Research Article

AL- ANBAR JOURNAL OF VETERINARY SCIENCES

Vol. 13 Issue:2, (2020) ISSN: P-1999:6527 E-2707:0603

Using of *Saccharomyces Cerevisiae* and *Lactobacillus Acidophilus* as Probiotic Against *Salmonella Enterica Serovar Typhimurium* Isolated from Poultry

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Doi: https://doi.org/10.37940/AJVS.2020.13.2.10

Received: 17/9/2020 Accepted:13/11/2020

Abstract

The study aimed to determine the effect of S. cerevisiae and L. acidophilus as probiotics against S. Typhimurium isolated from poultry, for this purpose (50) fecal samples were collected from poultry to isolate S. Typhimurium, while fermented milk was used for isolation of Lactobacillus acidophilus. Results of the current study showed that S. Typhimurium was isolated at a rate of 6%. The in-Vivo adhesion Index test showed a high ability of L. acidophilus to adhesion to rat intestine endothelium in compare with Saccharomyces cerevisiae. The inhibitory zone occurred by S.cerevisiae filtrate, L. acidophilus filtrate, (S .cerevisiae and L. acidophilus mixture) filtrate were (10, 16, 19 mm) respectively. The results of the experimental study showed the high activity of (S.cerevisiae and L. acidophilus mixture) filtrate in protecting experimental animals. The main pathological change that occurred by S.typhimurium was the infiltration of inflammatory cells.

Keywards: Probiotic, *S. typhimurium*, **of *Saccharomyces Cerevisiae, Lactobacillus* ,** Poultry

استخدام خميرة الخبز الجافة و العصيات اللبنية الحمضية كمعززات حيوية ضد سالمونيلا التيفوئيدالمعزولة من الدجاج

الخلاصة

هدفت الدراسة الى تحديد تأثير خميرة الخبز الجافة (Saccharomyces cerevisiae) والعصيات اللبنية الحمضية(Lactobacillus acidophilus) كمعززات حيوية ضد سالمونيلا التيفوئيد (Salmonella typhimurium) المعزولة من الدجاج، لهذا الغرض تم جمع (50) عينة براز دجاج لغرض عزل سالمونيلا التيفوئيد، بينما استخدم الحليب المخمر لعزل العصيات اللبنية الحمضية.أظهرت نتائج الدراسة الحالية عزل سالمونيلا التيفوئيد بمعدل 6%، كما أظهر اختبار مؤشر الالتصاق في الجسم الحي قدرة عالية لعصيات اللبنية الحمضية على الالتصاق على بطانة أمعاء الفئران مقارنة مع الخميرة.نطاق التثبيط حدث بواسطة ارتشاح الخميرة، العصيات اللبنية الحمضية، (خليط الخميرة و العصيات اللبنية الحمضية) كانت على التوالي (10،16،19ملم)، اوضحت نتائج الدراسة التجريبية فعالية عالية لخليط (الخميرة والعصيات اللبنية الحمضية) في حماية الحيوانات المختبرية. ان التغيرات المرضية الرئيسية التي حدثت بواسطة سالمونيلا التيفوئيد كانت ارتشاح الخلايا الالتهابية.

Introduction

Antibiotics are less effective against several bacterial pathogenic microbes , like Salmonella strains, because of antibiotic-resistant bacteria so there is need for methods of preventing and treating infections caused by

enteric pathogenic bacteria (1). Lactobacillus and Bifidobacterium Species are the most commonly probiotics used for treatment the infectious diseases, including travellers' diarrhoeas and antibiotic-associated. Other microrganisms, including Saccharomyces boulardii, Enterococcus faecium, Streptococcus thermophilus, Leuconostoc species, Bacillus species and Escherichia coli Nissle strain are researched in vitro or in human and animals trials, are being used prophylaxis or therapeutic purposes(2,3). The first mention of probiotic term is by Lilly and Stillwell in 1956 which describe growth factors produced by microorganisms (4). Probiotic defined by Schaafsma and Guraner as a microorganism that have good effects on human and animal health (5), while (6) defined probiotic as any production contain diagnostic microorganisms in adequate number which have ability to change numbers and types of microflora in the body. Main characters of microorganisms that using as probiotic are: not has pathogenic or toxic effects, able to grow and multiply in the intestine, resistant to bile duct product, constant genetically and easily storage (7).

