NEW DEVELOPMENTS IN COMPOSITION AND PROPAGATION OF CHEESE STARTERS

1259

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Composition of Starters

During the past few years, the composition and propagation of cheese starters have been receiving increased attention from cheesemakers, commercial starter makers, and research workers. Several factors have stimulated the recent interest. Foremost among these have been: the widespread occurrence of starter failures resulting from bacteriophage; the frequent occurrence of residual antibiotics in milk; and the general use of pasteurized milk.

Four general kinds of lactic starters have been available, as follows:

1. Single-strain starter. It contains only one strain of either *Streptococcus lactis* or *Streptococcus cremoris*.

2. Mixed-strain starter. It contains two or more strains of one species, that is either *S. lactis* or *S. cremoris*.

3. Multiple-type single-strain starter. It contains one strain of *S. lactis* and one strain of *S. cremoris*.

4. Multiple-type mixed-strain starter. It contains two or more strains of *S. lactis* and two or more strains of *S. cremoris*. Usually this kind of starter contains initially at least four strains of each species.

It is generally assumed that multiple-type mixed-strain starters are being used most commonly in this country, and that, in addition to *S. lactis* and *S. cremoris*, they also contain either or both *Leuconostoc citrovorous* and *Leuconostoc paracitrovorus*. The last two species are commonly known as "the citrate fermenters," "aroma producers," or "the associative bacteris."

An ideal cheese starter would be resistant to bacteriophage and antibiotics, consistently form the desired amount of acid at the proper rate, and uniformly develop the desired intensity of characteristic flavor. Also, and of great practical importance, the ideal starter would maintain its desired characteristics and activity through repeated propagation in the cheese factory. Unfortunately, such an ideal starter has not been developed.

Individual strains of *S. lactis* and *S. cremoris* differ in their susceptibility to individual strains of bacteriophage--commonly known as phage. Some strains of these bacteria are susceptible to only one strain of phage, while others are susceptible to many strains of phage. In terms of cheese starters and phage, this means that infection with one strain of phage can cause complete starter failure when the starter contains only susceptible strains of bacteria. On the other hand, one strain of phage might have little or no

apparent effect when the starter contains many strains of bacteria and they differ in their susceptibility to phage. Some strains of phage, however, attack so many strains of starter bacteria that they can ruin a starter even though it contains many strains of bacteria. Also, there is always a possibility of infection with more than one strain of phage.

Individual strains of S. lactis and S. cremoris also differ in their sensitivity to antibiotics and in their rate and amount of acid production.

Recently, more and more cheesemakers have been carrying several multiple-type mixed-strain mother starters. In some factories, the starters are rotated systematically with the aim of preventing a build-up of phage. In other factories, from two to four such complex starters are mixed in preparing the bulk starter. Both practices seem to be giving much better results than the use of either single-strain cultures or mixtures of two strains which require more protection, care, and control. Bulk starters prepared from a mixture of several multiple-type mixed-strain mother cultures have given more uniform acid development and less difficulty due to phage and antibiotics than those prepared from simpler starters. However, to obtain maximum success with complex starters, the following procedures must be followed:

1. The individual starters must be proved to be compatible when they are mixed, because some strains of lactic bacteria are definitely antagonistic to others.

2. The individual starters should be propagated separately as mother cultures or intermediates and mixed only in the preparation of bulk starter. If several starters are mixed and propagated repeatedly as blend, certain strains may soon become dominant, and after a few propagations the blend may actually contain only a few strains. Thus the benefits of a complex starter would be lost.

3. All mother starters, intermediates, and bulk starters must be protected against phage infection.

4. Any individual starter that shows either loss of activity or changed characteristics during continued propagation must be discarded and replaced with a new one. Some factories obtain fresh, commercial, freeze-dried, "seed" starters each day for making intermediate starters. The intermediates are mixed in preparing the bulk starter.

A much different procedure is being followed in some factories, particularly in certain foreign countries. Sufficient single strains of lactic bacteria are carried as mother cultures to provide two different strains for use each day in the preparation of bulk starter during a period of at least a week. A somewhat similar procedure is being tested by at least one commercial laboratory in this country. Each one of many selected single strains of lactic bacteria is being combined with a compatible strain of associative bacteria. Each single strain with its associate would be propagated as a mother culture, and three or four such strains would be mixed in preparing the bulk starter. The associate is being included to improve the activity of lactic bacteria and to aid in the development of flavor.

In general, attempts to improve cheese flavor through the use of supplemental starters, such as lactobacilli and micrococci, have either failed or yielded inconclusive results.

Starter Media

Selected and properly heated whole milk has been used most frequently for propagating starters. Skim milk has been used also. The results of practical experience and research show that such milks, if of high quality, are usually satisfactory for starters. However, frequent and unpredictable variations in growth-supporting properties of fluid milk have led some cheesemakers to use pretested, spray-dried, nonfat milk (NFS) reconstituted in water to give about 10 per cent solids. Both the NFS and the water must be free of inhibitory substances. Although pretested NFS are available commercially, it is important that every cheese factory test each lot with each of its own starters before it is put into actual use. The factory starters should be propagated daily for at least a week to make certain that they will maintain their activity, flavor, and body characteristics. Some water supplies are not suitable for reconstituting NFS for starters. Methods for reconstituting NFS for starter and for testing the suitability of NFS and water have been published by Horrall, et al. (J. Dairy Science, 33, 245-249 and 250-256, 1950).

