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How to Develop a New Functional Probiotic

Yeon-Hee Lee

Culture Collection of Antimicrobial Resistant Microbes, Department of Biology,
Seoul Women's University, Seoul 151-877, Korea; E-mail: yhlee@swu.ac.kr

INTRODUCTION

Probiotics are defined as live microorganisms which, when administered in adequate amounts confer a health benefit on the host (FAO/WHO 2001) and expected to be the replacement of antimicrobial agents for human as well as nonruminant anmals (1,20). And prebiotics are non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria. Even though the term "probiotics" has been defined as "living microorganisms, which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition", the characteristics that are currently used for the identification of probiotic bacteria have not been clearly established. Recently, dead lactic acid bacteria (LAB) showed better immunoenhancing activity. In this talk, health benefit, identification methods, and safety tests of probiotics will be presented and several examples for.

Health Benefits of Probiotic

Health benefits of probiotics are from their possible modes of action, i.e., production of compounds that are inhibitory toward pathogens, competition with harmful microorganisms for nutrients and energy, competition with deleterious species for adhesion sites, enhancement of the immune response, and more. Besides of well-known functions such as supporting intestinal health, new functions have been reported. Health benefits of probiotic and their mechanism of action are listed in Table 1.

When one wants to develop a new functional probiotic, the first necessary step is to develop the right assay system for the function, i.e. ELISA method to detect cytokines, assay method for growth inhibition of pathogens, assay methods for various chemicals produced by LAB. After the candidate LABs has been chosen with in vitro assay methods, the next step is a test with appropriate animal model system (21). And the final step must include human test with a control group.

Requirements as a Probiotic for Human Use

Lactic acid bacteria (LABs) need several common characteristics to be commercially used as probiotics (10). These are abilities to adhere to cells (2), ability to exclude or reduce pathogenic adherence, persist and multiply constituting the human intestinal microflora (8), produce acids, hydrogen peroxide, and bacteriocins to suppress the growth of pathogens (5), survive the upper gastro-intestinal tract. In addition to these, LABs must be safe and therefore noninvasive, noncarcinogenic, and nonpathogenic, genetically stable without mutations, lack of potential for making with pathogenic bacteria. To be commercialized, it needs to be easily mass-produced in vitro as well as remaining viable during procession, storage and mixing in food/feed (13). Most of all, LABs should be safe to be used even to immunocompromised people at high concentration (6).

Table 1. Health benefits of probiotics and their mechanism

Health benefit	Mechanism	Reference
Lactose tolerance	Degrading lactose by producing lactase	20
Intestinal health	Producing immune enhancing chemicals Colony resistance Produce unfavorable environments for intestinal pathogens by producing acid, short fatty-acid, and bacteriocins, etc. Change the toxin adhesion site Change the intestinal microflora Compete with pathogen for the binding site Increase intestinal mucosa preventing the adherence of pathogen to intestinal epithelial cells Change the intestinal environment unfavorable to intestinal pathogens	14, 15
Anti intestinal cancer effect	Bind to the carcinogen Deactivation of carcinogen Decrease the production of carcinogen by intestinal microflora Increase immune enhancing activity Affect the concentration of the secondary bile salt	11, 19
Immune enhancing activity	Increase the unspecific protection to infection and cancer Increase the antigen-antibody interaction Increase the production of sIgA	6, 9
Suppress allergic reaction	Prevent antigen to infiltrate into blood	17
Decrease the lipid level in blood and heart problem	Catabolism of cholesterol reducing the conc. of cholesterol Degrdation and excretion of bile salt by producing bile salt hydrolase Anti-oxidation	22, 23
Reduction of reproductive and bladder infection	Compete with genitourinary pathogens to adhere Colonization resistance Production of inhibitory chemicals such as hydrogen peroxide, biosurfactant, and bacteriocin	3, 4, 11, 12, 18
Reduction of gastritis and gastric cancer	Decrease the growth of Helicobacter pylori by producing bacteriocin, hydrogen peroxide, and lactic acid Decrease the adherence of *Helicobacter pylori*	15, 16, 19
Decrease tooth caries	Decrease the growth of causative agents for tooth caries including Strep. mutants by producing bacteriocin, hydrogen peroxide, and lactic acid Decrease the infiltration of causative agents into bone cells Increase bone formation	This work

Identification

A LAB for human use needs to be identified to the strain level. Just like identification for any other bacterium, the microscopic observation after Gram-staining must be the first step. And then biochemical and physiological tests using such as API kit is useful for identification. Or 16S rRNA sequencing and comparison with database can be used. Genus and sometimes species can be differentiated with group-specific PCR. In case of differentiation of strains, randomly amplified polymorphic DNA determination (RAPD) and/or pulse-field gel electrophoresis (PFGE) need be performed.

In Vitro Tests for Safety

Every probiotic needs to be tested for its safety with animals before human use. Because animal tests needs

times (at least more than one month) and money, several in vitro tests can be performed to screening out the unsafe LABs (7). These are hemolysis test, gelatin liquifaction test, production of harmful chemicals (i.e. ammonia, indole, phenylalanine), production of harmful enzymes (i.e. β-glucuronidase, 7 α-dehydroxylase, nitroreductase), platelet agglutination, degradation of intestinal mucus. These tests are mainly aimed to detect any virulence factors such as toxin and toxic enzyme. Besides of the direct safety problem, the antimicrobial resistance should be checked. Even though, more antimicrobial resistant LABs have been preferred as a probiotic, there are many concerns about the possibility of transfer of antimicrobial resistance from probiotics to the intestinal microflora. As a result of this, the transferability of the antimicrobial resistance should be checked with broth mating or filter binding methods.

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