The first isolation of lactobaccilus was done by Lister in 1878, its gram positive bacteria, Facultative anaerobic, non-motile , non-spore forming , negative to catalase production test, indol test , and H_2S production test (4). Lactobacillus used as antibiotics alternatives because it safe, efficient, and widely used in chickens feeding and has been noticed a simulated growth of chicks, the inhibition the pathogens of intestinal microbes, a promoted immune function decreased morbidity, vitamin synthesis,
reduced serum cholesterol levels and anticarcinogenic effects (8,9,10,11).

Saccharomyces cerevisiae, is a nonpathogenic yeast, have positive effects on poultry production such as in egg production, reproduction, feed efficiency, growth rate, reduce liver toxicity and residual aflatoxin B1. In addition, supplementation of yeast, yeast cultures and yeast extracts to feed has gived environmental and economic benefits in poultry diets for the past 40 years (12,13.14). *S. typhimurium* is gram negative bacteria, non-motile , non-spore forming lactose non ferment, caused many diseases to human and animals like diarrhea and typhoid fever (15). Studies have indicated that *Saccharomyces cerevisiae* can be used in the prevention and treatment of bacterial infectious diseases, including
Paratyphoid, typhoid and nontyphoidal Salmonella (3).

(16) mention when chicks were given Salmonella spp at10^4 cfu/chick and then were treated with 10 kinds of Lactobacillus at 10^8 cfu/chick, the results indicate that Lactobacillus can decrease the cecal Salmonella counts, decrease the mortality of dieased in chicks and booster the balance of intestinal flora. The aim of this study was to Use the *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* as probiotic against *Salmonella typhimurium* isolated from tikrit poultry.

Materials and methods

The current study conducted in animal house of College of Veterinary Medicine, University of Tikrit in Slah aldeen Province In period from February to August 2019.

- Isolation of *S. typhimurium*: 50 fecal samples were collected from chicken infected with diarrhea, the samples cultured in selenite F broth and cultivated at 37°C for 24h, then subcultured on Salmonella – Shigella agar, Xylose Lysine Deoxycholate agar and MacConkey agar. After colony appearance, gram stain and groups of biochemical tests were applied according to (15). Then confirmed by API 20.

- Isolation of *Lactobacillus acidophilus*: lactobacillus isolated from fermented milk on Deman Regosa Sharp Broth (MRS) (Himedia- India), (by add 1ml of fermented milk to 9ml of MRS broth) then

transport to laboratory and culture on Deman Regosa Sharp agar with 1% Ca CO3 and cultivated in 5-10% CO2 at 37C for 24h. (17). Gram stain and group of biochemical tests were applied according to (18).

- Preparation of *Saccharomyces cerevisiae*: 1gram of *Saccharomyces cerevisiae* (Pakmaya- France) add to tube contain 10 ml of Glucose yeast extract peptone broth (GYEP) then cultivation aerobically at 37C for 24h.
- Preparation of *S. cerevisiae and L. acidophilus* filtrated fluid
  - a- Cultivation of *S.cerevisiae* and *L. acidophilus*: *S.cerevisiae* cultivation on GYEP in concentration of $1X^{9}10$ Cell/m then aerobically at 37C for 24h. while *L.acidophilus* cultivation on MRS $1X10^{6}$ Cell/ml anaerobically at 37C for 24h (19)
  - b- The two culture centrifuged (6000 cycle/ minutes for 10 minutes). The supernatant has been taken and filtrated by Millipore (0.22 micrometer) the filtrated fluid has been concentrated by Lyophilizer.
  - c- Determination of inhibitory zone: 0.1 ml of $1.5X10^{8}$ CFU/ml of *S. typhimurium* suspension were disseminate in agar media. Holes in plate were done by cut aseptically with sterile cork borer, then 100µl of filtrated fluid were put in hole and incubation at 37ºC for 24h, the inhibition zone were measured using caliber.

