Phosphatase Production in Dairy Products by Microorganisms *

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The value of the phosphatase test in detecting inadequate pasteurization of market milk naturally suggests its use with other dairy products. With certain of these products, for example butter and cheese, the situation is quite different than with market milk because in normal commercial and consumer channels they may be held for extended periods at temperatures which do not prevent the growth of organisms. This raises the question of whether positive phosphatase tests necessarily indicate inadequate pasteurization of the milk or cream used for the products or whether phosphatase can be formed in the products.

Various investigators have noted the production of phosphatase by microorganisms. In the work herein reported a variety of organisms was considered but most of the attention was directed to species known to grow in butter. The organisms were first tested for phosphatase production in sterile milk, and then selected cultures were tested in butter.

LABORATORY PROCEDURE

A modified Scharer short test was used and the results considered positive only when they were very definite. With the cultures that were positive in milk, tests also were run with heated cultures and again in a buffer substrate containing no disodium phenyl phosphate in an attempt to exclude growth products which would react with the indicator; these trials were regularly negative. For the most part no serious attempt was made to evaluate the activity of the phosphatase, as indicated by the phenol liberated, because this was greatly influenced by the amount of inoculation, time and temperature of growth, and other factors. Phosphatase production was tested only at the normal pH of the buffer substrate employed, rather than at various pH values, as was the case in certain studies that have been reported. A culture definitely was considered phosphatase negative only after a 7-day culture grown at a reasonably satisfactory temperature failed to give a test. Many of the species studied grew very well at 21° C. but with some of them 37° C. was employed. With butter, the tests were run on serum recovered by melting and centrifuging the butter.

BACTERIAL PRODUCTION OF PHOSPHATASE

Of the species studied, the most active phosphatase production in milk occurred with those belonging to the genus *Pseudomonas*. *Ps. putrefaciens* is an excellent example. Each of 52 cultures, no two of which came from the same source, gave strong reactions in 24 hours, although there was some variation in the reactions, presumably because this species does not readily initiate growth in milk or on agar. Up to a certain point, the phosphatase increased as the culture aged. The production of phosphatase is now used at the Iowa Agricultural Experiment Station in the tentative identification of *Ps. putrefaciens;* that is, cultures which give the typical reaction in litmus milk are next tested for phosphatase production.

Ps. nigrifaciens also rapidly produced phosphatase. *Ps. mephitica* was less active but still conspicuously positive. *Ps. cyanogenes* was even less active. The other species of *Pseudomonas,* which in-

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cluded *Ps. aeruginosa, Ps. fluorescens, Ps. synxantha, Ps. fragi, Ps. graveolens, Ps. mucidolens* and some others, were negative.

In the *Escherichia-Aerobacter* group, certain cultures of *Aerobacter* gave a positive reaction while the *Escherichia* cultures were negative. A single culture of *Flavobacterium fecale* was strongly positive. Some of the cultures of *Alcaligenes* were positive and some negative, the positive cultures being unidentified species from sewage and negative cultures being *Alc. lipolyticus* and *Alc. viscosus.*

Each of five cultures of *Oospora lactis* gave a positive reaction, although the reactions were not as strong as with some of the bacterial species.

Only negative results were obtained with organisms of the genus *Streptococcus,* which included *S. lactis, S. citrovorus, S. paracitrovorus, S. citrophilus, S. diacetilactis* and *S. liquefaciens.* and with organisms of the genus *Lactobacillus,* which included *L. acidophilus, L. bulgaricus* and *L. casei.* Twelve cultures of the genus *Propionibacterium* gave negative results. Nine cultures of the genus *Bacillus* from stock collections, each representing a different species, also gave only negative results. Of seven cultures of the genus *Clostridium,* one gave a slight reaction. *Proteus ichthyosmius, Proteus vulgaris, Serratia indica, Serratia marcescens* and *Staphylococcus cremoris-viscosi* were negative. *Torula sphaerica, Torula cremoris, Mycotorula lipolytica* and various species of the genus *Sporobolomyces* also were negative.

Various cultures of bacteria isolated from butter on different occasions, but not identifiable on the basis of published descriptions, gave positive phosphatase reactions, some of them being strong.

The active production of phosphatase by certain species of organisms has a significance from the standpoint of phosphatase in dairy products that are held for extended periods at temperatures above the freezing point because most of these cultures can grow at relatively low temperatures. This is particularly true of some of the *Pseudomonas* species which are known to grow in butter.

