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

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**Abstract**

The study aimed to determine the effect of *S. cerevisiae* and *L. acidophilus* as probiotic against *S. typhimurium* isolated from poultry, for this purpose (50) fecal samples were collected from poultry to isolated *S. typhimurium*, while fermented milk used for isolation of *Lactobacillus acidophilus*. Results of current study showed that *S. typhimurium* isolated in rate of 6%. In-Vivo adhesion Index test showed high ability of *L. acidophilus* to adhesion on 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 experimental study showed that high activity of (*S.cerevisiae* and *L. acidophilus* mixture) filtrate in protect experimental animals. Main pathological changes occurred by *S.typhimurium* were infiltration of inflammatory cells.

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

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 microorganisms, 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 H2S 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 gave 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 at 10^4 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 diseased 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 1X10⁹ Cell/ml aerobically at 37C for 24h. while L.acidophilus cultivation on MRS 1X10⁶ 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 1.5X10⁸ 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 37⁰C 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 |
|---------------------------|--------------|--------------------|
| 1<sup>st</sup> group      | Non          | Non                |
| 2<sup>nd</sup> group      | (4% S. cerevisiae filtrate) | S. typhimurium |
| 3<sup>rd</sup> group:     | (4% of L. acidophilus filtrate) | S. typhimurium |
| 4<sup>th</sup> group      | (2% of L. acidophilus filtrate + 2% S. cerevisiae filtrate) | S. typhimurium |
| 5<sup>th</sup> group      | Non then infected experimentally with S. typhimurium | Fed by diet contain 2% of L. acidophilus filtrate + 2% S. cerevisiae filtrate |
| 6<sup>th</sup> group      | Non          | S. typhimurium      |

The fed continuous for seven days before and seven days after exposure to 1X 10<sup>6</sup> 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 *Salmonella 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<sub>2</sub>S), 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](image)

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, MnSO<sub>4</sub> and MgSO<sub>4</sub> (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                   |

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*.

Figure 2: Inhibitory zone occurred by L. acidophilus

Figure 3: Inhibitory zone occurred by S. cerevisiae.
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) |
|----------------------------|--------------|------------------|-------------------|-----------------|----------------------------------|
| 1<sup>st</sup> group       | Non          | Non              | Non               | Non             | 0                                |
| 2<sup>nd</sup> group       | (4% *S. cerevisiae* filtrate) | Non | *S. typhimurium* | Diarrhea, fever continuous for 3day | 1-5                              |
| 3<sup>rd</sup> group:      | (4% of *L. acidophilus* filtrate) | *S. typhimurium* | Non | 1-3                          |
| 4<sup>th</sup> group       | (2% of *L. acidophilus* filtrate + 2% 4% *S. cerevisiae* filtrate ) | *S. typhimurium* | Non | 1-2                          |
| 5<sup>th</sup> 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                          |
| 6<sup>th</sup> 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 lactobacilius able to decrease intestine pH, and produce inhibitor substance (H<sub>2</sub>O<sub>2</sub>, CO<sub>2</sub>, acetyldehyde, 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).](image)

![Figure 5: Cross section of Rat spleen infected with S.typhimurium, showing infeliteration of inflammatory cells. (H&EX40).](image)

Figure 4: Cross section of Rat spleen infected with S.typhimurium, showing infeliteration 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 inhibition effect. Results suggest use L. acidophilus filtrate + *Saccharomyces cerevisiae* filtrate in protective of experimental animal from *S.typhimurium* infection.

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