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# Antimicrobial Activity of Metabolites of Various Strains of *Lactobacillus acidophilus*

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***Abstract*— The antimicrobial activity of metabolites of eight strains of *Lactobacillus acidophilus* (FTDC 2804, FTDC 0785, FTDC 8592, FTDC 1295, FTDC 4793, FTDC 4462, FTDC 0582 and FTDC 2916) against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative), was examined and compared using agar well diffusion method. *Lactobacillus acidophilus* was cultivated in two different types of dairy growth medium namely, full cream milk and skim milk. The results showed that the metabolites of all the eight strains had significant antimicrobial effect based on zone of inhibition results when compared to control. There was a statistically significant difference in the zone of inhibition data for *Staphylococcus aureus* and *Escherichia coli* among the metabolites of the eight strains cultivated in the two different growth medium. Certain *L. acidophilus* strains were more effective against *Staphylococcus aureus*, while other strains were more effective against *Escherichia coli*. On the other hand, the growth medium had no significant influence on the antimicrobial effect of metabolites of seven strains except *L. acidophilus* FTDC 4462 against *Escherichia coli*. As for *Staphylococcus aureus*, the growth medium only affected the antimicrobial effect of metabolite of strain *L. acidophilus* FTDC 1295, but did not affect the antimicrobial effect of metabolites of the other seven strains. It can be concluded that *L. acidophilus* cultivated in dairy products produced metabolites with antimicrobial property, which could provide beneficial medicinal values to human.**

***Keywords*: *Lactobacillus acidophilus*, growth medium, antimicrobial activity.**

## I. Introduction

Probiotics is a broad term that refers to the concept of feeding live microbial organisms to human beings with the goal of preventing and treating diseases. The concept of probiotics progressed around 1900, when Elie-Metchnikoff hypothesized that the long and healthy lives of Bulgarian peasants were the outcome of their consumption of fermented milk and milk products [1]. The ability of probiotics to withstand the normal acidic conditions of the gastric juices and the bactericidal activity, as well as the production of lactic acid that inhibits the growth of other microorganisms, allow them to be established in the intestinal tract was reported [2]. Members of the genera *Lactobacillus* and *Bifidobacterium* are the most common probiotics used in commercial fermented and non-fermented dairy products today [3]. In these days, full cream milk and skim milk are the popular probiotic food supplements among all. Beneficial effects conferred by *Lactobacilli*, including inhibition of gram negative and positive pathogenic bacteria, were described by [4]–[6]. The presence of antimicrobial activities among the probiotics incorporated into these different food products against common microbial pathogens. Substantiating the antimicrobial activities of probiotics will affirm their use in the development of functional foods for the betterment of the health of the consuming public [7].

The aim of this study was to investigate the effect of *Lactobacillus acidophilus* strains cultivated in two different types of dairy growth medium namely, full

cream milk and skim milk against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) pathogenic bacteria. The metabolites of *Lactobacillus acidophilus strains* were used for the study.

## II. MATERIALS AND METHODS

The materials used to study the antimicrobial activity of *Lactobacillus acidophilus strains* were Man-Rogosa–Sharpe (MRS) broth (Hi-media, India). Nutrient agar, nutrient broth (Merck, Darmstadt, Germany). Full cream milk (Nestlé, Malaysia), and Skim milk (Sunlac, New Zealand). Sterile distilled water was used as negative control.The other reagents used in the experiment were analytical grade.

### *A. Test Microbial Isolates Culture*

The test pathogenic microorganisms, *Escherichia coli* and *Staphylococcus aureus* were obtained from General Hospital. Penang, Malaysia. The bacterial species were cultivated and maintained in nutrient agar slants in screw-capped tubes. The agar slants were preserved in a refrigerator at 4 °C until use.

### *B. Probiotic lactic acid bacteria*

Strains of *Lactobacillus acidophilus* (FTDC 2804, FTDC 0785, FTDC 8592, FTDC 1295, FTDC 4793, FTDC 4462, FTDC 0582 and FTDC 2916) were obtained from the culture center of Universiti Sains Malaysia, Penang. The strains were inoculated in sterile MRS broth (dextrose 20.0 g/L; meat peptone 10.0 g/L; beef extract 10.0 g/L; yeast extract 5.0 g/L; sodium acetate 5.0 g/L; disodium phosphate 2.0 g/L; ammonium citrate 2.0 g/L; tween 80 1.0 g/L; magnesium sulfate 0.1 g/L, manganese sulfate 0.05 g/L) at 37° C for 36 h. The organisms were activated three successive times prior to use. The culture in MRS was centrifuged at 5000 rpm (Beckman, USA), the supernatant was discarded and the pellet was washed by normal saline. The centrifugation and washing process was repeated twice.

