Synergistic Effect To Two Type Of Lactic Acid Bacteria And Their Synbiotic Effect With Prebiotic Raisin Against Some Pathogenic Bacteria

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Abstract :

The present study demonstrated the potential of using raisins as prebiotic a result of antibacterial activity shown by the aqueous extract of raisins against different types of bacteria .

Also this study conducted on two types of probiotic to know the synergistic effect to both of them together. Synbiotic (prebiotic and probiotic) also tested against test bacteria.

Results revealed that the highest activity in synbiotic & synergistic effect against *E.coli* and *Listeria* the zones of inhibition showed the range of (24-25) mm for this effect., While *staphylococcus aureus* only could resist this synergistic effect.

Prebiotic had the lower activity compared with others, so we conclude that the synbiotic effect is the better against all pathogenic bacteria in the study.

Keywords : Raisin, synergistic effect, Lactic Acid Bacteria and synbiotic.

Introduction:

Raisins are one of the dried fruit products made from grapes (Tabrizi *et al.*, 2014; Farah bakhsh *et al.*, 2015). The history of consumption of Raisin is very old, the Bible mention provides the first written of raisin around 1000 B.C (Alaskari *et al.*, 2012A).

Raisins contain polyphenols, antioxidants, flavonoids, vitamins, minerals and large amount of iron, that may benefit overall human health (Rivero-cruz *et al*.,2008).

The various phytochemicals reported in raisins include : triterpenes, fatty acids, flavonides, amino acids, hydroxycinnamic acids and 5 – hydroxymetheyl – 2 – furaldehyde and suppress the growth of pathogens (Williamson and carughi, 2010). Also the presence of oleanolic acid in raisins enhance antibacterial activity against pathogen (Yoon and Choi, 2010). Many of substances like tartaric acid, fructooligosaccharides (Fructans) and oleanolic acid (which Raisins are major dietary source) act as prebiotic (Altaee *et al*., 2014).

A prebiotic is a non-digestible food ingredient that affects beneficially host by selectively stimulating growth and ctivity of one or a limited number of bacteria in colonic micro flora, So prebiotics are a valid approach to dietary manipulation of colonic microflora (Agte *et al*., 2010). In the other side, Lactic Acid Bacteria (LAB) are a group of gram-positive bacteria including many of genera, within the group of LAB are (*Lactobacillus* and *Lactococcus)* species due to their potential beneficiary properties as probiotics (Baqer *et al.,* 2014 and Muhsin *et al*., 2015).

Probiotics are live microorganisms present in food and dietary supplements that beneficially affect the individual by improving the intestinal microbial balance properties (Mohsin et al., 2013). Also Lactobacillus and Lactococcus have been introduced as probiotics due to their health – promoting effects through their ability to increase the natural defenses of the host against enteropathogens by the production of antimicrobial substances or through competitive inhibition and expulsion of these pathogens (Jamalifar et al., 2011; Muhsin et al., 2005).

There are much studies about antimicrobial activities of raisins and L-A,B, but much less attention paid to use the raisins syrup activity as prebiotic and comparative with LAB activity (as probiotic) on pathogen, to be the beginning to use these natural materials (prebiotic and probiotic) as an alternative to antibiotics after extracted and purified.

To our knowledge, this is the first report of raisins and mixed growth of two Genus of LAB in this field of Research.

Methods :

1- **Food material:** Raisins (seedless) were purchased from local market to use as a prebiotic.

2- **Preparation of Raisins juice:** Raisins were cleaned and sterilized in a hot air oven at 50Cº for 24hr. Raisins (100g) were dipped in warm Distill water (100)ml for 1 Day, then were crushed by mixer and afterward filtered by filter paper. (Ghavide *et al.*, 2013). Stock solution (juice) was saved in refrigerator to use as prepiotic in antibacterial activities, in Agar well diffusion method.