Study of adhesion Index:

- a- The broth of (*S.cerevisiae* and *L.acidophilus)* centrifuged (2000 cycles/ mints) for 10 minutes, the sediment has been taken and resuspension in normal saline
- b- The endothelium of Rats intestine were taken after 24h of fastening. The Rats killed and endothelium has been taken by aseptic glass slide and resuspended in normal saline
- c- two mixture were prepared (*S.cerevisiae* suspension with endothelium suspension 1:50) and (*L.acidophilus* suspension with endothelium suspension 1:50) for 10-15 minutes. Smear from each mixture were prepared and stain by Wright stain (20).

- Determination of Curative and preventive effectiveness of *L. acidophilus* and *S.* cerevisiae in Rats: 36 adult male Rats divided in to six groups. Each group includes six Rats, the design of experimental as in table 1.

Table 1: experimental design of current study

No. of experimental groups	Type of diet	Type of treatments
1st group	Non	Non
2nd group	(4% *S. cerevisiae* filtrate)	*S.typhimurium*
3rd group:	(4% of *L. acidophilus* filtrate)	*S.typhimurium*
4th group	(2% of *L. acidophilus* filtrate + 2% *S. cerevisiae* filtrate)	*S.typhimurium*
5th group	Non then infected experimentally with *S.typhimurium*	Fed by diet contain 2% of *L. acidophilus* filtrate + 2% *S. cerevisiae* filtrate
6th group	Non	*S.typhimurium*

The fed continuous for seven days before and seven days after exposure to 1X 10^6 CFU. Of *S.typhimurium*, the clinical signs recorded every day, bacterial shedding applied by fecal collection daily then culture on S-S agar.

Results and discussion

Isolation of salmonella: out 3 of 50 fecal samples *Sallmonella typhimurium* isolate in rate 6% , which isolated appear as lactose non ferment on MacConkey agar, on S-S agar appear as large transparence colony with black center (produce H_2S). positive to catalase test, citrate utilized test on Simmen citrate and Methyl Red test, while negative to Urease , Voges Proskauer's, Oxidase , Indole. Also it compatibility to stander *S typhimurium* isolates as in figure 1 .

Figure 1: Results of API 20 for *S typhimurium*

Isolation of *L. acidophilus* : *L. acidophilus* isolated on MRS media, the early diagnosis depend on transparent zone round colony, negative to (catalase and oxidase test and urease) test. On gram stain the bacteria appear as gram positive bacteria arrangement as signal or pear or short chains. Ferment to glucose, manitol , lactose, fructose, maltose while non-ferment to xylose and arabinose sugar. The isolation of *L. acidophilus* isolated on MRS media due to compound of this media like acetate, nitrate, tween20, MnSO4 and MgSO4 (21).

In-Vivo adhesion Index: the result showed high ability of L. acidophilus to adhesion on rat intestine endothelium which to (18-24 cells) while *Saccharomyces cerevisiae* has not this feature. The ability of *L. acidophilus* to adhesion due to presence of Lipoteichoic acids and Surface Protein Layer which consist from more than 30% of hydrophobic amino acid (22).

Results of inhibitory zone: table 2 showed that clear effects of (*L. acidophilus* and *S. cerevisiae*) filtrate in inhibition *S. typhimurium* and the highly inhibition effect occur by mixture of *S.cerevisiae* and *L. acidophilus* filtrate. Figure 2, 3.

