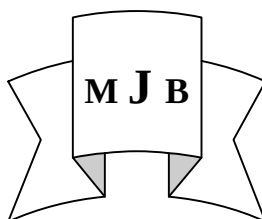


Extracellular Products of *Lactobacillus acidophilus* as Probiotic Against Multi-drugs Resistant *Pseudomonas aeruginosa*.

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**Abstract**

In this study in vitro potencies of some antibiotics were compared singly and in combination with probiotic released by *Lactobacillus acidophilus* isolated from local yoghurt against multi-drugs resistance *Pseudomonas aeruginosa*. Doxycycline, chloramphenicol and ciprofloxacin were randomly selected for this study. *Lactobacillus acidophilus* had characterized according to its specific characteristics; e.g. colonial morphology, cellular morphology and biochemical tests. Crude product of this organism in Modified Rogosa sharpe broth medium was used as probiotic against multi-drug resistant *P.aeruginosa* being isolated from sever infected burns. The probiotic and antibiotics included in this study were used separately and in combination against *P.aeruginosa*. Combination of probiotic with chloramphenicol resulted in inhibition zone of 24 mm. in diameter compared with 9mm and zero for each of probiotic and chloramphenicol alone respectively. It is of synergy action. Combination of probiotic with doxycycline revealed an inhibition zone of 19 mm in diameter versus 9 mm for each of probiotic and doxycycline alone when used separately indicating for synergy action as well. A third combination included ciprofloxacin with probiotic yielded an inhibition zone of 24 mm. in diameter while ciprofloxacin alone revealed an inhibition zone of 30 mm. indicating for antagonistic action.

This paper is a part of M.Sc. thesis, College of Medicine, University of Babylon. The comprehensive data are included in the thesis (in press).

Introduction

Lactobacillus acidophilus is Gram-positive facultative anaerobic or microaerophilic bacterium. It is the major species among lactic acid bacterial group and has the ability to tolerate the high acidity [1]. This organism named as such because it converts lactose and other sugars to lactic acid which is the principle end products of carbohydrates metabolism [2]. In

humans *L. acidophilus* is present as symbiotic normal flora in the vagina, oral cavity and gastrointestinal tract [3]. The production of lactic acid makes its environment acidic, which inhibits the growth of harmful bacteria [4]. Moreover some studies demonstrated the protective effects of some strains of *Lactobacilli* against some syndromes, since they act as anti-tumor [5].

Lactobacillus acidophilus is regarded as probiotic, since it competes and interferes with adhesion of other microbial pathogens in addition to its ability to produce H_2O_2 , bacteriocin, lactic acid and other molecules which act as inhibitors for other microorganism. According to the currently adopted definition by food and agricultural organization /world health organization [6], probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". Today, specific health effects of probiotics are being investigated and documented including alleviation of chronic intestinal inflammatory diseases [7], prevention and treatment of pathogen-induced diarrhea, and urogenital infections [8]. Thus, there is a need to find new ways to control evolving drugs resistant *P. aeruginosa* infections and embark on the need for a continued search for new antimicrobial compounds. In present study investigate the use of probiotic in combination with antibiotics as a therapeutic strategy.

Materials and Methods

Three isolates of *Lactobacillus acidophilus* isolated from local yoghurt and fully identified and characterized according to the routine procedures [9]. The crude extracellular products of *Lactobacillus acidophilus* grown anaerobically in Modified Rogosa Sharpe (MRS) broth at 37 Co

for 24 hrs were separated by centrifugation at 6000 rpm for 15 min. The supernatant was Millipore filtered and the PH was adjusted at 6.5 [10], and used as probiotic alone and together with some selected antibiotics against one isolate of *P. aeruginosa* isolated from severe infected burns and diagnosed as a multi-drugs resistant [11, 12].

Culture media: MRS broth and MRS agar (Himedia) were used as selective media for *Lactobacillus* spp [13]. Muller-Hinton agar (Oxoid) was used for detection the antibiotics susceptibility by wells diffusion in agar [1] and the results were interpreted according to CLSI documents (CLSI, 2010). The following antibiotics were used Doxycycline, chloramphenicol and ciprofloxacin (BIDOX- India).