3- **Probiotic source:** Reference Lactic Acid bacteria strains including *Lactobacillus* and *Lactococcus* were obtained from AL-Mustansiriay university / science collage / biology department / high studies / Laboratories that isolated from yoghurt and identified by Forbes and Danniel, (2007).

4- **Extraction of cell – free supernatant (CFS):** both of LAB isolates were cultured in MRS broth (MRS: Man – Rogosa – Sharpe) separately at 37Cº/24h.; cells were removed by centrifugation at 10,000 xg for 15min, pH of supernatants was adjusted to 6.5, supernatants were filtered through 0.22mm filter paper. CFS was prepared according to Al-Askari, *et al.*, (2012B) and used to as antibacterial activity agents using Agar well diffusion method.

5- **Pathogenic tested bacteria:** (16) isolates of Identified pathogenic bacteria were included in this study, they had been obtained from Imam – Ali hospital / Baghdad, these isolates were identified again by chemical test and the stock culture were kept in refrigerator 4Cº.

6- **Synergistic interactions Between CFS of *Lactobacillus* and *Lactococcus* Against pathogenic bacteria**: The_mixing (2.5)ml of each of the CFS (*Lactobacillus* and *Lactococcus*) in sterilized tube to use in antibacterial activity as synergistic effect between probiotic groups in Agar well diffusion method.

7- **Synbiotic effect between prebiotic and probiotic against pathogenic bacteria:** The mixing (2.5)ml of each of the probiotic (prepared above) and prebiotic (Raisins juice) in sterilized tube to use in antibacterial activity as synbiotic effect between prebiotic and probiotic against pathogenic bacteria in Agar well diffusion method.

8- **Antimicrobial activity:** antimicrobial screening of synbiotic and synergistic effect were detected by Agar well diffusion method of MH agar against following (16) bacterial cultures include *Pseudomonas, Acinetobacter, E.coli Klebseilla, Salmonella* (each of them 2 isolate), and *Streptococcus vibrio cholera , Serratia , Listeria , S.epidermedis , Saphylococcus aureus* (1 isolate).

The bacterial cultures were inoculated on Muller Hinton agar plates using sterilized cotton swabs. In each of these plates, wells were made using a sterilized pork borer. The 100*µl* of each of prebiotic, CFS probiotics (*Lactobacillus* + *Lactococcus)* and (prebiotic + probiotic) [to show the probiotic alone, Synergistic and Synbiotic effect respectively] inoculum were loaded into each well. Plates were incubated at 37Cº for 24hr. After incubation, all plates were examined for the presence of zone of inhibition around the wells and measured with mm. This method prepared according to sharma *et al*., (2013).

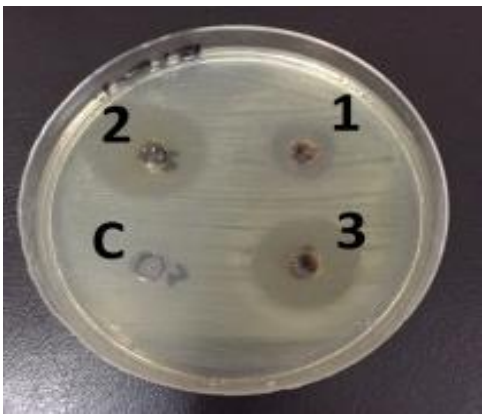
Result :

Results. The present study for in vitro antimicrobial activity was carried out on probiotic and prebiotic. Theses revealed the presence higher activity in Synbiotic effect against [***E.coli*** (1) and ***Listeria***] growth (25, 24)mm respectively. The zones of inhibition showed the range of (13-25mm) for Synbiotic effect. (Table and Figure 1)

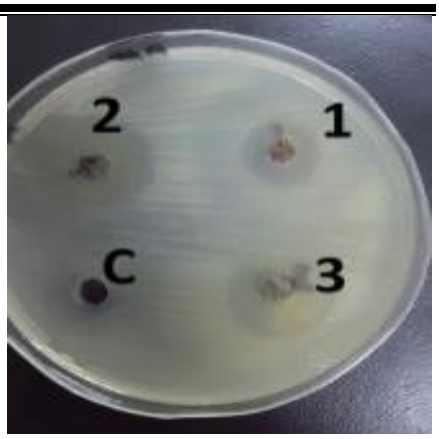
Table 1: Synbiotic, Synergistic and prebiotic effect against pathogenic bacteria.

