National Research Programme NRP 69
Healthy Nutrition and Sustainable Food Prodution

Organic food preservation with inhibitory bacteria

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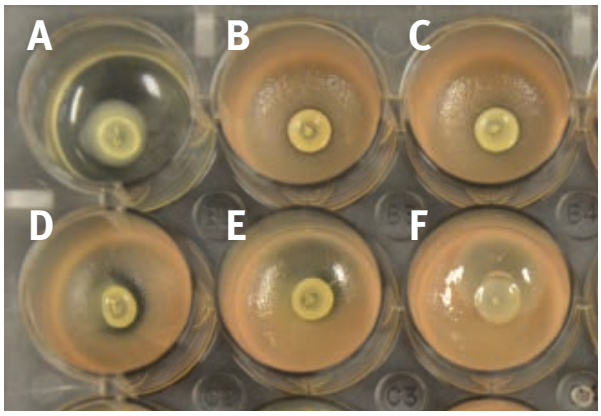
Customised bacteria extend food's storage life

Humans have been using alcohol or lactic acid as biological food preservatives for thousands of years. But there are a lot of other biological methods of preservation, including micro-organisms that are capable of inhibiting moulds or undesirable germs. A research group taking part in NRP 69 has developed a new procedure for categorising preservative bacteria. They tested hundreds of strains of *Lactobacillus* bacteria, investigating the conditions under which they could delay the deterioration of food such as salami or cheese. Certain *Lactobacilli* also have development potential as protective micro-organisms that could help prevent the spread of antibiotic resistance through food.

For thousands of years, humans have been using fermentative micro-organisms to keep food edible for longer periods – using yeast or lactic acid bacteria to preserve beer and cheese, for example. The metabolic products formed during the fermentation process, such as alcohol or lactic acid, act as biological inhibitors of moulds and micro-organisms that could spoil food. Besides these well-known preservatives, there are many other micro-organisms that are not harmful to humans and act as preservatives even in small quantities. As part of NRP 69, a research group from ETH Zurich examined the characteristics of new strains of *Lactobacillus* bacteria that possess inhibitory properties. The aim of the project was to develop a procedure for identifying bacterial cultures suitable for delaying the deterioration of foodstuffs such as salami or cheese. This type of organic preservation makes food safer and helps reduce food wastage.

In the first stage, the researchers examined more than 500 strains of *Lactobacillus* bacteria that they had previously isolated from fermented foods. Using a specially developed method known as a microtiter plate test, they tested the strains for antimicrobial properties. Testing identified 65 *Lactobacillus* strains with antibacterial properties and 154 with antifungal properties. The researchers then sequenced the genomes of over 70 strains to gain a better understanding of why and how they inhibit other bacteria.



The picture shows the extent to which various *Lactobacilli* (white colony in the centre of the container) inhibit the growth of food-spoiling yeasts (red lawn). The bacterial strain at the top left (A) had a strong inhibitory effect. No inhibitory effect could be detected for B and C, while D and E had a moderate effect, and F inhibited growth to a small extent.

Suitable protective cultures

With a view to their future use in food production, the research group investigated how well the 500 or so strains fare under production conditions. They did so by simulating various conditions and seeing how the bacteria reacted – for example when exposed to high salt concentrations of the type that are common in the meat and cheese maturation process. This identified the most suitable strains for use in the production of specific foodstuffs.

In a further step, the researchers put the potential inhibitors through practical testing in a laboratory environment, using suitable bacterial cultures to provide protection throughout the production and subsequent storage of salami and unpasteurised cheese. The trials with salami confirmed that four strains used possessed antibacterial properties. The *Lactobacillus* strains reduced concentrations of pathogenic Listeria to below the limit of detection. Since Listeria can cause dangerous infections in humans, reducing them is of great interest to the industry.

Lactobacillus strains were successfully used in unpasteurised cheese fermentation to inhibit the Enterococci in the cheese. Enterococcus concentrations fell by 96% compared with cheese with no antibacterial cultures. Enterococci are bacteria with various antibiotic resistance genes that they transmit to other bacteria around them. Inhibiting Enterococci in food is thus relevant to efforts to combat antibiotic resistance (see box below).

Further information: *www.nrp69.ch*

Application

Protective cultures in food production

Based on a better understanding of the preservative properties of bacterial strains, bacterial protective cultures can be used for specific purposes in industrial food production. The researchers believe this type of biological preservation has particular potential in the industrial production of fermented meat and milk products. Before the concept that has been developed can be widely used, its application in practice will need to be refined. The cost of classifying and analysing the possible negative properties of bacteria using whole genome sequencing is low. The procedure applied to *Lactobacillus* in NRP 69 is therefore also suitable for detailed analyses of various strains of other bacterial genera.

The classification of *Lactobacillus* strains prepared during the project will make it possible to select individual strains for the particular properties they possess. The process thus provides a foundation for using customised bacteria to specifically provide protection during food production. Because they have preservative properties, inhibitory bacteria can extend the storage life of food and help reduce food wastage due to impurities or premature deterioration.

Recommendation

Combating antibiotic resistance in food

The spread of bacterial resistance to antibiotics is a global health issue. Unpasteurised milk products and ready meals are among the foods that contain resistant bacteria such as Enterococci, which are capable of transferring resistance genes to other bacteria around them. The risk that these bacteria represent in terms of spreading antibiotic resistance is underestimated. The research group therefore recommends introducing legal limits for Enterococci in food. It also recommends using suitable *Lactobacillus* strains against Enterococci.