Recent research shows that the suitability of NFS for starters is affected greatly by the relative intensity of preheat treatment used in making them (Greene and Jezeski, J. Dairy Science, 40, 1046-1052, 1053-1061, and 1062-1071, 1957). A series of stimulation-inhibition-stimulation effects was found to accompany progressive increases in heat treatment. On the basis of the data obtained in these studies, it is reasonable to expect that manufacturers of NFS will be able consistently to produce a more suitable product for use with starters.

A special culture medium for starters has attracted considerable attention since it was announced at the 14th International Dairy Congress in September, 1956, by Fisons Milk Products Limited, Coleraine, North Ireland. It was designated as "Phage Resistant Medium," or simply as PRM. The makers claimed that "phage will not multiply in PRM solution and if initially present some of the phage will die out during incubation," and that "any starter culture that will grow in PRM will develop normally in spite of the heaviest phage contamination." PRM was developed on the basis of previous knowledge that phages against S. lactis and S. cremoris will not multiply in the absence of calcium. It is a calcium-free, spray-dried powder prepared from milk and is to be reconstituted as a 10 per cent solution in distilled water. Most supplies of water contain sufficient calcium for the development of phage.

Babel reported that PRM "does not permit development of bacteriophage and certain cultures contaminated with bacteriophage have been made phage-free with not more than three propagations in PRM." (Proceedings, EURDD Research Conference on Cheese, Beltsville, Maryland, 1957.) He also found that most cultures grew faster and produced more acid in skim milk than in PRM. All of the cultures tested by him grew in PRM, but many of our strains have failed to grow.

Although this medium seems to offer certain distinct advantages, much additional information is desired, particularly regarding its suitability for use with multiple-type mixed-strain starters and its effectiveness under commercial cheesemaking conditions in this country. Furthermore, on the basis of a preliminary presentation concerning the manufacture of PRM, the Food and Drug Administration had reservation regarding its use in cheesemaking.

Facilities and Equipment for Making Starters

Separate starter rooms, new types of equipment, and improved procedures for transferring and propagating starters are being employed more generally to provide better protection against contamination, particularly protection against phage.

In many instances, a starter room has been built within the factory but located as far as possible from the cheese vats and whey separator. In other instances, a separate building has been constructed. In either case, the room should be constructed and equipped so that it is easily cleaned and kept clean. Also, it should be equipped with an independent filtered-air system, and there should be sufficient air pressure to force the air outward when the door is opened. However, the mere existence of an elaborately equipped starter room is not sufficient to protect against contamination. Success or failure depends chiefly on the techniques of the starter maker.

Two relatively new types of protective equipment are being adopted for transferring and propagating starters. They are as follows:

1. Water-sealed, air-filtered starter tanks with protected inoculating spouts. They are similar to those used for many years in New Zealand and Australia. Briefly the basic principles and protective features of this equipment are as follows: The lid, agitator shaft, and inoculating spout are water-sealed to prevent entrance of air. While the milk is being heated, the expanded air escapes through a small opening at the top of the tank. At the end of the heating period, but before cooling begins, an air filter is attached to the opening. Thus, only filtered air is drawn into the starter tank. At the time of inoculation, a steam ring is first placed around the inoculating spout, then the water-sealed cover is removed through a jet of steam which is directed upward and across the opening. The culture is introduced through the steam-protected spout, and the water-sealed cover is replaced.

2. Air-tight containers plugged with rubber stoppers. Flexible polyethylene bottles sealed with serum-type, rubber stoppers are used for the mother and seed cultures. Stainless steel cans equipped with specially designed rubber stoppers are used for bulk starter. The rubber stopper or seal is forced into the neck of the can be means of a clamping device, and a serum-type, rubber stopper is inserted into a small opening provided for inoculation. The cans are completely submerged in water while the milk is being heated and cooled. A revolving cradle with wheels is used for mixing the inoculum and for moving the 10- to 40-gallon cans.

The starters are transferred from bottle to bottle and from bottle to can through the serum-type, rubber stoppers with specially designed inoculating needles which are the equivalent of two hypodermic needles connected end to end. The culture is forced out of the flexible polyethylene bottle by simply squeezing it.

The basic principle of this equipment and procedure is to prevent contamination from the air. More specifically, they were designed to prevent phage contamination. Outstanding improvement has been obtained following their use in England.

The importance of protection against phage has been emphasized during this discussion because most cheese makers and research workers believe

that it is the most important problem in the composition and propagation of cheese starters. However, the new developments in the composition of starters and in the preparation and use of NFS for propagating them have been aimed chiefly at improved uniformity and activity of starters free of phage.

Uniformly good-quality cheese cannot be made without uniformly good-quality starters.