

Synbiotic Effect of Probiotic (*Bifidobacterium sp*) and Prebiotics (Chicory and Inulin) against some pathogenic bacteria

Jehan A. S. Salman*

Date of acceptance 4 /12 / 2008

Abstract

The Antimicrobial activity of Probiotic *Bifidobacterium sp* and Prebiotics : chicory roots (Hot water extract) and Inulin (10 %) against some Pathogenic bacteria (*Escherichia coli* , *Proteus mirabilis* , *Klebsiella sp* , *Pseudomonas aeruginosa* , *Serratia marcescens* was studied .

The combination of Probiotic and Prebiotics (Synbiotic) (*Bifidobacterium sp* + chicory) and (*Bifidobacterium sp* + Inulin) also tested for their antimicrobial activity against Pathogenic bacteria .

Results showed that *Bifidobacterium sp* had good antimicrobial activity against all the Pathogenic bacteria tested , followed by chicory and inulin .

The synergistic inhibitory effect of Synbiotic (*Bifidobacterium sp* + chicory) and (*Bifidobacterium sp* + inulin) on Pathogenic bacteria was higher than the effect of *Bifidobacterium sp* alone, chicory alone and inulin alone.

Key words: Synbiotic,*Bifidobacterium sp*,Chicory,Inulin,Pathogenic bacteria

Introduction

In the last few years great attention was dedicated to Probiotics and Prebiotics or their combined use (Synbiotics) in the importance of human health in natural way [1].

Probiotics—a word derived from Latin and Greek meaning literally "for life"—has been defined in many ways since it was first coined 50 years ago. [2][3] The usefulness of probiotics is rapidly becoming apparent. Probiotics are usually bacterial components of the normal human intestinal flora, for example lactobacilli and bifidobacteria, that produces as an end products of metabolism lactate and short chain fatty acids such as acetate and butyrate [4].

Prebiotics are a more recent concept, first defined less than 10 years. [5] They are chemical substances, usually oligosaccharides, that act as substrates specifically for the host's intrinsic probiotic bacteria, and thus encourage their growth.

Prebiotics are selected as being non-digestible by the host and not metabolised by non-probiotic gut flora such as *Bacteroides spp* and *Escherichia coli*. *Prebiotics* serve as a food supply for the friendly bacteria of the large bowel (bifidobacteria and lactobacilli), enhancing their growth and cell division rate. The official definition of prebiotics is: "Nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one species or a limited number of species of bacteria in the colon " [6]

Prebiotics are available naturally in breast milk and in certain vegetables (for example, Jerusalem artichokes and onions), and as synthetic oligosaccharides based on fructose or galactose, known as FOS and GOS respectively [7]. Many foods naturally high in inulin or oligofructose, such as chicory , garlic and leek , have been

*Lecturer/ Department of Biology – College of Science - AL- Mustansiriyah University

seen as "stimulants of good health" for centuries [8].

Chicory (*Cichorium intybus*), a member of the sunflower family, produces a large tapered root which has been used for many years for its beneficial effect on the human digestive system. Chicory is prebiotic which becomes a food source for the growth of probiotics and in this way supports the natural functioning of the digestive system and can help reduce levels of harmful bacteria [9].

A *synbiotic* refers to a product in which a Probiotic and a Prebiotic are combined. Both probiotics and prebiotics may be helpful in malnutrition, particularly in lactose intolerance and calcium absorption, and in constipation [5], and prevent gastrointestinal diseases in human and animals [1]. Combining probiotics and prebiotics into "synbiotics" will further enhance the immunosupportive effects [10].

The aims of this study were to assess the effect of a combination of prebiotic and probiotic (Synbiotic) on some pathogenic bacteria

Materials and Methods

***Bifidobacterium* isolate :**

Bifidobacterium sp. was isolated from Activia yoghurt product. It was identical according to [11] by using the cultural, microscopical and biochemical examinations. The isolate was grown in De Man Rogosa Sharpe (MRS) broth for 24 h. at 37 °C.

Pathogenic Bacteria :

Isolates of *Escherichia coli*, *Proteus mirabilis*, *Klebsiella sp.*, *Pseudomonas aeruginosa*, *Serratia marcescens* were collected from different infection sources from Central Medicine City hospital in Baghdad. Isolates were identified according to [12] by classical microbiological methods and API 20-E system.