Table 2: Inhibition effect by filtrates

Type of inhibitor filtrate	Diameter of inhibitory zone (MM)
L. acidophilus	16
S. cerevisiae	10
S.cerevisiae and *L. acidophilus*	19

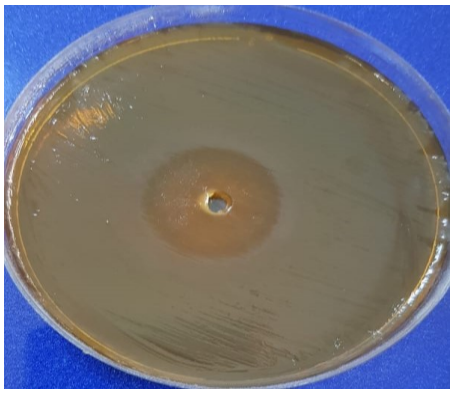

Figure 2: Inhibitory zone occurred by L. acidophilus

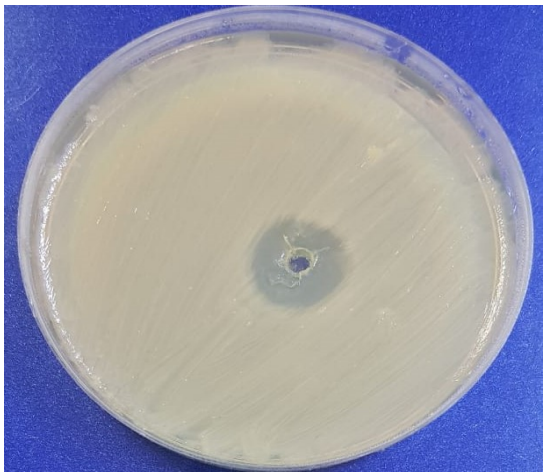

Figure 3: Inhibitory zone occurred by S. cerevisiae.

The inhibitory effect of *L. acidophilus* is agreed with (23) that's due to its ability to produce (Acetic, Propionic and Lactic acid which decrease in pH and killed the salmonella (24). The inhibition effect of *S. cerevisiae* is due their ability to produce endotoxin, acidic compounds and proteolytic enzyme (25).

Result of experimental study: from table 3 showed that ability of *L. acidophilus* filtrate and *L. acidophilus* filtrate + *S. cerevisiae* filtrate in protective of experimental animal from infection by *S.typhimurium* , while *S. cerevisiae* filtrate unable to protective of experimental animals from infection by *S.typhimurium*. also the results shows ability of *L. acidophilus* filtrate + *S. cerevisiae* filtrate in treatment of diarrhea that caused by *S.typhimurium*.

Table 3: Curative and preventive effectiveness of *L. acidophilus* and Saccharomyces cerevisiae filtrate

No. of experimental groups	Type of diet	Clinical singes	Type of treatments	Clinical singes	Time of bacterial isolation (day)
1st group	Non	Non	Non	Non	0
2nd group	(4% *S. cerevisiae* filtrate)	Non	*S.typhimurium*	Diarrhea, fever continuous for 3day	1-5
3rd group:	(4% of *L. acidophilus* filtrate)		*S.typhimurium*	Non	1-3
4th group	(2% of *L. acidophilus* filtrate + 2% 4% *S. cerevisiae* filtrate)		*S.typhimurium*	Non	1-2
5th group	Non then infected experimentally with *S.typhimurium*		Fed by diet contain 4% of *L. acidophilus* filtrate + 4% 4% *S. cerevisiae* filtrate	Diarrhea, continuous for 4day	1-3
6th group	Non	Non	*S.typhimurium*	Diarrhea, fever, continuous for 7day	1-7

Lactobacillus may be effect on salmonalla by Competitive exclusion mechanism by competition on food or adherence site. Also lactobacillius able to decrease intestine pH, and produce inhibitor substance (H_2O_2, CO_2, acytayle dehade, Bacteriocin , Lactocidin, Acidolin, Acidophilin, Lactolin , Lactobacillin, Lactobrevin) which kill the salmonella (26).

Lactobacillus have ability to stimulation of immune response by activation of Natural Killer cells, macrophage , plasma cell, interferon production, interleukin production ((IL-12,IL-6,IL-5,IL-2,IL-1) , IFN-δ, INF-α (27). *S. cerevisiae* has ability to stimulation IgA , IFN-δ, NF-α , IL-18, IL-12 (23).

