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The Antagonism of *Enterococcus Fecalis* Isolated from Kefir with *Salmonella Typhimurium* Caused Poultry Diarrhea in Vitro

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Abstract: ***This study was conducted to investigate the role of Enterococcus Fecalis which isolated from kefir in treatment of diarrhea chicken caused by Salmonella Typhimurium.Enterococcus Fecalis isolated from kefir on azide blood agar, Bile esculin agar several physiological and biochemical tests were conducted to identify of Enterococcus Fecalis. Intestinal samples were collected from chicks which were suffering from diarrhea from veterinary clinics in Baghdad and AL-Muthana province. Salmonella isolated on S.S agar and X.L.D agar three isolates were obtained belonging to the Salmonella out of (50 intestinal samples) based on the (Rapid ONE system) was identified as one Salmonella group and for complete serotyping identification the three isolates sent to the Central Public Health Laboratory. The result was one isolate was diagnosed as Salmonella serovarTyphimurium.Thetwo isolates of Enterococcus Fecalis appeared approximately similar antagonistic effect against Salmonella Typhimurium with (25 mm) inhibition zone of the A isolate and (22 mm) inhibition zone of B isolate.***

Keywords: Kefir, antagonism, probiotic, poultry diarrhea, *Enterococcus*

1. Introduction

The fowl manufacturing is now in front of a prohibition for the use of antibiotic feed additives for disease prevention and growth promoters where the presence of antibiotic residues in poultry products cause a threat to public human health when it consumed by human (Inborr, 2001), their application impairs the protective microflora of the gastrointestinal tract and significantly reduces the effectiveness of the intestinal barrier to infection. An alternative, is the use of probiotics which are products made from living microorganisms (Lee *et al*., 2008)defined probiotics as live microbial food supplements, which beneficially influence humans and animals, and that can promote bird health by reducing pathogen colonization. The microorganisms which considered as probiotics bacteria are non-pathogenic factor of microbiota and possess highly adherence ability on the gastrointestinal tract, also resistance to gastric juice and bile juice, such as lactic acid bacteria (Fuller, 1995; Ashraf *et al*., 2009). Kefir is a fermented milk produced by the inoculation of kefir grains into milk, followed by a fermentation period of about 1 day at room temperature. The grains contain a mixture of complex microflora such as lactic acid bacteria and yeast which are lodge by apolysaccharide matrix calls " kefiran", these microorganisms called probiotic. The kefir is respected one of the oldest probiotic dites. It possible that kefir was part of the sour milk preparation discussed by Metchnikoff in his early work describing probiotic and their advantages on the raised long life of some community of Bulgarian people (Powell, 2006). So aim of the study is study the role of *Enterococcus Fecalis* isolated from kefir in the treatment of the chicken diarrhea caused by *Salmonella Typhimurium*in vitro.

2. Material & Method

2.1 Kefir Sample

Kefir grains used were of Indian origin. Fermentation of milk by kefir grains was achieved by adding 50 g of kefir grain into 500 ml sterile cows milk insterile jar, incubation for 3 days at 28°C was achieved. The kefir grains from the fermented milk were collected by using a sterile sieve and rinsed with sterile water to be used as a starter for the next cycle of the fermentation process (Burcu and Alper, 2010)

2.2 Isolation of *Enterococcus Fecails*

Fermented kefir grain homogenates and kefir liquid were diluted in MRS broth medium and appropriate dilutions were spread on MRS agar and azidblood agar, then incubated an aerobically at 37°C for 24 h. A single colony was selected and cultivated on the azide blood agar at 45°C for 24 hr to obtain of pure colony according to (Holt *et al*., 1994).

2.3 Identification of*Enterococcus*

Identification of *Enterococcus* tested biochemically according to (Cowan, 1974; Sharpe, 1979; Manero and Blanch, 1999) in addition to it diagnosed byRapID™ STR System according to (Frauenhoffer and Duffett, 1986).

2.4 Isolation of *Salmonella Typhimurium*

The terminal part of the infected chicken intestines was taken and scrup the liner of intestine and placed in 10 ml pepton water medium, after incubation period at 37°C for 24 hr transferred 1ml of pepton water to 9 ml of selenite broth and incubated in 42°C for 24 hr. Drop from this medium was transferred by sterile loop to streaking on S.S. agar and incubated in 37°C for 24 hr. Single pure black center colony was taken and cultivated on (XLD,

MacConky agar) as selective media of *Salmonella* according to (Quinn *et al*., 2004).

2.5 Identification of *Salmonella Typhimurium:*

The isolates were identified biochemically and serologically according to (Cheesbrough, 2002).