Results and Discussion

Figure-1 below shows the result of doxycycline, probiotic and doxycycline-probiotic combination against *P. aeruginosa*. Doxycycline and probiotic alone revealed weak action against this organism when tested per se since the inhibition zone were 9 mm in diameter for each, while the inhibition zone increased up to 19mm. in diameter when doxycycline and probiotic combined together, which indicates that the effect of this combination was synergistic against *P. aeruginosa* which exhibited remarkable sensitivity according to standard records (CLSI, 2010).

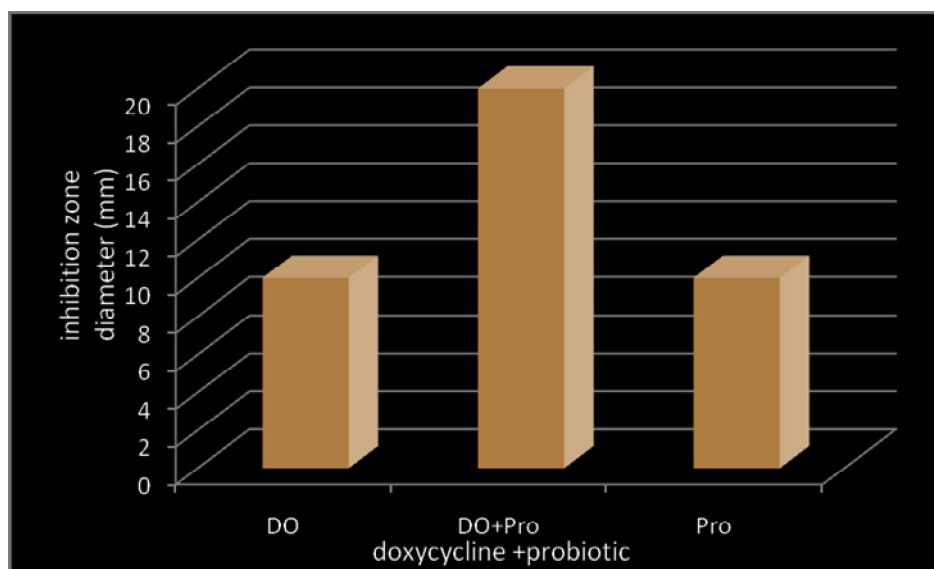


Figure 1 The effect of doxycycline and probiotic alone and in combination against *P. aeruginosa*.

The test organism (*P. aeruginosa*) revealed fully resistance (no inhibition zones) towards Chloramphenicol (figure-2). However, it became quite sensitive, and the inhibition zone went up to 20 mm in diameter when chloramphenicol combined with probiotic and therefore the action of this combination is clearly synergy as well [14]. Results also revealed that ciprofloxacin showed antagonistic action when combined with probiotic, since the inhibition zones to ciprofloxacin and probiotic were 30 mm and 9 mm in diameter respectively but the inhibition zone to combination of ciprofloxacin and probiotic decreased to 24mm in diameter as shown in (figure-3). The synergistic effect resulted from combination probiotic with each of

ciprofloxacin and chloramphenicol attributed to bacteriocin that forming pore in cell membrane channels. through the 'barrel-stave' mechanism. That has a bactericidal effect on sensitive organisms by dissipating the proton motive force, inhibiting the transport of amino acids, and causing the leakage of essential compounds by the formation of pores in the cytoplasmic membrane [15]. Although some of the bacteriocins have been shown to act in a bacteriostatic manner. Bactericidal activity of bacteriocins may be accompanied by lysis of sensitive cells (bacteriolytic bacteriocins), which allow pass the antibiotics through pores formed in cell membrane which increase concentration of antibiotic inside the cell [16].