No. of Isolate	Test bacteria	Zone of Inhibition (mm)		
		Synbiotic effect	Synergistic effect	Prebiotic effect
1	*Pseudomonas1*	20	21	18
2	*Pseudomonas2*	16	20	17
3	*Acinetobacter1*	18	17	15
4	*Acinetobacter2*	23	23	15
5	*E.Coli1*	25	25	13
6	*E.Coli2*	17	16	15
7	*Klebseilla1*	17	20	17
8	*Klebseilla2*	21	22	19
9	*Salmonella1*	19	22	12
10	*Salmonella2*	14	11	10
11	*Streptococcus spp*	18	19	18
12	*Vibrio cholera*	15	14	10
13	*Serratia*	16	15	14
14	*Listeria*	24	25	-
15	*S.epidermidis*	13	11	-
16	*S.aureus*	20	-	18

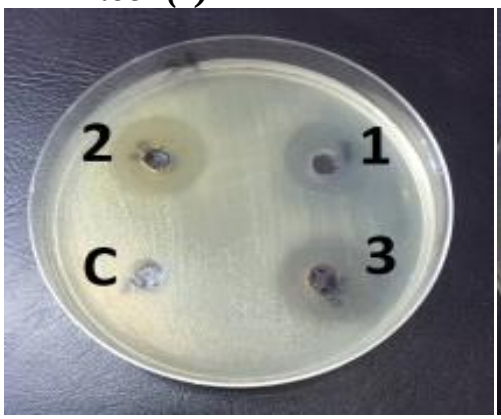
Synbiotic effect: (probiotic + prebiotic) effect
Synergistic effect: probiotic (*Lactobacillus* + *Lactococcus)*
Prebiotic effect: Raisins syrup
Inhibition zone: mm .



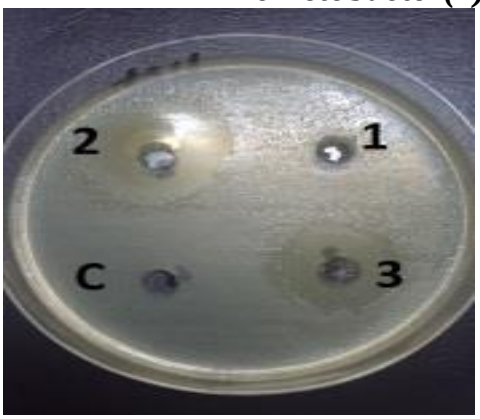
E.coli(1)



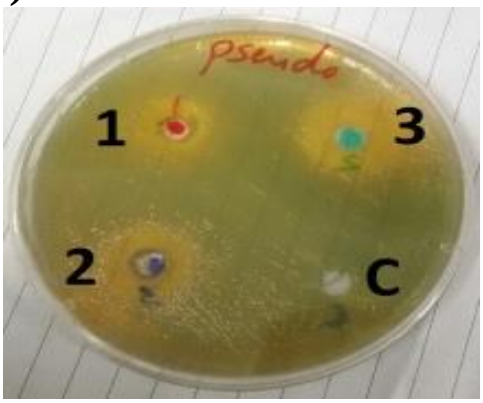
Acinetobacter(2)



Klebseilla(2)

