Antagonistic Effects of Lactobacilli on Gram-Negative Bacteria

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Abstract: Three lactobacilli strains were examined for the inhibitory activity against some gram-negative bacteria with a well diffusion method. Lactobacilli have strongest antagonistic activity against P. aeruginosa in well diffusion method; it was the least sensitive tested bacteria. In spite of E. coli is inhibited secondly in well diffusion method (16mm), the value of the inhibition is lower than spot on lawn method (26mm). These results showed that spot on lawn method is a better method than the well diffusion method.

Keywords: Antagonism, Gram-negative bacteria, Lactic acid bacteria, Well diffusion method.

1. Introduction

Antagonistic effects of some microorganisms against others in vivo and in vitro have been reported by many investigators. On many raw foods, the bacterial microbiota is often composed of mixed species. The activities of one of bacterial species may be influenced by the growth activities of others (1). Bacterial antagonism could arise from the combined effects of several mechanisms during their growth in the media. For example, one group of microorganisms may remove a growth factor required for the growth of another, synthesize a substance inhibitory to another or produce an adverse pH or Oxidation Reduction Potential (Eh) (2). Lactobacilli are fermentative and saccharolytic microorganisms. Their production at least half of the end product carbon is lactate. Major fermentation products from utilizable carbohydrates are mainly lactate, may give some acetate, ethanol, CO₂ [3], hydrogen peroxide, di-acetyl [4] and bacteriocins [5] which have inhibitory effects towards other bacteria especially against pathogen bacteria like E. coli [6], Pseudomonas aeruginosa [4]. While inorganic metabolites like di-acetyl inhibit gram-negative bacteria by reducing the pH, bacteriocins are one of the organic metabolites which inhibit mostly gram-positive bacteria [4]. Bacteriocin-like substances may be defined as an extracellularly released bacterial peptide or protein molecules that in low concentrations are able to kill some closely related bacteria by a mechanism against which the producer bacterium itself exhibit some specific immunity [6]. In this study, we examine to determine the antagonistic effect of lactobacilli against some gram-negative bacteria by a comparison of spot on lawn and well diffusion assays which are commonly used methods for the measurement of antagonistic activity.

2. Materials and Methods

2.1 Bacterial Strains and Culture Media

The lactic acid bacteria strains used in this study are Lactobacillus casei, Lactobacillus plantarum and Lactobacillus fermentum. As indicator bacteria strains, Pseudomonas aeruginosa, Escherichia coli, Enterobacter cloacae, Salmonella, and Proteus mirabilis were used. L. casei and L. plantarum was maintained anaerobically in de Man, Rogosa and Sharpe (MRS) broth at 37°C, L. fermentum at 42°C for 24 hours and then transferred to MRS agar slants and stored at +4°C. Pathogen indicator microorganisms were maintained on Brain Heart Infusion (BHI) agar, others on nutrient agar [10].

2.2 Well Diffusion Method

Well diffusion method of Kivanç [11] was followed with modifications. 16 h washed cells of indicator bacteria, had inoculums of 103 and 106
cells/mL, were added 800 CL in 10mL nutrient agar-
tween 80 mixtures (0.2% Tween 80) and poured on
plates. After solidification, 6mm diameter wells were
opened and covered with soft agar (0.75% agar) then 30
CL cell-free supernatant was filled [10]. After
supernatant’s diffusion, plates were incubated at 37°C
for 24 h, anaerobically. Non-cultured nutrient agar-
tween 80 mixtures were used as a control. After
incubation, a clear zone around the wells is an evidence
for antimicrobial activity. All of these investigations
repeated for 24, 48 and 72 h lactic acid bacteria’s cell-
free supernatant.

2.3 Spot lawn method
Inhibitory activities of lactic acid bacteria on 16
washed hours cells of indicator bacteria were
determined by spot lawn method, as described by
Schillinger and Lucke. The inhibition zone after 24
hours and 37°C, anaerobically incubation, is measured
in millimeters.

3. Result and Discussion
The inhibitory activities of lactobacilli against some
gram-negative bacteria were compared with well
diffusion and spot on lawn method (Table 1, 2). In both
methods, L. fermentum and L. plantarum strains
exhibit significant inhibitory activity against indicator
microorganisms mostly in our study, in a spot on lawn
method, P. aeruginosa was determined as the most
sensitive tested bacteria followed by P. mirabilis and P.
aeruginosa with 30-33mm inhibition zones (Table 2).
On the other hand, in well diffusion assay, P.
aeruginosa was the most inhibited indicator
microorganisms with 23mm inhibition zones (Table 1).
Although E. coli is inhibited secondly in well diffusion
method (16mm), the value of the inhibition is lower
than spot on lawn method (26mm) (Table 2).

The most resistant indicator microorganisms were
in a spot on lawn method, E. cloacae, and in well
diffusion assay, P. aeruginosa (Table 1 and 2). In spite
of Salmonella was one of the most resistant strains in
well diffusion method, it was inhibited 28mm in spot
on lawn method. P. aeruginosa was sensitive in spot
on lawn method while it was resisted in well diffusion
method. These results might be due to the cells present
in spot on lawn method. According to Schillinger and
Lucke [10], spot on lawn method is more effective
method than a well diffusion method for measuring
antimicrobial activity. Similar results were found by
Con and Gokalp [11]. They showed that L. plantarum
inhibited C. perfringens, C. botulinum and B. cereus
with spot on lawn method but, there was no inhibition
zone with the well diffusion method. As a result, the
inhibitory activity of lactobacilli on tested bacteria
under spot on lawn test could be due to all metabolites;
lactic acid, acetic acid, di-acetyl, bacteriocin etc. In the
well diffusion method, supernatant of lactic acid
bacteria were used, anaerobic conditions were prepared
to decrease H₂O₂ inhibitory activity and pH was
adjusted to 4.5. So, the inhibition zone which had been
seen around wells could be a result of bacteriocin. We
conclude that spot on lawn method has several
advantages towards well diffusion method by means of
the efficiency of the inhibition and the facility of the
application of the method.

Table 1. Antagonistic effect of lactobacilli against various gram-negative bacteria by well diffusion method.

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*Measured in millimeters

Table 2. Antagonistic effect of lactobacilli against various gram-negative bacteria by spot on lawn method.

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References


