be extracted and used in biopreservation of fish diets during 90 days of storage.

## MATERIALS AND METHODS

**Culture:-**

The investigated bacterial strain (*Lactobacillus plantarum* DSMZ 20191) was obtained from the collection of food Science Department, Faculty of Agriculture,Ain Shams University, Egypt. The culture re activated on MRS broth medium.

**Production of the antifungal metabolite(s):-**

The bacterial strain was cultivated on 2000 ml of de Man, Rogosa and Sharpe (MRS)(de Man *et al.*, 1960) broth medium divided into 20 flask, each containing 100 ml and inoculated with 2% of the bacterial cell suspension, then incubated at
32° C for 48h.

**Extraction of antifungal metabolite(s):-**

A cell free extract was obtained by centrifugation at (10.000 xg at 4°C for 20 min) the extract was adjusted to pH 7.0 by means of 1M NaOH to exclude the effect of organic acids. The extract then filtrated through a 0.2 mm pore size cellulose acetate filter (Schilllinger and Luke, 1989). The extract then dialyzed for 12h and added to the fish diet by volume of (1-2-3-4 and 5ml/100 g of the fish diet and then the different diets were stored in sterilized plastic bags at 4°C.

**Testing of antifungal activity:-**

The activity was investigated by using the pour plate method using potato dextrose agar medium (PDA), in which serial dilution was carried out using 0.8% Nacl sterilized saline solution and only dilution of $10^4$ was selected for total fungal count in each volume Volumes of the antifungal metabolites then 1ml of this dilution was used for testing the antifungal activity, then the plates were incubated at 32° C for 3 days. The total count of fungal colonies was then carried out for different plates.

**The experimental feeds :-**

Three diets were formulated, the first diet was the dietary protein derived mostly from animal sources (fish meal, meat meal, bone meal and poultry by product meal).The second diet was derived from plant sources (soybean meal,

sunflower meal and corn gluten meal. Finally the third diet was a mixture of animal and plant.

**Preparation of diets:**

All ingredients were prepared by successive grinding through a commercial feed grinder (1/ 16 mesh), without any additional heat. Before mixing we add different concentrations of the antifungal supernatant (1, 2, 3, 4 and 5%) to all diets. Then diets were mixed mechanically by horizontal mixer, the feed mixture was processed into a Califomia pellet meal (CPM) machine.

## RESULTS AND DISCUSSION

**Demonstration of antifungal activity**

*Penicillum* and *Aspergillus* species have been reported as spoilage organisms during storage of food and feeds and *Fusarium* species are often found on cereal grains, where they might produce mycotoxins (Filtenborg *et al.*, 1996). Studies on the effect of LAB on fungi are complicated by the fact that some fungi are sensitive to the normal by products of lactic acid bacteria (LAB) metabolism, most notably lactic and acetic acids (Bonesteroo *et al.*, 1993). In our study we avoided the effect of organic acids to demonstrate the effect of low molecular weight pertinacious antifungal metabolitesfound in the extract produced by *Lactobacillus plantarum* DSMZ 20191 against moulds contaminated plant animal and mixed fish diet.

Fig. (1) demonstrate antifungal activity of *Lactobacillus plantarum* DSMZ 20191 against moulds contaminated animal fish diet. According to microscopic examination the isolated moulds are belonging only to *Aspergillus sp.* At Zero time (A) the antifungal activity was estimated, at 1% it was recorded (75%), while the concentrations of (2, 3, 4, and 5%) were recorded activity of 100%. The antifungal activity at 15 and 30 days of storage, (B) was recorded the same results, at 1% of our extract the activity was (72%), also the activity at 2% recorded (78%). Finally the antifungal activity at (3-4 and 5%) recorded 100%. After 45 days,(C) the antifungal activity was decreased to recorded at 1% (68 %), on the other hand the activity at 2% was (70%). Finally the activity at 4 and 5% was recorded 100%. Moreover, after 60 days of storage, (D) further decrease in activity was recorded. The activity at 1% was (66%), while at 2% it was (70%). Finally at the concentrations of 3-4 % the activity recorded (73%), (75%) and at concentration of 5% the activity was 100%. The antifungal activity after75 days, (E) recorded more decrease at 1% it was (63%), while at 2% it was (67%) furthermore the activity recorded (74%), (77%) and (81%) for 3-4 and 5% respectively. Finally at 90 days of storage, (F) the antifungal activity recorded (61%).LAB are well known for their antifungal activity, which is related to the production of a variety of compounds including acids, alcohols, carbon dioxide, diacetyl, hydrogen peroxide, bacteriocines and cyclic peptide (Gerez *et al.*, 2009). These compounds were added to several foods in order to conserve them from food- borne and spoilage organisms (El-Ziney and Debevere, 1998).