Preparation of chicory extract :

Chicory root (*Cichorium intybus*), were obtained from North of Iraq. Roots samples were homogenized with water (1:2 w/v) and heated at 120 °C for 20 min. (1 atm.); the treated plant material was then filtered [13].

Synbiotic effect on Pathogenic bacteria:

Antimicrobial activity of Probiotic (*Bifidobacterium sp.*), Prebiotics (chicory roots (Hot water extract)) and Inulin (10%) and a combination of probiotic and prebiotics (Synbiotic): (*Bifidobacterium sp.* + chicory) and (*Bifidobacterium sp.* + Inulin) against Pathogenic bacteria was tested by using agar diffusion assay according to [14]:

Pathogenic bacteria cultures were plated on fresh Nutrient agar plates (10⁵ CFU/ml per plate), and wells were prepared into the agar by using sterile Pasteur pipettes. 50 µl aliquots of fresh *Bifidobacterium sp.* culture alone, chicory extract alone, Inulin solution (10%) alone, *Bifidobacterium* culture + chicory extract (1:1), *Bifidobacterium* culture + Inulin (1:1) were suspended in the agar wells. Plates were incubated for 24 h. at 37 °C, and the diameters of inhibition zones around the wells were measured.

Statistical analysis:

Results have been analysed statistically using ANOVA test. Acceptable level of significance was considered to be below 0.05.

Results and Discussion

The Antimicrobial activity of Probiotic *Bifidobacterium sp.* and Prebiotics (chicory and Inulin) and the Synbiotic effect of Probiotic and Prebiotics (*Bifidobacterium sp.* + chicory), (*Bifidobacterium sp.* + Inulin) against Pathogenic isolates was tested. Antimicrobial activity of

Bifidobacterium cell , chicory extract and inulin was observed against *E.coli* (Inhibition zones , 17 , 12 , 11 mm) respectively . The antimicrobial activity increased to (20 , 19 mm) with significant differences ($P > 0.05$) when Synbiotic (*Bifidobacterium sp* + chicory) and (*Bifidobacterium sp* + Inulin) was used (Fig . 1).

Figure 2 shows the antimicrobial activity of *Bifidobacterium sp*, chicory , inulin and Synbiotics [(*Bifidobacterium sp* + chicory) and (*Bifidobacterium sp* + Inulin)] against *Proteus mirabilis* . *Bifidobacterium sp* had good inhibitory effect with significant differences ($P > 0.05$) (inhibition zone 15 mm) , followed by chicory and inulin (inhibition zones 11 mm). Synbiotics (*Bifidobacterium sp* + chicory) and (*Bifidobacterium sp* + Inulin) had the highest inhibitory effect , as observed by the formation of a large inhibition zones (22 mm) with significant differences ($P > 0.05$).

Figures 3,4,5 shows the inhibitory effect of *Bifidobacterium sp* , chicory , inulin and Synbiotics [(*Bifidobacterium sp* + chicory) and (*Bifidobacterium sp* + Inulin)] against *Klebsiella sp* , *Serratia*

marcescens and *Pseudomonas aeruginosa* . *Bifidobacterium sp* showed inhibitory effect , as observed by the formation of inhibition zones (16,16,17 mm) against pathogenic bacteria respectively .Among the chicory and Inulin tested for their antimicrobial activity against tested pathogenic bacteria , chicory shows inhibition zones (15,11,11) mm respectively ,and inulin shows inhibition zones (14, 11,10) mm respectively , no significant differences was observed between antimicrobial activity of *Bifidobacterium sp*, chicory and Inulin against *Klebsiella sp* while significant differences was found against *Serratia marcescens* and *Pseudomonas aeruginosa* .

The synergistic effect of Synbiotic (*Bifidobacterium sp* + chicory) (inhibition zones 21,26,21 mm) and (*Bifidobacterium sp* + inulin) (inhibition zones 19,21,20 mm) was higher than the effect of *Bifidobacterium sp* alone, chicory alone and inulin alone with significant differences ($P > 0.05$) against *Klebsiella sp* , *Serratia marcescens* and *Pseudomonas aeruginosa* respectively .

