

Aim of the Experiment:**ANTIMICROBIAL ACTIVITY OF PROBIOTIC CANDIDATES****Introduction**

One of the mechanisms by which commensal and probiotic bacteria provide colonization resistance to pathogens is by directly competing for the same niche. Some beneficial microbes acquire similar nutrients as pathogens, often more efficiently, thus hindering the replication and colonization of infectious agents. *Lactobacillus* is one of a number of probiotics considered to be biological therapeutics and host immune-modulating biologicals that are generally recognized as safe (GRAS). Recent studies demonstrated several antimicrobial mechanisms of *Lactobacillus* such as nutrient competition, production of inhibitory compounds, immune-stimulation and competition for binding sites. In addition, *Lactobacillus* can produce lactic acid, acetic acid, formic acid and other acids to reduce intestinal pH, which may be the most important mechanism. These bacteria can also secrete certain antimicrobial molecules, such as ethanol, fatty acid, hydrogen peroxide and bacteriocins to exert the antimicrobial activity.

Materials

1. Probiotic strain
2. *Bacillus cereus*, *E. coli*, *Listeria monocytogenes*
3. MRS agar and Broth
4. Test tubes
5. PBS buffer
6. Centrifuge
7. Gel Borer
8. Membrane filter (0.22 μ)

Procedure

1. The antagonistic ability of lactobacilli, against *Bacillus cereus* and *E. coli* was estimated by an agar well diffusion assay.
2. For this purpose, following media were used-Tryptone glucose yeast extract (TGE) agar for microbiological plate preparation, MRS broth for lactobacilli and Brain heart infusion for pathogens.
3. Overnight activated cultures of lactobacilli, adjusted to OD₅₉₅ at ~1.6 with MRS, were centrifuged for 15 min at 4°C, 8000 rpm and the cell spent were filtered through 0.22 μ m to remove residual cells.
4. A 100 μ L of prepared supernatant was placed in a 10 mm well punched in TGE agar

plates of 140 mm, inoculated with 200 µL of test pathogen at 45°C, before plating.

5. Before inoculating pathogens, to TGE agar their OD were adjusted to MacFarland 1 in PBS (pH 7.2). Subsequently, the plates were incubated at 37°C for 18 h to monitor the zone of inhibition around wells, and a zone of 2 mm or more were reported as positive.

Observations

Antimicrobial Activity (+/-)

Name of the Isolate	<i>Bacillus cereus</i>	<i>E. coli</i>	<i>Listeria monocytogenes</i>
Control (MRS)			
Isolate A			
Isolate B			
Isolate C			

Interpretation