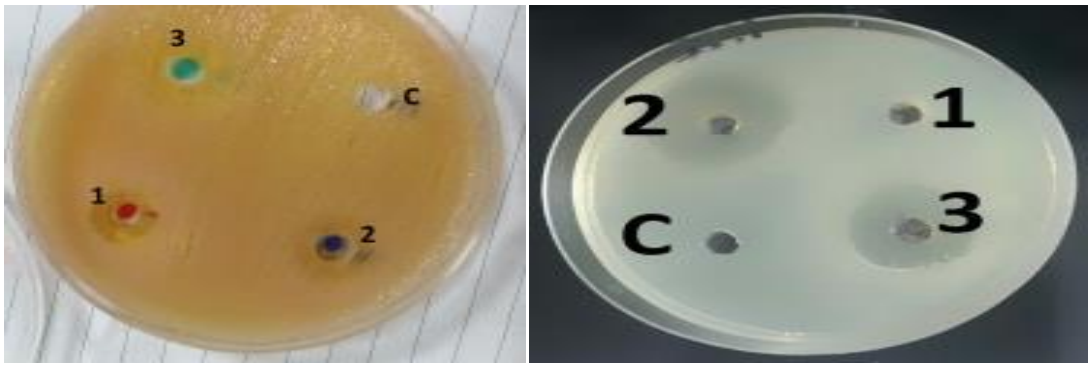


Listeria(2)



Pseudomonas(1)

Salmonella (1) ***Staphylococcus aureus***



Serratia ***Staphylococcus epidermidis(1)***

Streptococcus spp ***Vibrio cholera***

Figure 1: Synbiotic, Synergistic and prebiotic effects against pathogenic bacteria.

1. Prebiotic.
2. Probiotic ; Synergistic *(Lactobacillus + Lactococcus).*
3. Prebiotic + probiotic (Synbiotic) .
4. Control .

In the other side, the antibacterial activity of the synergistic effect was screened against 16 pathogenic bacteria which was found to be the highest inhibition against *E.coli* and *Listeria* growth also with (25) mm for both of them whereas that poor activity of Synergistic effect against *Salmonella* and *staphylococcus epidermides* with (11) mm just. All of test bacteria showed zones of inhibition ranged (11-25)mm. but *S.aureus* could resist this Synergistic effect.

There is no study about our results in the Synbiotic effect to can be compared with others, but our result, can be compared with study of sharma et al., 2013 and Dixit et al., 2013 that Confirmed the antimicrobial activity of probiotic against pathogenic bacteria can be ranged from moderate to high, because of the result of present investigation highlights that the antibacterial potentiality of the Symbiotic and Synergistic effect of probiotic and prebiotic.

Also our results agreed with many sources (Chowdhury *et al.*, 2012; Chakraborty and Bhowal, 2015)

Viability of *E.coli* and *Listeria* grown in Synbiotic and Synergistic effect from that *E.coli* and *Listeria* grown in prebiotic effect alone, whereas *E.coli* did not submit to this effect, but with 13mm, while *Listeria* and *S. epidermidis* did not response to its. The antibacterial activity of prebiotic against tested bacteria ranged (10-19) mm and the highest effect was against *Klebseilla* (19)mm. The prebiotic had activity (14) out of (16) test bacteria, include weak zones of Inhibition against *Salmonella* (10)mm. as in the table and figure above.

This results were agreed with Nirmala and Narend hira kannan., (2011) and serio, et al., (2014) about raisins antimicrobial activity against pathogenic bacteria that use as prebiotic.

Discussion:

The mechanisms of antibacterial activity in probiotic strains appears to be multi factorial and is due to bacteriocins and organic acid-produced by them (Cha M . et al., 2012; Takagi A. , *et al* 2015 & Dixit et al., 2013), also Sharma et al., (2013) showed that the probiotic activity due to the ability to prevent the growth of other pathogenic bacteria in the same media and its can prevents premature ageing and damage to cells.