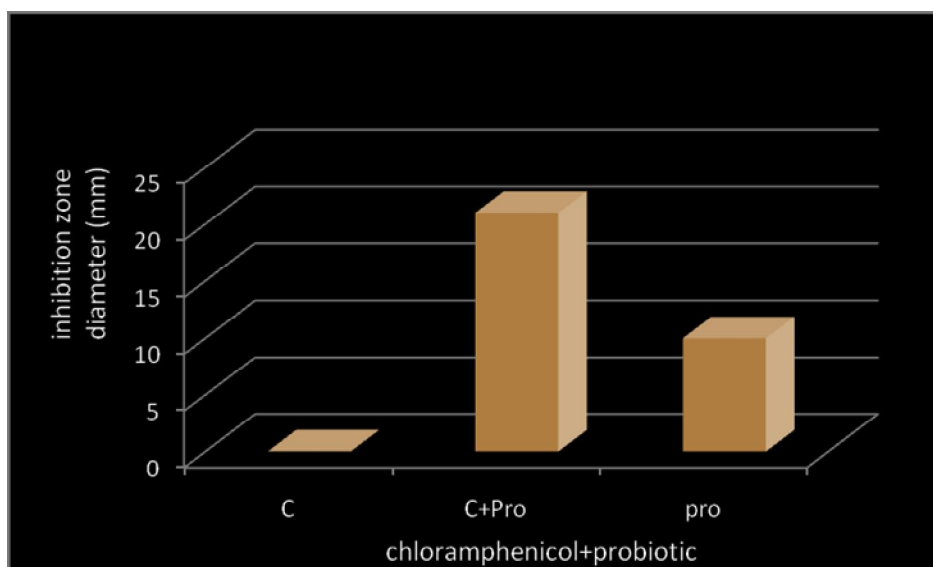


Figure 2 The effect of chloramphenicol and probiotic alone and in combination against *Pseudomonas aeruginosa*.

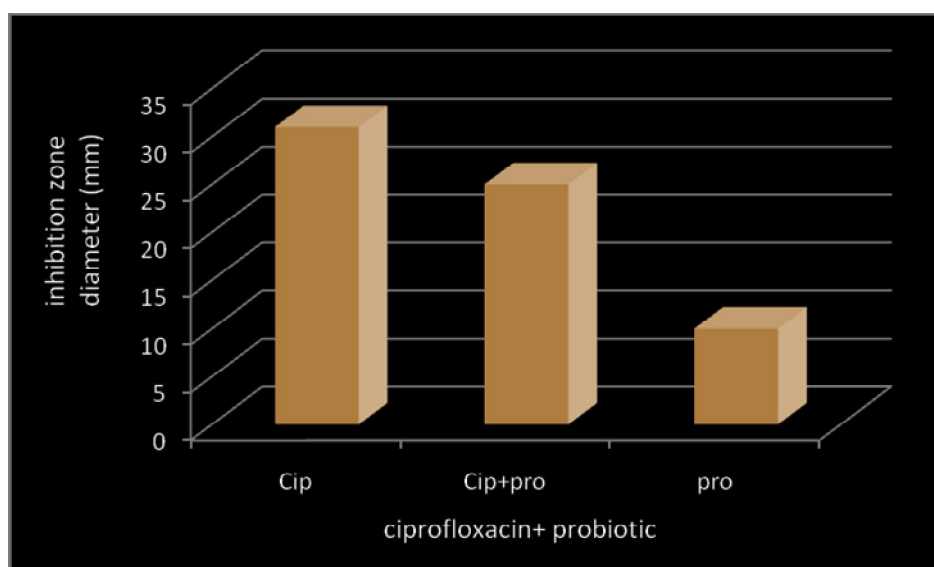


Figure 3 The effect of ciprofloxacin and probiotic singly and in combination against *P.aeruginosa*.

