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# How to Develop a New Probiotic

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There are three factors to be satisfied to become a probiotic. These are the right identification, safety, and scientifically proved function.

## Identification

Bacteria other than lactic acid bacteria (LABs) can grow on MRS. It is not recommended to consider every bacterium grown on MRS is a LAB. Especially enterococcus can grow on MRS. So there must be a screening method to be taken to eliminate enterococcus. Gram-staining and various biochemical and physiological tests must be performed. And the identification needs to be confirmed with 16S rRNA sequencing. If a new strain had a new characteristic (i.e. new function), then it has to be differentiated from strains in the same species, which are already on the market under a patent or at least the type strain, with molecular methods such as pulse field gel electrophoresis.

## Safety

LABs existing in food and baby feces are generally considered as safe (GRAS). However, recently more and more reports have been published the possibility of a LAB as a pathogen. This suggests that it is necessary to check the virulence of LABs. Moreover, the antimicrobial resistance of LABs provokes a concern, especially in case of Enterococcus. Vancomycin-resistant Enterococcus (VRE) becomes a big problem worldwide. Since VRE itself is dangerous and vancomycin-resistance can be transferred from Enterococcus to *Staphylococcus aureus* and produce the superbug "Vancomycin resistant *S. aureus* (VRSA)", the presence of genes responsible for vancomycin-resistance in all Enterococcus probiotic should be checked. As a preventive measure, every new LAB needs to be checked for the presence of virulence and antimicrobial resistance which can be transferred to other species before they are on the market.

## New function

If anybody wants to develop a new functional probiotic, he/she needs to have a method to test the

function *in vitro, in situ,* and then in animal, and finally in human experiments. Since the animal and human experiments cost a lot of money and time, it is essential to use *in vitro* and *in situ* test method. If there is no method available, then he/she has to develop the method to prove the mechanism of the new function. For example, if one wants to develop a probiotic for oral health, he/she can show the inhibition on the adherence and growth of *Streptococcus mutans* on bone cells without harming the bone cells. If one wants to develop a probiotic for gastric health, he/she can show the same preventive mechanism against *Helicobacter pylori.* In this talk I will present several newly developed probiotic in my lab and how their functions were proved.