The plant fish diet is highly contaminated by moulds and the isolated molds were belonging to *Aspergillus sp.*, *Fusarium sp.* and *Penicillium sp.* Fig.2 (A) demonstrating that, the activity at 1% was (38%)at Zero time, by increasing the volume to 2%, the antifungal activity was raised to (45%). Additionally the activity at

(3,4 and 5%) was 50, 41 and 48% respectively. After that, the results at 15 and 30 days (B), were the same, at the start the activity was (45%) at 1%, then it was recorded (53%) at 2%. The activity at 3% was (58%), on the other hand the activity at 4 and 5% was (64%) and (77%). Decrease in activity toke place after 45 days (C) and it was (35%) at 1%. At 2% the activity up to (44%), moreover the activity at 3, 4 and 5% was (53%), (66%) and (75%) respectively. The activity was little decreased after 60 days (D) and recorded at 1% (36%). As noted from the Fig. the antifungal activity was increased to (44%) at 2%, the activity again increased to (52%) at 3%. Finally the activity at 4 and 5% was (61%), (73%) respectively. There was a little decrease in activity after 75 days of diet storage (E) and the activity recorded at 1% (34%) , and at 2% (41%). By increasing the concentration to 3%, the activity raised to (50%). Furthermore the activity at 4 and 5% increased to (54%) and (70%) respectively. After 90 days of storage, (F) the activity recorded (33% ) at 1%, at 2% the activity was (39%), Finally the activity at 3, 4. and 5% recorded (48%), (58%) and (65%) respectively .*Aspergillus* and *Penicillium* species are the most common spoilage fungi for many foods and feeds while *Fusarium* species are reported to attack cereal grains in the field (Samson *et al.*, 2000).The antifungal activity of strain *Lb. plantarum* against *Aspergillus sp.* And *fusarium sp.* was reported by many researchers . Hikara *et al.*, (1994) reported on the discovery of strain of *Lb. plantarum* (*Lb. planetarium* 601) with antifungal capabilities towards *Fusarium sp.* And *Aspergillus niger.*

Fig. (3) demonstrated the antifungal activity of metabolites added to the mixed fish diet and it recorded good activity against the contaminants of *Aspergillus sp.* and *Fusarium sp.* The activity at Zero time (A) was (40%) at 1%, by increasing the concentration the activity gradually increased. At 2% the activity was (49%). Also at 3% the activity recorded (56%). The antifungal activity was (65%) and (66%) for 4 and 5% respectively. After 15 days of storage (B), the activity was little increased at the different concentrations and recorded (46%) at 1%. On the other hand the activity at 2% raised to be (53%). Furthermore the activity at 3,4 and 5% was (61%), (66%) and (90, 87, 84, 75 and 60%) respectively. By time the activity was little decreased after 30 days (C) and still as it was after 45 days. The activity at 1% was (44%), while the activity at 2% recorded (50%), the activity further increased to (53%), (58%) and (68%) for 3, 4 and 5%. After 60 days (D), the activity decreased to (42%) at 1% and it was (48%), (51%) and (56%) at 2, 3, 4%. Moreover the activity at 5% was (66%). On the other hand the activity little down again at 75 and 90 days (E), at 1% the activity was (40%), the activity at 2% up to (46%), at 3, 4 and 5% the activity was (49 %), (55%) and (62%). From the previous results we can say that our tested bacterium relatively exhibited high antifungal activity against molds contaminated mixed fish diet at Zero and 15 days, then the activity slightly decreased by time but we can considered it good activity at high concentration of the antifungal extract. The use of protein-like compounds are preferred over the use of acids because their activity is present over a wide range of pH and they are heat stable (Muhialdin *et al.*, 2011). Lavermicocca *et al.*, (2000) could isolated a metabolite from *Lb. plantarum* strain 21Bwhich exhibited abroad spectrum activity against *Aspergillus flavus* and *Aspergillus niger.* In a related study carried out by Latilla *et al.*, (2002) they studied the antifungal potential *Lb. plantarum* strain (E98) against *Fusarium sp.*The results indicated that *Lb.plantarum* cell free extracts were effective against *fusarium sp.*