2.6 Antagonisitic Activity of *Enterococcus Fecails* against *S. Typhimurium*in vitro by agar spot method

The test was conducted by agar spot method according to (Jacobsen *etal*., 1999) method. The *Enterococcus* isolates were propagated in MRS broth medium and incubated anaerobically at 37°C for 48h. For the agar spot test, 4 µL of the propagated *Enterococcus* isolates were spotted on the center of the surface of MRS agar medium, in triplicate and incubated anaerobically for 24 h at 37°C to allow colonies to grew. 100 µl of heavy growth from *S.Typhimurium*(approximately 10^7cfu/ml) in 15 ml of Nutrient agar were poured on the plate in which *Enterococcus* were grown, then incubates for 24 h at 37°C, after that measure the diameter of the inhibition zone around *Enterococcus.* The clear zone was used as an indication of the ability of theisolated *Enterococcus* to antagonize *S.Typhimurium.*

3. Results and Discussion

It was obtained two isolates belonging to *Enterococcus Faecalis*named as (*Enterococcus Faecalis* A and *Enterococcus Faecalis*B) from homogenized kefir grain and it liquid. *Enterococcus Faecalis*bacterium appear under the microscope as : Gram positive diplo cocci or short chain and sometimes appeared as ovoid cocci, non-motile when examined the motility. Cultivation of *Enterococcus Faecalis*bacterium on Azid blood agar appeared as round, white or creamy color colonies, have slightly convex with smooth margin whereas the colonies on Bile esculin agar appear as dark brown round colonies because of esculin presence which being complicated compound with ferrous in Ferric Ammonium Acetate compound one of constitute this medium. Bile esculin agar considered selective medium of the Streptococcus group D especially when incubation at 45°C the main two differentiated characters between the *Streptococcus* species (do not grow at 45°C) and *Enterococcus.*On MacConky agar the bacterium appear as cercal small pink colonies. MacConky agar cannot grow positive bacteria with the exception of *Enterococcus* genus because have ability to resistance of bile salt and crystal violate.

The biochemical tests that *Enterococcus Faecalis*was catalase negative, oxidase negative, highly tolerance with 4, 5, 6.5%NaCl, and grown at different temperature (10°C, 45°C) as well as pH=3 and 1% of bile salt and the production ammonia from argenin hydrolysis, the fermentationtests appeared the bacteria was fermented to (lactose, sucrose, mannitol, trehalose, maltose, rhamnose, ribose)and non-fermented to (melibiose, arabinose, raffinose).This results agree with (Yasin, 2009). The result of RapID™ STR panle test revealed that the microcode (60643) which gave diagnosis (99.9 %) in the ERIC(Electronic RapID™ Compendium) as *Enterococcus Faecalis* isolate.

Three bacterial, isolates obtained which belong to the Salmonella genus and one bacterial strain belonging *to Salmonella*serovar *Typhimurium* out of 50 intestinal sample of chicken suffered from diarrhea. Stained *S.Typhimurium* with Gram's stain appeared as Gram negative rod shape. *Salmonella Typhimurium* colonies appeared rounded, convex, pale with black center on Salmonella-Shigella agar whereas appeared red, smooth, with black center on Xylose-Lysine Deoxycholate agar. On MacConky agar the colonies appeared small, rounded pale color colonies because not ferment lactose sugar in medium. Results of the biochemical tests of *S. Typhimurium* showed that this bacterium was catalase negative, oxidase negative, indol negative, TSI (triple sugar iron) a positively indicator for *Salmonella spp*. in a result : pink (in slant) /yellow (in bottom) with gas and H2S production, urease negative, lactose non fermentive, in addition to utilization of citrate on Simmon Citrate agar by transformation medium color from green to blue, motile on SIM medium because of the defused growth in the medium. The result slide agglutination test is matching with the characterization of genus *Salmonella* as fixed in (Quinn *et al*., 2004) and (Brenner *et al*., 2005).

The antagonistic activity of *E.Faecalis*against *S.Typhimurium*:

The antagonistic assay of the two *E.Faecalis*isolates (*E. Faecalis*A and *E. Faecalis*B showed in (Table: 1). *E. Faecalis*A appeared inhibitory effect (Fig: 1) similar to that caused by *E.Faecalis*B.

Pathogen	Zone of inhibition (mm)	
Salmonella Typhimurium	*Enterococcus Fecalis A*	*Enterococcus Fecalis B*
	25	22

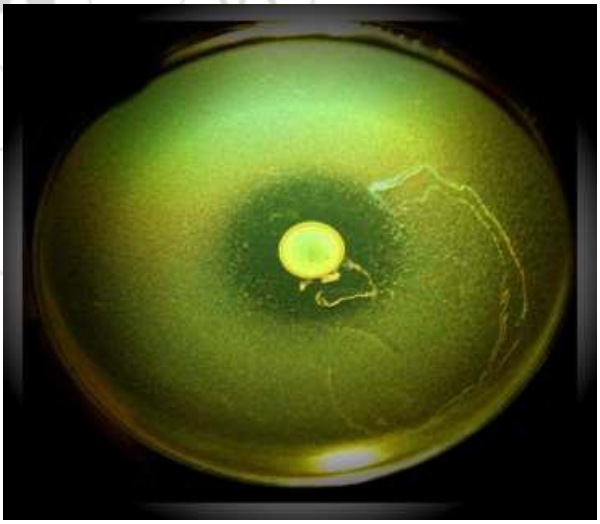

Figure 1: Positive antibacterial effect of *E.Fecalis*against *S.Typhimurium*

Enterococci may create antimicrobial proteins named bacteriocins(enterocins) which possess the possibility to limit the growth of microbes (Franz *et al*., 2007), these enterocins involved a variety of proteins in terms of size, microbial targets, manner of action and protection Mechanism (Riley and Wertz, 2002). In addition to that the production of enterocins, *E. Faecalis*also eliminated pH

value and made the gastrointestinal environment acidic by sugars fermentation and transformed to the lactic acid which inhibit pathogenic bacteria.

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