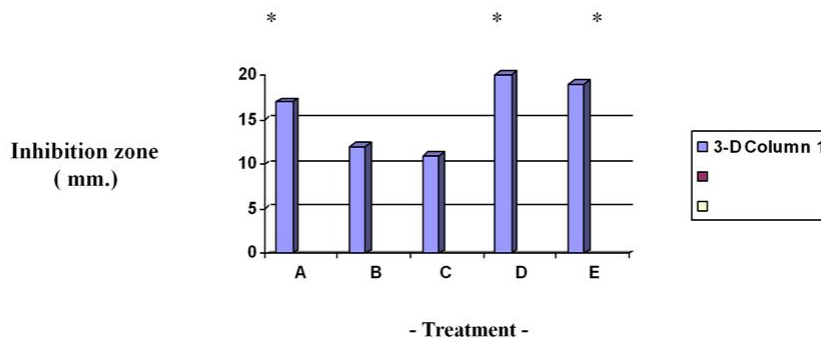


FIG. 1. Synbiotic effect against *E.coli* (A) *Bifidobacterium sp*. culture (B) chicory extract (C) Inulin(10%) (D) *Bifidobacterium* + chicory (E) *Bifidobacterium* + Inulin

* Significant differences ($P > 0.05$)

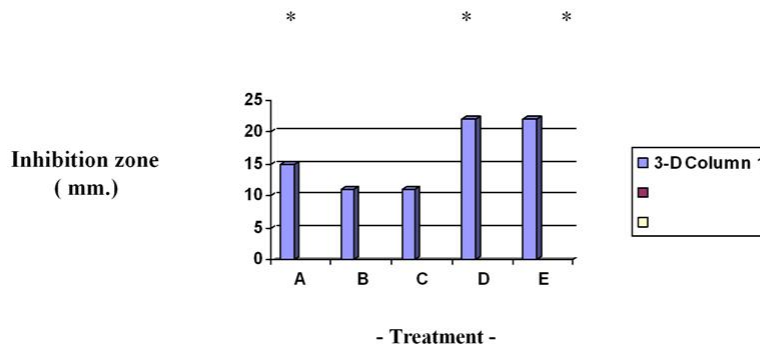


FIG.2. Synbiotic effect against *Proteus mirabilis*(A) *Bifidobacterium sp.* culture (B) chicory extract (C) Inulin(10%) (D) *Bifidobacterium* + chicory (E) *Bifidobacterium* + Inulin
* Significant differences (P >0.05)

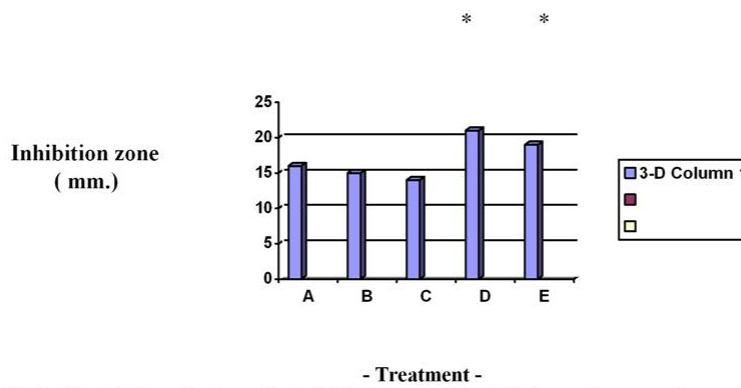


FIG. 3. Synbiotic effect against *Klebsiella sp.*(A) *Bifidobacterium sp.* culture (B) chicory extract (C) Inulin(10%) (D) *Bifidobacterium* + chicory (E) *Bifidobacterium* + Inulin
* Significant differences (P >0.05)

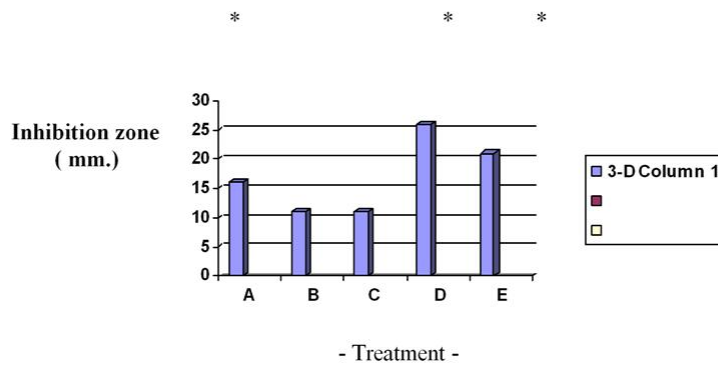
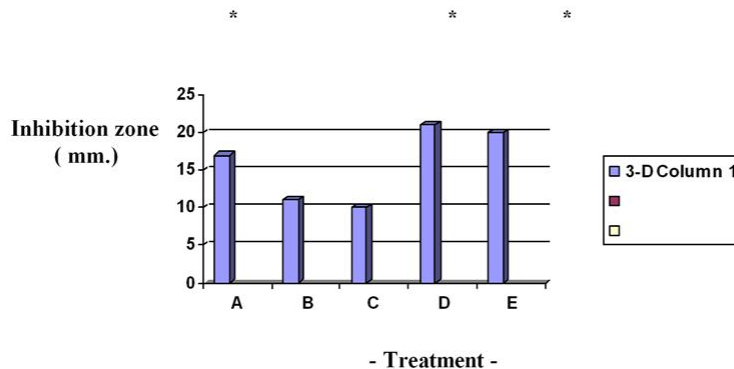