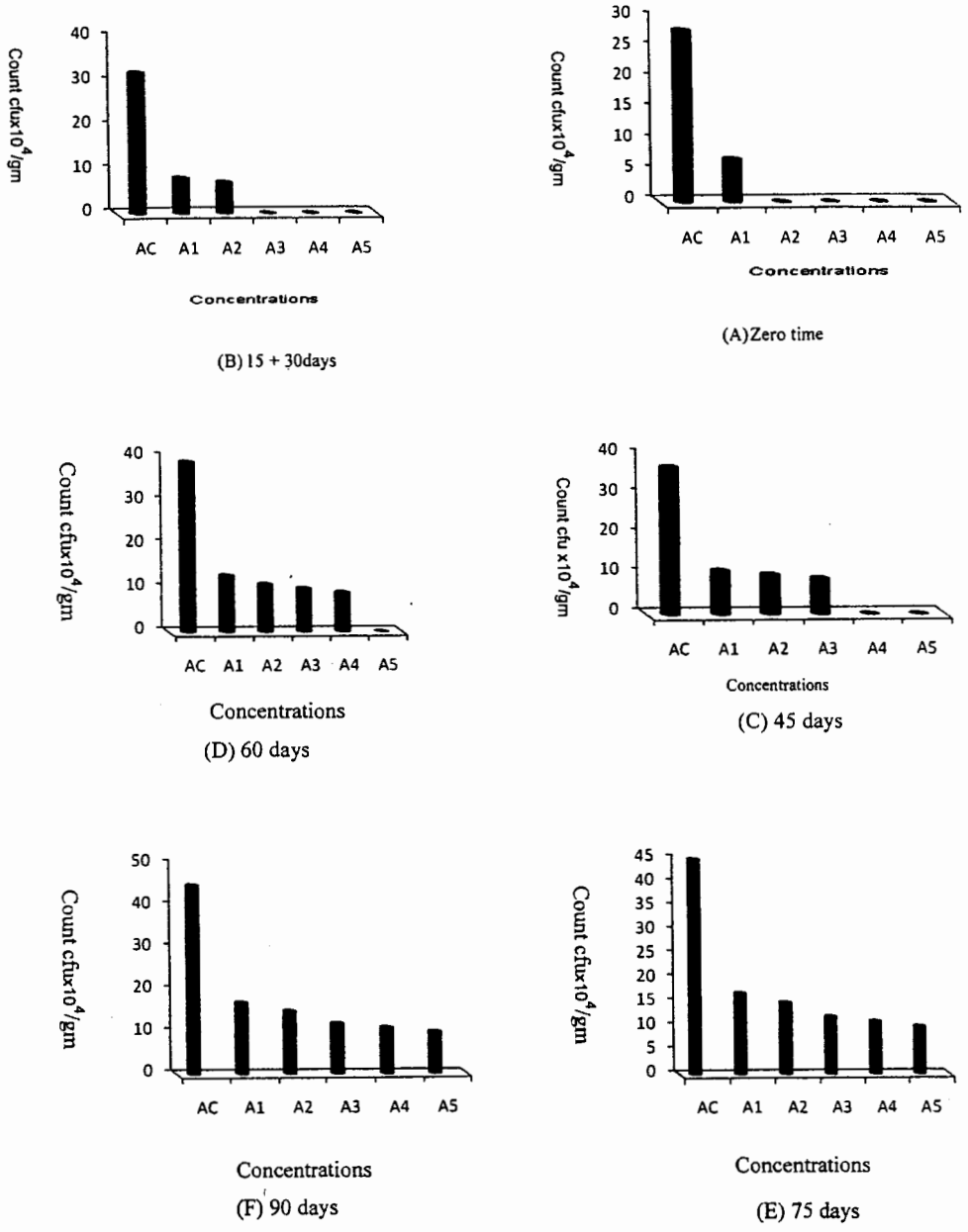


**Fig.(1): Demonstration of antifungal activity of *Lactobacillus plantarum* DSMZ 20191 against fungi contaminated animal fish diet using different metabolites concentrations.**
**where: Ac= Control sample, A1= 1%, A2= 2%, A3= 3%, A4= 4% and A5=5%**