The results on phosphatase production by organisms also are of interest from the standpoint of identification of species, a point that has been suggested by various investigators. Certainly, tests that are not too time-consuming and which have a real significance from the standpoint of the identity of organisms are needed. While many cultures of the various species must be investigated before adequate information can be formulated, interesting points along this general line are developing. Since different genera apparently include both phosphatase negative and phosphatase positive organisms, it appears that the test can be of value only within a genus.

Various cultures that produced phosphatase in sterile milk also produced it in other liquid media in which the particular species developed satisfactorily, while cultures that were phosphatase negative in sterile milk also were negative in other suitable liquid media. No detailed study was made of the numbers of organisms required in the media to give a positive phosphatase test.

PRODUCTION OF PHOSPHATASE IN BUTTER

In testing the phosphatase production of selected species in butter, the following procedure was used. Sweet cream was pasteurized at about 77° C. for 30 minutes. After cooling to about 17° C., portions of the cream were put into sterile jars, inoculated with the test organisms, and held over night at about 6° C. The cream was churned in the jars with a laboratory churn and the butter washed with sterile water and worked with sterile equipment. The butter, some of which was salted and some unsalted, was held at 21° C. This is a common temperature for keeping quality tests on butter. Although the changes at 21° C. sometimes are different from those at the temperatures to which butter is exposed in commercial channels, there is a general similarity and the changes occur more quickly; 7 days at 21° C. are roughly equivalent to a considerably longer period at 5° or

10° C., at least for many of the species that grow in butter.

The organisms which actively produced phosphatase in milk also rapidly produced it in unsalted butter. These included *Ps. putrefaciens, Ps. nigrifaciens, Ps. mephitica* and *Fl. fecale.* Certain cultures that were less active in milk, such as *O. lactis* and an *Aerobacter* strain, also gave definite reactions in unsalted butter. Some of the unidentified cultures gave conspicuous reactions in the butter.

In general, when the butter was salted, the production of phosphatase was less rapid and less extensive than with the corresponding unsalted butter but was still very definite with various organisms.

Portions of some lots of the experimental butter were held at temperatures considerably below 21° C. and tested for phosphatase at various intervals. Phosphatase production occurred in these portions, although it was less rapid than at 21° C., as would be expected from the temperature relationships of the organisms.

PHOSPHATASE DEVELOPMENT ON HOLDING

Various investigators have reported that butter which was originally phosphatase negative may become positive on holding. In studying this point, 28 samples of salted butter and 74 of unsalted butter were used, the latter being churned from highly ripened cream in order to get the desired flavor. The butter was made in a number of plants that varied greatly in the general methods of operation. Seven of the salted samples and 16 of the unsalted samples changed from negative to positive or from slightly positive to more strongly positive. Serums from these samples were streaked on the agar used for milk analysis and representative colonies picked into milk. After purification the cultures were tested for phosphatase production in milk. Three of the salted samples and nine of the unsalted samples readily yielded organisms that produced phosphatase.

The failure to isolate phosphatase positive organisms from all the samples streaked may have been due to the difficulty with which certain species develop on plates, although growing readily in butter. An example of such an organism is *Ps. putrefaciens;* ordinarily it can be isolated from butter in which it is present only with special procedures.

In general, the phosphatase positive organisms isolated from butter produced little or no acid in milk and were proteolytic. No attempt was made to identify them but studies along this line are under way. Several of the cultures were added to pasteurized cream and the cream churned, as already outlined. The resulting butter became phosphatase positive on holding.

CHEESE

Although trials were not carried out, in general it appears that there is less danger of phosphatase producing organisms causing positive phosphatase reactions in cheese than in butter. The most active phosphatase producing organisms encountered are relatively sensitive to acid and would have less opportunity for significant growth in cheese than in butter because of the rapid acid production during the manufacture and ripening of cheese. In a limited number of experimental cheddar cheese made from pasteurized milk, the phosphatase tests were still negative after ripening for several months.

SUMMARY STATEMENT

Various organisms produced phosphatase in sterile milk. Of the species studied, some of those belonging to the genus *Pseudomonas* were the most important in this respect. These and other phosphatase-producing organisms also produced phosphatase in both unsalted and salted butter, although the production was less rapid in the salted product. This general relationship suggests that phosphatase production by organisms in butter during the relatively long, normal holding period should be considered in applying the test to butter. An important point in this connection is the ability of various *Pseudomonas* organisms to grow at relatively low temperatures. In general, it appears there is less danger of phosphatase production causing positive tests in cheese than in butter.