Pseudomonas aeruginosa has been selected in this study because it possesses intractable chemotherapeutic problem as it has unusually

high intrinsic resistance to many antibiotics. This inherent resistance seems to be associated with the permeability of the complex outer

layers of the cell envelope to some drugs, which prevents the attainment of an inhibitory concentration within the cell. The non-specific resistance of Gram-negative bacteria is recognized as a limitation in the treatment of infections of these organisms. However, the general pattern of resistance is well known and stable, so that drugs are prescribed of which the infecting organism are not inherently resistant and releasing B-Lactamases that destroy the antibiotic. It seems that doxycycline, chloramphenicol and probiotic act against *P.aeruginosa* by same mechanism when used separately and *P. aeruginosa* easily resist or tolerate this action but this action became synergistic when probiotic combined with each of doxycycline or chloramphenicol and the mechanism resulted in sensitivity of this test organism. In conclusion, extracellular products of *lactobacillus acidophilus* can be used as probiotic to support the potency of antibiotics against bacterial pathogens.

References

1. Forbes, B.A., Daniel, F.S., and Alice, S.W. (2007). *Bailey and Scott's diagnostic microbiology*. 12th. ed. ,Mosby Elsevier company , USA.
2. Abedon, S. T.; (1998). "Supplemental Lecture".mansfield.osu.edu. sabedon.bio:12020.
3. Dick, L.T.M.; Silvester, M.; Lawson, P.A. & Collins, M.D. (2000). *Lactobacillus fornicalis* sp. nov., isolated from the posterior fornix of the human vagina. *Int. J. Syst. Evol. Microbial.*, 50: 1253-1258.
4. Ljungh, ?; Wadstr?m, T.; (2009). *Lactobacillus Molecular Biology: From Geno. to Probiotic*. Cai .Aca. Pr. 1:41-47
5. Lacto Chen, X.; Fruehauf, J.; Goldsmith, J. D.; Xu, H.; Katchar, K. K.; Koon, H. W.; Zhao, D.; and Kokkotou, E. G.(2009). "Saccharomyces boulardii Inhibits EGF Receptor Signaling and Intestinal tumor growth inapcmin mice". *Gastroenterology*. 137 : 914-923.
6. FAO/WHO. (2001). "Health and Nutritional Properties of Probiotic in food including Powder Milk with Live Lactic Acid Bacteria". Food and Agriculture Organization of the United Nations, World Health Organization.
7. Mach,T., 2006). "Clinical usefulness of probiotics in inflammatory bowel diseases" *Official. J. of the Pol. Phy. So.* 57: 23–33.
8. Reid, G., (2008). "Probiotic Lactobacilli for urogenital health in women". *J. Clin. Gastroenterol.* 42: S234–s236).
9. MacFaddin, J.F.(2000). *Biochemical tests for the identification of medical bacteria*. 3rd ed. The Williams. and. Wilkins-Baltimore, U.S.A.
10. Schillinger, V. and Luch, F. K. (1991) Antibacterial activity of *Lactobacillus sake* isolated from meat. *J.APPL. Environ. Microbial.* 55:(8) 1901-1905.
11. Weinstein, R. A.; Nathan, G. and Gruen, S. (1980). Endemic amino glycosides resistance in gram-negative bacilli: epidemiology and mechanisms. *J. Infect .Dis.* 141: 338 – 345.
12. Gotoh, N.(2001). Antibiotic Resistance Caused by Membrane Impermeability and Multi-Drug Efflux

Systems. Nippon . Rinsho, 59: 712 – 718.

13. DeMan, J. C., Rogosa, M. and Sharpe, M. E. (1996). A medium for the cultivation of Lactobacilli. J. APPI. Bacteriol., 23 (1): 130-135.

14. Aboud, Z.M.(2001).The effect of antibiotic combination on Staph.aureus and P.aeruginosa .M. Sc. Thesis in Microbiology.College of Science.Kufa Univ.(In Arabic).

15. Tahara, T and Kanatani, K. (1996). Isolation, partial characterization and mode of action of a bacteriocin produced by Lactobacillus acidophilus JCM 1229. J. Appl. Bacteriol. 81: 669-677.

16. Deraz, S., Karlsson, N.E., Khalil, A. A and Mattiasson, B. (2007). Mode of action of acidocin , a bacteriocin produced by the potential probiotic strain, Lactobacillus acidophilus DSM 20079. J. Ind. Microbiol. Biotechnol. 34: 373-379.