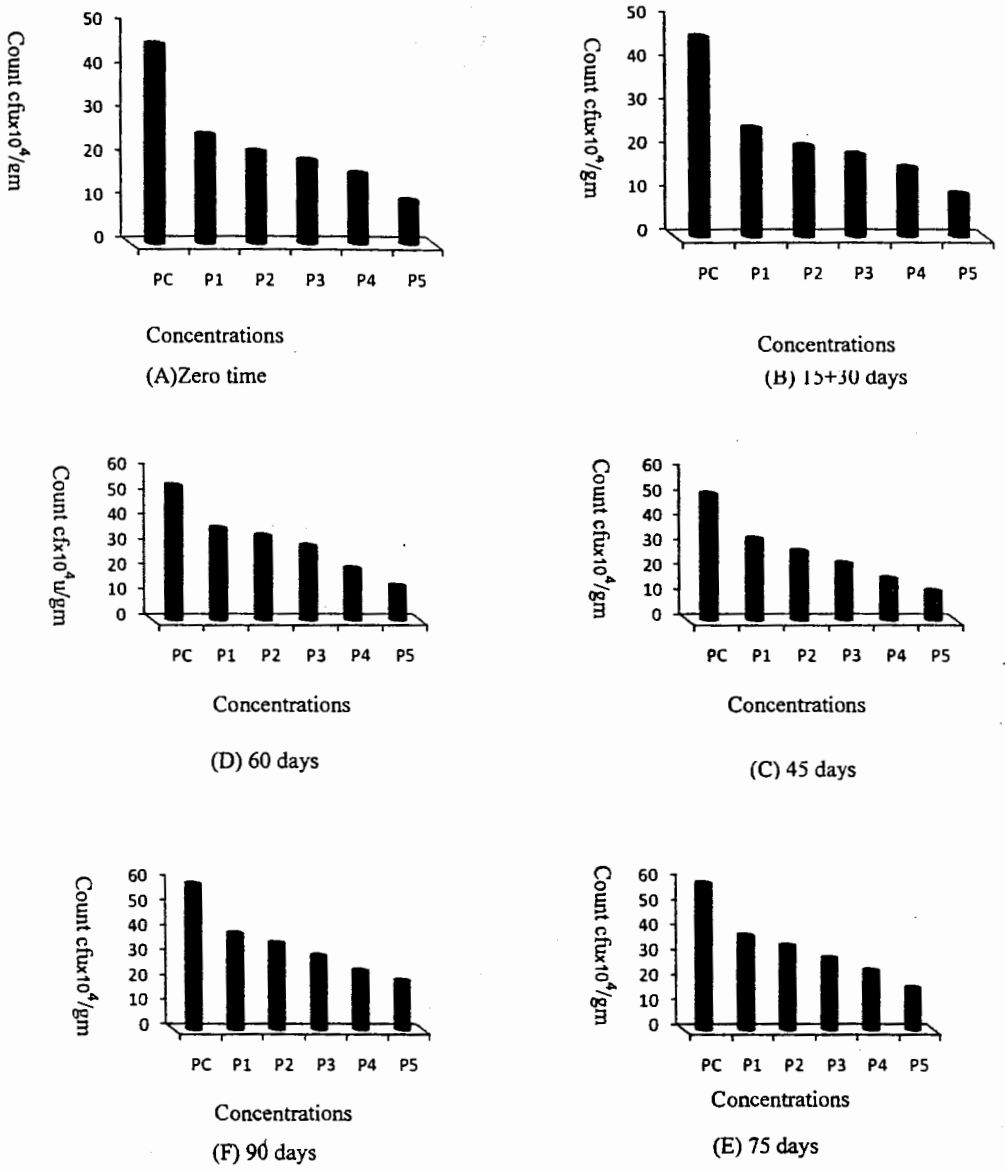


**Fig.(2): Demonstration of antifungal activity of *Lactobacillus plantarum* DSMZ 20191 against fungi contaminated plant fish diet by using different metabolites concentrations.**
**Where: Pc= control, P1= 1%, P2= 2%, P3=3%, P4=4%and P5= 5%**