The cells re-suspended in the two different dairy media full cream milk and skim milk (1 % v/v inoculum size). Both media were incubated at 37 °C for 36 hr. After incubation the bacterial suspension was centrifuged at 4 °C for 15 min with a rotation speed of 5000 rpm . the metabolites within the supernatant was collected . The metabolites were evaluated for antimicrobial activity.

### *C. Evaluation of antimicrobial activity*

Agar well diffusion method is widely used to determine the antimicrobial activity against different types of pathogenic microorganisms [8] - [11].

Two flasks with 100 ml of nutrient broth were used. In each flask, the selected micro organisms were cultivated for 18 hours. Serial dilution was then performed for each micro organism and viable count was obtained. The concentration estimated for the bacterial strains was $1\times10^5$ CFU/ml. 100 µL of each grown bacteria was mixed with 5 ml of nutrient broth to produce bacterial suspensions. 0.1 ml of each bacterial suspension was inoculated in a 15 ml cooled molten nutrient agar. These inoculated media were poured into sterile disposable petri dish and mixed gently. The mixture was allowed to solidify for one hour at ambient room temperature (28 °C).

Three wells were made using sterilized cork borer (diameter 6 mm) on each plate that contained the test micro organisms. Two wells were filled with 20 µL of the metabolites of *Lactobacillus acidophilus* strains and the third well was filled with 20 µL of sterile distilled water as a negative control. The plates were incubated at 37 °C for 24 hours. The antimicrobial activities were determined by measuring the diameter of growth inhibition zone formed around the wells observed after incubation.

To further check whether the pathogens were inhibited or killed, a swab was taken from the growth inhibition zone and then inoculated into nutrient broths and incubated at 37°C for 24 hours. The broth tubes were then checked for growth. Presence of growth in the broth was interpreted as an inhibitory activity, while no growth was interpreted as microbicidal. Each of the tests in the determination of antimicrobial activity of the probiotics was conducted in two individual trials, each in triplicate.

### *D. Statistical analysis*

The results were analysed statistically using one-way analysis of variance (version 13.0, SPSS, USA). The result also analysed by using Paired-samples T-test for comparison between two media for the same strain. When there was a statistically significant difference, post-hoc Tukey Honestly Significant Difference test was applied. ANOVA data with $P < 0.05$ were classified as statistically significant.

## III. RESULTS AND DISCUSSION

The anti-bacterial effect of metabolites from different strains of *L. acidophilus* grown in full cream milk and skim milk on *E. coli* and *S. aureus* growth, is shown in figures 1 and 2 respectively.

The results showed that the metabolites of all eight strains exerted considerable antibacterial effect as zone

of inhibition results were significant when compared to the negative control ($P < 0.05$). Among the eight strains cultivated in the two different growth medium, there was a statistically significant difference in the zone of inhibition data for the metabolites of *S. aureus* and *E. coli*. Certain *Lactobacillus acidophilus* strains were more effective against *S. aureus*, while other strains were more effective against *E. coli*. The inhibition zone diameters were in the range of 9.83-10.68 mm in full cream milk and 9.78-10.63 mm in skim milk. The results indicated that the agar diffusion method was comparatively more superior in the study of antimicrobial activity of *L. acidophilus* strains. This could be explained by a good diffusion of metabolites from *L. acidophilus* strains in the well method and therefore resulted in the growth inhibition of the pathogenic microorganisms. On the other hand, the growth medium had no significant influence on the antibacterial effect of metabolites of seven strains except *L. acidophilus* FTDC 4462 against *E. coli*. As for as the effect of *S. aureus* is concerned, the growth medium only affected the antibacterial effect of metabolite of strain *L. acidophilus* FTDC 1295, but did not affect the antibacterial effect of metabolites of the other seven strains (Fig. 2) . The results showed the pathogenic strains were microbicidal as there was no growth among all strains. It is obvious from the results of the measurement of the inhibition zone diameters that the metabolites are effective. This can be explained from the fact that the metabolites contained bioactive products such as organic acid, hydrogen peroxide ($H_2O_2$) and bacteriocins during the metabolism of the microorganism in its life cycle. It was reported that the cell-free supernatant solution from strains of lactic acid bacteria exhibited antimicrobial activity which prevented the growth of different strains of *S. aureus* and *E. coli* [12].

