Enumeration of Viable Lactobacillus acidophilus Organisms in Dairy Products

M. L. SPECK

Department of Food Science
North Carolina State University, Raleigh, North Carolina 27607

(Received for publication August 17, 1977)

ABSTRACT

Pasteurized low fat milk to which is added Lactobacillus acidophilus has now become a product of major volume on the U.S. market. The product contains millions of L. acidophilus per ml and is considered to be in the general category of cultured milk products for application of regulatory monitoring. There is a growing interest in developing standards for allowable minimum numbers of L. acidophilus in such products at point of sale. This can be accomplished best by use of a medium selective for lactobacilli such as Lactobacillus Selection Agar (LBS); non-selective media, such as APT agar, can also be used to enumerate L. acidophilus. The latter would provide less assurance that the counts were due to lactobacilli. The non-selectivity of APT, however, usually results in higher counts of L. acidophilus. There is need for application of accurate monitoring of such products to ensure the numbers as well as the identity of the culture used as the milk supplement.

The microbiological monitoring of noncultured pasteurized dairy products is accomplished primarily by performing the Standard Plate Count and the coliform count, although tests to detect other groups of microorganisms that may affect the shelf-life of the products also may be applied. In cultured milk products, the Standard Plate Count is not used because the starter bacteria are so numerous that they would obscure the much lower numbers of nonstarter bacteria present. In such products, only the coliform count is done along with the phosphatase test. It is generally agreed that regulatory procedures currently in use satisfactorily evaluate the sanitary quality of milk and cultured milk products.

A little over two years ago, a noncultured pasteurized milk product to which Lactobacillus acidophilus is added appeared on the American market. Understandably, this presented some immediate problems to regulatory officials who were charged with evaluating the sanitary quality of this product, and yet were faced with the possibility of having to condemn the product because the L. acidophilus bacteria often would form pin point colonies on media used for doing the Standard Plate Count. On October 28, 1975, a notice was sent from the Chief of the Dairy and Lipid Products Branch, FDA, to all regional food and drug directors for attention of the regional milk specialist. This memorandum in part states:

"In order for the requirements of the Grade A Pasteurized Milk Ordinance to apply to this fluid milk product the words 'safe and suitable bacterial culture' need to be inserted in Definition Q, Optional Ingredients, following the words 'similar ingredients.' Definition Q would now read, 'Optional ingredients shall mean and include Grade A dry milk products, concentrated milk, concentrated milk products, flavors, sweeteners, stabilizers, emulsifiers, acidifiers, vitamins, minerals and similar ingredients and safe and suitable bacterial cultures.' We believe this is the most expeditious way to handle this product as the addition of a pure Lactobacillus acidophilus culture to a milk product is analogous to the addition of other optional ingredients.

We recognize that certain other changes in the administrative and sanitation requirements of the Ordinance need to be made and we have these under consideration.

With respect to labeling, the name of the product is considered to be 'Lowfat milk with Lactobacillus acidophilus culture added' and the label should contain the necessary ingredient labeling. The words 'Sweet acidophilus' is considered simply a fanciful term and there would be no objection to the use of these words immediately preceding the name of the product.'

Subsequently, it was decided that this product should be microbiologically monitored in the same manner as cultured milk products, viz., by the use of coliform count and phosphatase test.

WHY ADD THE LACTOBACILLUS?

At this point, it should be understood that L. acidophilus is added to milk because of evidence in the literature that the microorganism can be of benefit to many people (for comprehensive reviews of the literature
on the roles of the intestinal microflora, see references 2, 3, 4, 7, 8). Information is still being obtained on how the lactobacilli may benefit humans. The role of the intestinal lactobacilli is now considered to be somewhat different from that when regular cultured acidophilus milk was being studied. Formerly, the thrust was to consume sufficient quantities of L. acidophilus whereby the predominant flora in the intestinal tract would consist of this microorganism and basically eliminate most of the others. Later, however, it was realized that the intestinal microflora in healthy individuals contains a balance that is favorable to the host. Efforts to have the intestinal microflora dominated by L. acidophilus have essentially been abandoned. In maintaining the balance among the groups present, it has been observed that the lactobacilli appear to be particularly vulnerable and often are lowered tremendously or are absent. In such instances, it is not unusual to find intestinal upsets occurring which are characterized by diarrhea, flatulence or a combination of the two. To re-establish the lactobacilli, consumption of moderate numbers of L. acidophilus seems to be sufficient. For example, the daily consumption of 1 x 10⁸ to 1 x 10⁹ viable lactobacilli was the most satisfactory level of intake (5). More recent studies have shown that the consumption of 1-2 x 10⁹ L. acidophilus per day was adequate for increasing the number of intestinal lactobacilli appearing in the feces (6). Consumption of these numbers of L. acidophilus has made it possible to prepare a milk product that is not altered in flavor or taste, but which contains approximately 1 billion cells of L. acidophilus per half pint. Furthermore, this can be accomplished with a very modest increase in cost of the product to the consumer. This type of product was developed in our laboratories and introduced on the market in 1975; subsequently, similar products have appeared.