FIG. 4. Synbiotic effect against *Serratia marcescens* (A) *Bifidobacterium sp.* culture (B) chicory extract (C) Inulin(10%) (D) *Bifidobacterium* + chicory (E) *Bifidobacterium* + Inulin
* Significant differences (P >0.05)



- Treatment -
FIG. 5. Synbiotic effect against *Pseudomonas aeruginosa* (A) *Bifidobacterium sp.* culture (B) chicory extract (C) Inulin(10%) (D) *Bifidobacterium* + chicory (E) *Bifidobacterium* + Inulin
 * Significant differences (P > 0.05)

Results of the study showed the antimicrobial activity of *Bifidobacterium sp.* against pathogenic bacteria. This may be due to the production of organic acids (acetic and lactic) that lowered the pH of the medium [15], and production of other antimicrobial compound such as bacteriocin that acted as antibiotic agent [14].

Elmer *et al.* [16], Chuayana *et al.* [17] and Reyed [18] showed that *Bifidobacteria* inhibit the growth of many harmful bacteria *Salmonella*, *Shigella*, *Clostridium*, *Staphylococcus aureus*, *Candida albicans*, *Campylobacter jejuni*, *E. coli*, *Klebsiella* and *Bacillus cereus*.

Result also showed the antimicrobial activity of chicory extract. This may be due to the chicory content, inulin, a form of dietary fiber and substances called oligosaccharides that are thought to stimulate the growth and/or activity of beneficial intestinal microorganisms [9].

The inulin acts as "prebiotic" promoting selective development of

beneficial microorganisms "probiotic" [5]. It support growth of *Bifidobacterium sp.* and enhanced it to produce antimicrobial compounds acetic, lactic and benzoic acid and Bacteriocin type compound [19]. On the other hand, inulin reduce the amount of harmful bacteria such as *Bacteroides*, *Fusobacteria* and *Clostridia* [20].

Our results demonstrated that *Bifidobacterium sp.* had the highest inhibitory effect against pathogenic bacteria, followed by chicory and Inulin. The synergistic effect of Synbiotic (*Bifidobacterium sp.* + chicory) and (*Bifidobacterium sp.* + inulin) on pathogenic bacteria were higher than the effect of *Bifidobacterium sp.* alone, chicory alone and inulin alone.

References

1. Suskovic, J.; Kos, B.; Goreta, J. and Matosic, S. 2001. Role of lactic acid bacteria and *Bifidobacteria* in Synbiotic effect. Food Technol. Biotechnol., 39 (3):227—235.