The clinical signs of diarrhea that appear on control group which infected with Salmonella is attributed to their ability to invasion of intestine epithelium and penetration them and cause inflammation and absorption disorder and

diarrhea, also the enterotoxin that produce from salmonella can cause diarrhea (28). Also Salmonella caused pathological changes include degeneration on the epithelium of intestine and inflammatory reaction in liver and spleen. As in figure 4,5,6

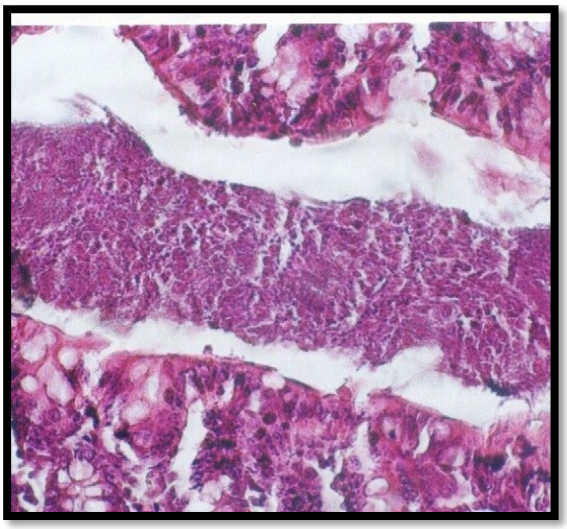

Figure 4: Cross section of Rat intestine infected with *S.typhimurium*, showing hyperplasia on cubic cells, degeneration on the epithelium and inflammatory exudate in intestine cavity. (H&EX40).

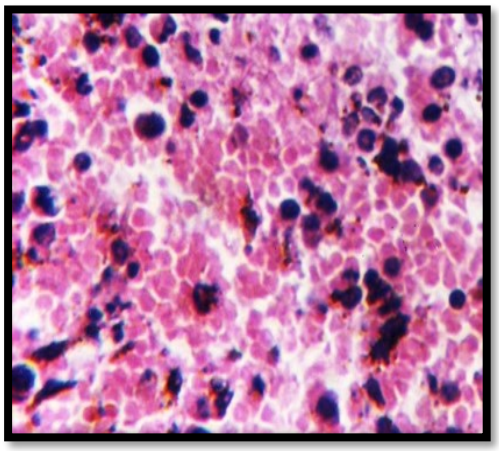

Figure 5:Cross section of Rat spleen infected with *S.typhimurium*

Showing infeliteration of inflammatory cells. (H&EX40).

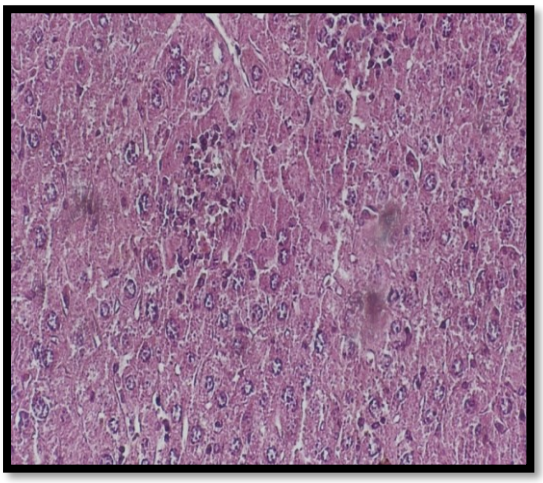

Figure 6:Cross section of Rat liver infected with *S.typhimurium* Showing infiltration of inflammatory cells. (H&EX40).

This results agreed with results recorded by (29, 30) the appearance of this pathological changes is due to bacteremia and reach to this organs (31).

Conclusions

It is concluded that the salmonella isolation rate were 6% . The result showed high ability of L. acidophilus to adhesion on rat intestine endothelium , while *Saccharomyces cerevisiae* has not. The inhibitory zone of mixture of *Saccharomyces cerevisiae* and L. acidophilus filtrate showed the highly inhibion effect. Results suggest use L. acidophilus filtrate + *Saccharomyces cerevisiae* filtrate in protective of experimental animal from *S.typhimurium* infection.

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