In the other side, the activity of raisins as prebiotic was reported by several authors' as Al-sakari er al., (2012A) who reported that presence LAB isolates like *Lactobacillus Fermentum* and *Lactococcus lactis* in raisins are the cause of Activity because of these isolate (LAB) produce

bacteriocin like niacin, which isolated from LAB as antimicrobial agent. Khemariya et al., (2013) was agreed with Alsakari et al., (2012B) about *Lactococcus lactis* activity and Niacin production. So, raisin syrup was used as starter of sourdough bread (yuttwang and shyu, 2015).

Or, this prebiotic activity as cleared by Agte et al., 2010 that prebiotic through promoting growth of probiotic may also exhibit protective and preventive effects.

But vimal et al., (2013) have attributed the reason for the effectivenss of raisins alone (as prebiotic) to the presence of essential oils and oleanolic acid which confirmed their efficiency against gram positive and negative bacteria.

Inhibited growth of gram positive bacteria may be related to impaired cell wall metabolism caused by oleanolic acid because of the triterpenoids of oleanolic acid may influence the enzymes related to peptidoglycan metabolism such as the proteins (Kurek *et al.,* 2014).

In the gram negative bacteria in addition to gram positive bacteria, the cell membrane was damaged in oleanolic acid treated cells, resulting that oleanolic acid can kill bacteria by destroying the bacteria cell membrane (Kim et al., 2015).

There are no study on the impact of cooperative (Synbiotic effect) in order to be comparative or discussion, but from our point of view is natural that the fact that the probiotic and prebiotic are effective inhibitory, high damping occurs when measured alone, combine both types of active substances, whether prebiotic or probiotic to become the most powerful influence on the pathogenic bacteria.

Also from our point of view the variation in the degree of pathogenic bacteria inhibition may b due the conditions of culture and incubation in addition to the nature of strains itself or genetic nature that may create a genetic mutation in a certain isolate does not exist in others in spite of their belonging to the same Genus.

All of the reasons above give clear reasons for the activity types that obtained in our results.

Conclusions:

1- the higher activity was found in synbiotic effect of lactic acid bacteria and raisins .
2- the synergism effect to *lactobacillus* and *lactococcus* together was lower than synbiotic but higher than prebiotic used (Raisins).

3- lower activity was revealed by raisins against test bacteria compared with synergistic and synbiotic effect.

Recommendations:

The discovery of a potent antibacterial Agent from natural origion will be a great advancement in bacterial infection, Include prebiotic and probiotic materials, including oleanolic acid in prebiotic.

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التأثير التازري لنوعين من بكتيريا حامض اللاكتيك وتأثيرهما التعاوني مع المقوم الغذائي الزبيب ضد بعض البكتيريا الممرضة

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الخلاصة:

بينت الدراسة الحالية إمكانية استخدام الزبيب كـــــــ بريبايوتك (أغذيه محفزة للمعززات الحيويه) نتيجة الفعالية التثبيطيه التي اظهرها المستخلص المائي للزبيب ضد انواع مختلفة من البكتيريا.

كما اجريت الدراسة على نوعين من البروبايوتك (معزز حيوي) لمعرفة التأثير التازري بينهما معا" , وايضا " تم اختبار فعالية التأثير التعاوني لكل من البروبايوتك والبريبايوتك وكشفت النتائج عن اعلى فعالية للتأثير التعاوني والتأثير التازري ضد بكتيريا (اي كولاي) وال (ليستيريا) كانت مناطق التثبيط للفعالية واضحة بمعدل(25–24)ملم .بينما استطاعت المكورات العنقودية الذهبية فقط ان تقاوم التأثير التازري.

اعطى البريبايوتك اقل تأثير مقارنة بالاخرين , لذا يمكن ان نستنتج ان التأثير التعاوني هو الافضل ضد جميع البكتيريا المرضية المستخدمه في الدراسة.

الكلمات المفتاحية :

الزبيب , التأثير الـــتأزري , بكتيريا اللبنية , التأثير التعاوني.