2. Jenkins, B.; Holsten, S.; Bengmark, S. and Martindale, R. 2005. Probiotics: A Practical Review of Their Role in Specific Clinical Scenarios. Nutrition. in Clin. Prac., 20(2): 262-270.
3. Reid, G.; Sanders, M.E. and Gaskins, H.R. 2003. New scientific paradigms for probiotics and prebiotics. J. Clin. Gastroenterol. 36:105-18.
4. Anderson, A.D.; McNaught, C.E.; Jain, P. and MacFie, J. 2004. Randomised clinical trial of synbiotic therapy in elective surgical patients Gut; 53:241-245.
5. Hamilton-Miller, J.M. 2004. Probiotics and prebiotics in the elderly. Postgraduate Medical J. ;80:447-451.
6. Biradar, S.S.; Patil, P.M. and Rasal, V.B. 2005. Prebiotics For Improved Gut Health. The Internet J. of Nut. and Well. 2 (1).
7. Rastall R.A. and Gibson, G.R. 2002. Prebiotic oligosaccharides: evaluation of biological activities and potential future developments. In: Tannock GW, ed. *Probiotics and prebiotics: where are we going?*. Wymondham: Caister Academic Press, :107-48.
8. Roberfroid, M. 2007. "Introducing inulin-type fructans." Br. J. Nutr. 93 Suppl 1: S13-25.
9. Hillestand, K. 2007. Pet food additives : Prebiotics, Probiotics, Kelp and Yeast. PedEducation. Com.
10. Stig Bengmark, S. and Martindale, R. 2005. Probiotics and Synbiotics in Clinical Medicine. Nutr. in Clin. Practice, 20(2): 244-261.
11. Wood, B.J. and Holzapfel, W.H. 1995. The Genera of Lactic Acid Bacteria.
12. Greenwood, D.; Slack, R.C. and Peutherer, J.F. 2002. Medical Microbiology. (Sixteenth ed.). Churchill Livingstone.
13. Brirao-de-Costa, M.L.; Januario, M.I.; Antonio, E.B. and Simao, F.M.L. 2005. Characterisation of inulin from chicory and salsify cultivated in Portugal. 2^{ed}. Mercosur Congress of Chemical Engineering.
14. Ibrahim, S.A. and Salameh, M.M. 2001. Simple rapid method for screening antimicrobial activities of *Bifidobacterium sp* of human isolates. Rapid Methods and Automation in Microbiology. 9: 52-63.
15. Beskorovainy, A. 2001. Probiotics : Determinants of survival and growth in the gut. Am. J. Clin. Nut., 73:399-405.
16. Elmer, G.W., Surawicz, M., and McFarland, L.V. 1996. Biotherapeutic Agents: Review, a neglected modality of the treatment and prevention of selected intestinal and vaginal infections. J. Am. Med Assoc. 275(11):870-876.
17. Chuayana, E.L.; Ponce, C.V.; Rivera, R.B. and Cabrera, E.C. 2003. Antimicrobial activity of Probiotics from milk products. Phil. J. Microbiol. Infect.
18. Reyd, R.M. 2007. The role of Bifidobacteria in Health Research of J. Med. and Med. Sci. 2(1):14-24.
- 19) **Tungland, B. C.** 2000. Inulin- A Comprehensive Scientific Review. [Duncan Crow HOME](#).
20. **COLONIC Plus -INULIN**. 2006. National Library of Medicine- PubMed.

تأثير الملتحقات للمعزز الحيوي (*Bifidobacterium sp*) والمقومات الغذائية
(الهندباء البرية والأنبولين) تجاه بعض البكتريا المرضية

جيهان عبد الستار سلمان*

*كلية العلوم /الجامعة المستنصرية/قسم علوم الحياة.

كلمات مفتاحية: الملتحقات ، بكتريا *Bifidobacterium sp*، الهندباء البرية، الأنبولين ، البكتريا المرضية

الخلاصة:

درست الفعالية ضد المايكروبية للمعزز الحيوي (Probiotic) *Bifidobacterium sp* والمقومات الغذائية (Prebiotics) التي شملت المستخلص المائي الحار لجذور نبات الهندباء البرية (*Cichorium intybus*) والأنبولين (10 %) تجاه بعض البكتريا المرضية : *Esherichia coli* , *Proteus mirabilis* , *Klebsiella sp* , *Pseudomonas aeruginosa* , *Serratia marcescens* , كما درست الفعالية ضد المايكروبية التآزرية للمعزز الحيوي والمقومات الغذائية الملتحقات (Synbiotic) والتي تضمنت (بكتريا *Bifidobacterium sp* + مستخلص جذور نبات الهندباء البرية (*Cichorium intybus*) و (بكتريا *Bifidobacterium sp* + الأنبولين) تجاه البكتريا المرضية . أظهرت النتائج أمتلاك بكتريا *Bifidobacterium sp* فعالية ضد مايكروبية جيدة تجاه جميع البكتريا المرضية قيد الدراسة ، تليها في ذلك الهندباء البرية والأنبولين، وكان التأثير التثبيطي التآزري للملتحقات (بكتريا *Bifidobacterium sp* + الهندباء البرية) و (بكتريا *Bifidobacterium sp* + الأنبولين) تجاه البكتريا المرضية أعلى من تأثير كل من *Bifidobacterium sp* لوحدها ومستخلص جذور نبات الهندباء البرية والأنبولين ، كلا على أفراد.