REGULATORY CONTROL

Since the lactobacilli were being added to milk to improve its nutritional qualities for the consumer, it was reasonable for regulatory agencies to be concerned that adequate numbers of the microorganism were present in the product. However, methodology for enumerating L. acidophilus is not contained in Standard Methods for the Examination of Dairy Products (1). Enumeration of L. acidophilus presents some problems not encountered in doing the Standard Plate Count. These are caused by L. acidophilus being more fastidious in its nutritional requirements than are the microorganisms normally present in milk that are enumerated by the Standard Plate Count procedure. To enumerate L. acidophilus, the medium, temperature of incubation, and, in certain instances, the gaseous atmosphere, must be specifically adjusted for obtaining accurate counts. These impose new procedures and equipment requirements on laboratories charged with performing such counts. It is important that accurate methods of enumeration be available since a number of states are now adopting a standard of not less than 2 million L. acidophilus per ml of milk for products in which the culture has been added to pasteurized low fat milk.

The procedure which we have used consists of the following:

1. Prepare and plate dilutions of the milk through the one to one hundred thousand dilution (1:100,000).
2. Pour the plates with Lactococcus Selection Agar (BBL).
3. Place the plates in an atmosphere of CO₂. This can be obtained by the Gaspack jar using the CO₂ generator, or by placing plates in a plastic container which is flushed with CO₂.
4. Incubate the plates at 37 C for 3 days.
5. Count all of the colonies and report as number of L. acidophilus per ml of the milk.
6. Incubation at 35 C can be substituted for 37 C if necessary.
7. It is possible that the Rogosa SL medium could be used as well as the Lactobacillus Selection medium, but we have gathered no data on this.

Microbiologists will quickly detect the differences in this procedure from that used for the Standard Plate Count (1).

Two other media have been used extensively for enumerating L. acidophilus and these are Lactobacilli MRS broth (Difco) plus 1.5% agar and APT agar. These two media are not selective for lactobacilli and their non-selectivity often allows higher counts to be obtained in enumerating L. acidophilus in a given milk sample. Also, when these media are used, it is not necessary to use a CO₂ atmosphere for L. acidophilus colonies to develop.

There may be an understandable reluctance to use the more selective medium and costly procedure for routine monitoring of the population of L. acidophilus in milk to which this culture is added. However, it must be realized that use of the Lactobacillus Selection (LBS) medium does permit more confidence in the count obtained as being caused by lactobacilli, rather than by other microorganisms that may have been present because of some undesirable practice(s) in handling of the milk.

It should be noted that the L. acidophilus which is used in preparing uncultured acidophilus milk has to be made with extreme care and a high level of sanitation. The product developed at our University and which is sold as Sweet Acidophilus™ milk must not contain any of the following: psychrotrophs, coliforms, staphylococci, salmonellae, or C. perfringens. Furthermore, through a monitoring program required of each licensed dairy, results must be submitted by the dairy routinely for the phosphatase test and for counts of L. acidophilus and coliforms. In addition, an approved independent laboratory must, on a quarterly basis, perform and submit results on the foregoing tests and test the milk for the presence of salmonellae and the number of staphylococci. It is believed that such a program benefits the dairy, the consumer, and also assists regulatory agencies in their monitoring responsibilities.
BILE-RESISTANT LACTOBAILLUS ACIDOPHILUS

Certainly it must be agreed that if the *L. acidophilus* is to have any benefit in the intestinal tract it must be viable when consumed. However, there are additional considerations that should be given to the quality of the product. *L. acidophilus* and certain lactobacilli found in the intestinal tract are able to grow in the presence of bile, and in our laboratories we have developed a procedure for enumerating these bile-resistant bacteria. The medium we have used is the LBS agar to which is added 0.15% oxgall, and is designated as LBSO agar. The plating procedure is done in the same manner as for the LBS agar cited earlier in this discussion. Cultures of *L. acidophilus* produce equal numbers of colonies on the LBS and LBSO media. However, we have found that when the culture has been subjected to certain stresses the LBSO count will frequently be markedly less than the LBS count. When the cells are placed in a favorable environment in the absence of bile, they regain their ability to grow in its presence. This is a typical manifestation of microbial injury. Some of the factors which promote injury are: storage of frozen concentrates at insufficiently low temperatures (for example, storage at -20°C will promote much more injury than storage at -86°C or -196°C); growth of the cultures in media that are inadequate nutritionally; and improper preparation and storage of the milk to which *L. acidophilus* has been added. It would seem that maintenance of a maximum level of bile-resistant cells in the milk would be in the best interests of those consuming milk containing *L. acidophilus*.

In marketing a milk to which many *L. acidophilus* cells are added, it is obvious that much care must be taken to maintain the viability and bile resistance of the culture; but, it is essential that the identity and purity of the culture must be maintained. Fortunately, culture supply companies have this capability and are accustomed to preparing large quantities of cultures in a pure form, which can maintain the desired qualities of the culture. Nevertheless, surveillance of the acidophilus milk products does involve certain procedures which are not currently used routinely. Possibly this is an area wherein industry monitoring programs can be shared with regulatory agencies to accomplish goals of mutual interest.

ACKNOWLEDGMENT

Presented at the 64th Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians, Sioux City, Iowa, August 14-18, 1977

REFERENCES