Lactic acid bacteria (Slide 2)

Lactic Acid Bacteria (LAB) or Lactics constitute a diverse group of microorganisms associated with plants, meat, and dairy. The Lactics are also important commercially in the processing of meats, alcoholic beverages, and vegetables. They are used in the manufacture of dairy products such as acidophilus milk, yogurt, buttermilk, and cheeses.

Bacteriophage - Host Interactions (Slide 3)

Lactic acid bacteria (LAB) comprise different groups of microorganisms, such as Carnobacterium, Enterococcus, Tetragenococcus, Vagococcus, Weissella as well as species of genera which constitute the "industrial" core of LAB, like Lactococcus, Lactobacillus, Streptococcus, Pediococcus and Leuconostoc. The genus lactococcus is the best characterized food-related LAB. As lactococcal strains are able to grow in milk and transform lactose to lactic acid, they are commonly used as starter cultures in industrial fermentations for cheese production (1). The first negative effect of bacteriophages on dairy fermentation was reported in the mid-30s of the 20th century (2). Regardless of sanitary precautions, starter strain rotations and constant development of new phage-resistant bacterial strains, phages remain one of the main and economically most serious sources of fermentation failures. Phage-induced bacterial cell lysis leads to failed or slow fermentation, decrease in acid production and reduction of milk product quality, which in effect cause profound economical losses (3).

Nightmares of a cheese marker (Slide 4):

An example of a time-lapse microscopy video show casing the detrimental effects of phages during the fermentation process. Infection of *Lactococcus lactis* with virulent phages can cause cell lysis and death within 1 hour after infection.

Phages of LAB (Slide 5)

Lactococcal phages are currently classified into ten groups based on their morphology and genetic relatedness. However, three main species 936, P335 and C2 dominate the dairy industry in terms of persistence and frequency of isolation. Members of the 936 and c2 observe a lytic cycle only, while P335 members may propagate lytically or may integrate their genome into that of the host and replicate in tandem with the host's chromosome, a scenario during which they are termed lysogenic or temperate phages [4].

Defense mechanisms of lactic acid bacteria (slide 6)

Lactic acid bacteria as well as other bacteria infected by phages have evolved defense systems against bacteriophages, which allow them to survive in an environment full of their predators (phages). These anti-phage systems have been organized into groups depending on the manner by which they operate. Some of these systems include:

(i) Inhibition of phage attachment/adsorption

(ii) Blocking of phage DNA injection

(iii) Restriction modification systems

(iv) Phage abortive infection systems

Bacteriophages in the dairy plant (Slide 7)

It is important to identify the potential sources of phage contamination and limit their entry to the fermentation process. Phages can originate from:

Raw milk

Raw milk is the most probable source of naturally occurring virulent phages at titers as low as (between 10^1-10^3 PFU ml^{-1}) and constitute a continuous supply of bacteriophages in dairy plants [5,6]. Phage concentrations in raw milk also depend on conditions of collecting, handling and storing of milk by the supplier (farm), on transport to the plant and, finally, handling of the milk in the plant itself. It is also important to remember that phage biodiversity is increased by combining milk collected from different farms and these numbers can be even higher in processed milk.

whey protein concentrates

Whey proteins are used to standardize milk before the fermentation process or to improve the taste and texture as well as the nutrient value of the final product. Whey protein concentrates can be sources of high temperature-resistant phages and can influence the quality of the final product [7,8]

Starter cultures

The starter culture itself can be a source of phages, when strains contain temperate phages. Temperate phages are incorporated into the bacterial chromosome and their genome replicates in synchrony with the bacterial genome. Prophages are carried in many LAB strains [9,10] and may be induced from lysogenic to lytic form by the manufacturing conditions such as heat, salts, acidity, bacteriocins, starvation or UV[11,12]. Serial subculturing of temperate phages in milk may result in their replacement by a virulent mutant. Prophage induction from multiple lysogenic starter culture strains has the potential to influence fermentation. The main source of lysogenic strains are undefined cultures, for two main reasons: i) the exact strain composition of these starters is unknown; ii) elimination of lysogenic strains from undefined culture is very difficult.

Equipment/air

Phages are commonly present on working surfaces and usually need the presence of their bacterial hosts. Due to this fact, they are usually found in places where conditions for LAB development are favourable such as valves, crevices and "dead ends" (difficult cleaning and disinfection places) of production lines. Sequentially, raw milk handling, cheese milk processed in open vats and whey handling can lead to spreading of phages in the air. Virulent phages can circulate through the air far away from their aerosolization source due to the ability to bind to small particles (< 2.1 µm) [13].

Phage control strategy

Cleaning and disinfection

The classical operations of cleaning and disinfection are an essential part of milk processing. Cleaning-in-place (CIP) procedures are usually applied in milk processing lines [14]. The cleaning process can remove 90% or more of microorganisms associated with the surface, but cannot kill all of them. The presence of LAB among the residual microorganisms increases phage risk contamination.

Design of starter cultures rotation system based on phage contamination control

Starter cultures are a key factor influencing the diversity of phage population in a dairy plant. Application of undefined multispecies and multistrain cultures was the main strategy to overcome production problems related to phages in many factories in the past. Rotation strategy of defined multiple strain cultures demands selection of strains resistant to a wide range of phages, which could replace infected strains. Moreover, continual rotation of multiple strains during fermentation processes has an effect on phage co-evolution and was shown to increase phage diversity and their abundance in the dairy environment [15]. It also requires constant selection of starter strains with specific fermentative properties. An alternative is the use of a single, highly specialized phage-resistant strain and its variants carrying phage resistance plasmids obtained from naturally resistant strains.

Production organization

An important element reducing the spread of phages in the dairy plant is the organization of production. The control of phage risk in dairy plants relies on development and implementation of a variety of procedures such as aseptic technique, closed vats, Phage inhibitory media and severe heat treatment of bulk starter media .

Concluding remarks:

Phages represent a constant threat of serious economic losses in the dairy industry. As a results of this, dairy microbiologists have attempted for almost 80 years to eliminate or, at least, bring under better control, bacteriophages that interfere with the manufacture of fermented milk products. Phages rapidly disseminate in dairy environment and are difficult to eliminate. Thus it is important to understand how the host and phages co-exist in order to gain a better understanding this complex relationship in the hope of finding sustainable solutions to the ongoing problem faced in the dairy industry.

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