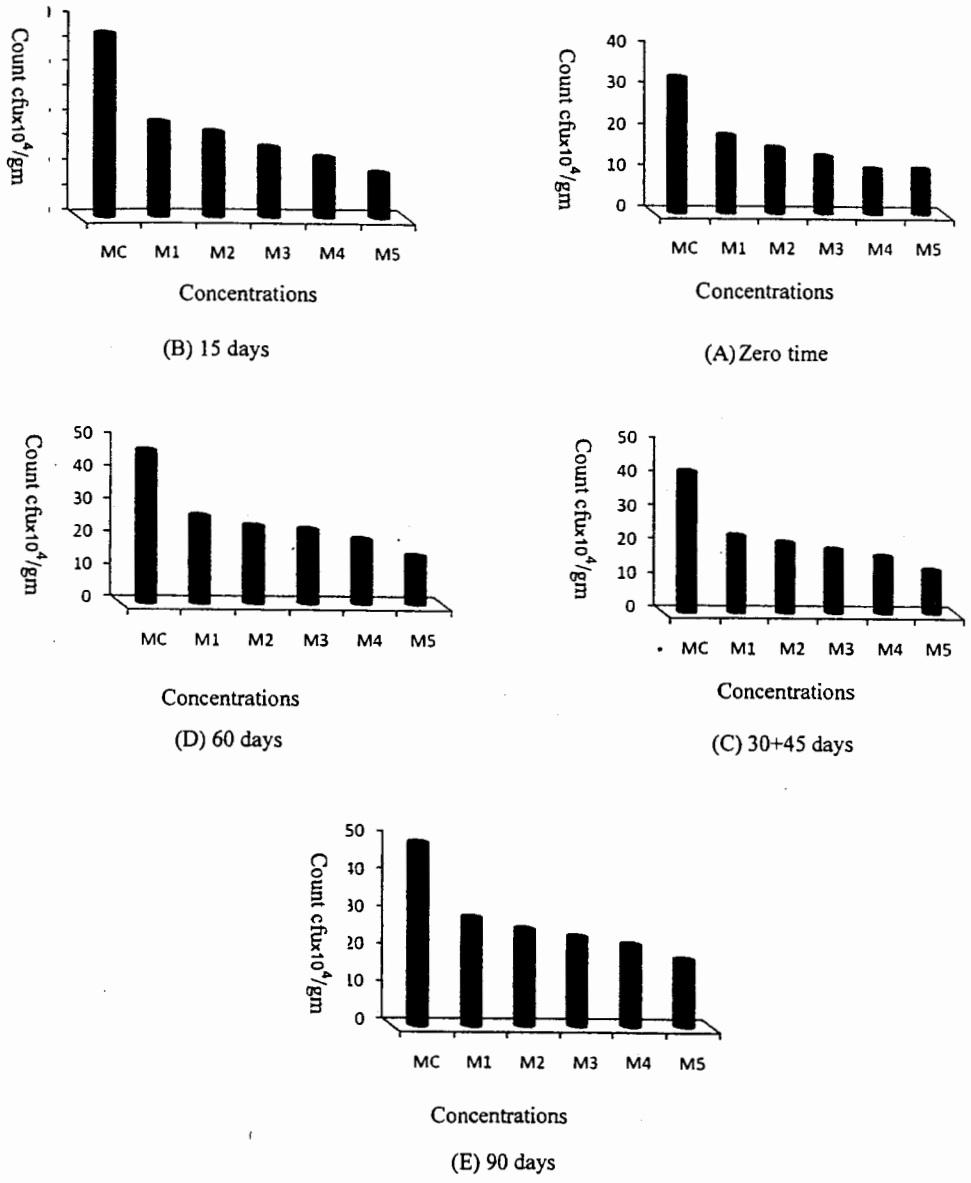


**Fig.(3): Demonstration of antifungal activity of *Lactobacillus plantarum* DSMZ 20191 against fungi contaminated mixed fish diet by using different metabolites concentrations.**
**Where: Mc= control, M1= 1%, M2= 2%, M3=3%, M4=4%and M5= 5%**

## CONCLUSION

Chemical preservatives and Fungicides which used to inhibit fungal growth and also mycotoxin production in different food and feed can be considered as serious potential health hazards for consumers. In addition to the effect of these chemicals on environment along with problems of microbial resistance favor the need for alternative methods in controlling fungal growth. Application of LAB in biopreservation of different food and feed is now has a very good interest.

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# استغلال قدرة ال *Lb. plantarum* DSMZ 20191 علي إنتاج بعض المضادات الفطرية في الحفظ الحيوي للأعلاف المستخدمة في تغذية إصبعيات البلطي (*Oreochroms niloticus*)