The results showed no significant difference in inhibition zone diameter of *L. acidophilus* cultivated in skim milk medium and full cream milk medium, the results of the inhibition zone diameter between the two media were comparable, which suggested good antimicrobial activity in the edible medium (full cram milk and skim milk) against pathogenic bacteria tested. In addition, the edible medium is not toxic and suitable for human consumption, which is the major advantage. In contrast, MRS medium was toxic to human. Fermented dairy products containing probiotics are becoming popular due to their health benefits [13]. However, milk is considered to be medium for the growth of probiotics due to presence of essential amino acids and low molecular weight peptides [14], [15]. Huttunen *et al*. [16] reported that Lactic acid bacterial strains are potentially promising because they generate bactericidal bioactive peptides (bacteriocins) and enzymes that are able to control bio-film formation and the growth of the pathogens. Certain LAB strains have been reported to be highly antagonistic to *S. aureus* [17]. *Lactobacilli* have been used widely in dairy products because of their health-promoting effects [18], [19].Therefore, it is obvious that *L. acidophilus* metabolites are effective against *E. coli* and *S. aureus*, and acted as an inhibitor against pathogenic bacteria. These results were consistent with the findings of other research groups [20]-[26].The genus *Lactobacillus* has a long history of safe use, especially in the dairy industry, and it plays a major role in fermented milk and other food products [27].

***Figure 1***

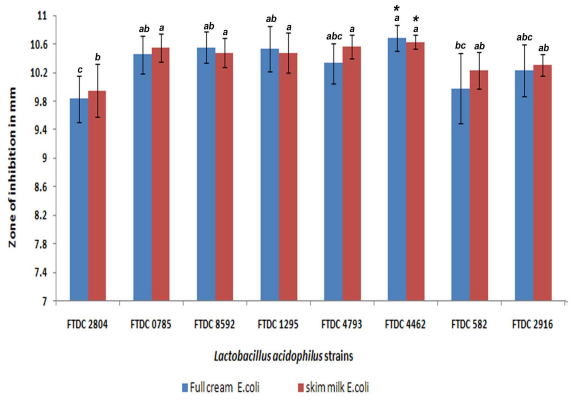


Fig. 1 The antibacterial activities of probiotic bacterial strains cultivated in full cream milk and skim milk against *Escherichia coli.*
Results are expressed as mean ± standard deviation; each data point is the average of measurement from six independent replicates, n=6. (abc) comparison between the strains grown in the same media ($P < 0.05$). * = comparison of the effect from same strain between the growth media ($P < 0.05$).

***Figure 2***

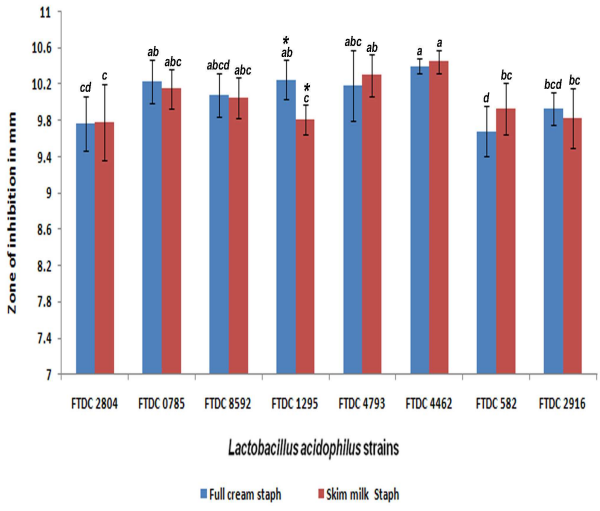


Fig 2: The antibacterial activities of probiotic bacterial strains cultivated in full cream milk and skim milk against *Staphylococcus aureus* a Results are expressed as mean ± standard deviation; each data point is the average of measurement from six independent replicates, n=6. (abc) comparison between the strains grown in the same media ($P < 0.05$). * = comparison of the effect from same strain between the growth media ($P < 0.05$).

## IV. CONCLUSIONS

The growth of the pathogenic bacteria was successfully inhibited as depicted by the zone of inhibition when metabolites of *Lactobacillus acidophilus* from full cream milk and skim milk medium were used, there was an inhibition of bacterial growth in well diffusion method and the effect of metabolites was found to be bactericidal on both pathogenic bacteria. It can be concluded that *L. acidophilus* cultivated in dairy products produced metabolites with strong bactericidal property, which could provide medicinally value added advantages to the human beings.


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