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صممت هذة الدراسة لإختبار مدي قدرة بكتيريا *Lb. plantarum* DSMZ 20191 علي تثبيط نمو الفطريات الموجودة بشكل طبيعي في مكونات اعلاف الأسماك والتي تؤدي إلي إتلافها والحد من فترة صلاحيتها. اثناء الدراسة تم استخدام تركيزات مختلفة من المركبات المضادة للفطريات المنتجة بواسطة البكتيريا (٥,٤,٣,٢,١ ملل/١٠٠جرام (حجم/ وزن) من الأعلاف، لمعرفة مدي قدرة هذة التركيزات علي منع نمو الفطريات في الأعلاف اثناء تخزينها. ومن نتائج الدراسة إتضح لنا أن *Lb. plantarum* لها قدرة عالية علي منع نمو فطريات *Aspergillus sp.* التي تلوث الأعلاف ذات التركيب الحيواني خاصة عند تركيز ٥ % . بينما يحدث تأثير تثبيطي أقل مع فطريات *Aspergillus sp., Fusarium sp.* And *Penicillium sp.* الملوثة للأعلاف ذات التركيب النباتي. واخيرا فإن نشاط البكتيريا المضاد للفطريات يوضح تأثير متوسط ضد فطريات *Aspergillus sp.* and *Fusarium sp.* في الأعلاف ذات التركيب الحيواني – النباتي المختلط.

وبذلك يمكننا استخدام قدرة بكتيريا حامض اللاكتيك علي إنتاج العديد من المركبات المضادة للفطريات في الحد من الفطريات الملوثة للعديد من الأغذية مما يحد من إستخدام المواد الحافظة الكيميائية واستبدالها بالحفظ الحيوي.