1939

Relationship of Achromobacter putrefaciens to the putrid defect of butter

Thomas Joseph Claydon
Iowa State College

NOTE TO USERS

RELATIONSHIP OF ACHROMOBACTER PUTREFACIENS TO THE PUTRID DEFECT OF BUTTER

by

Thomas Joseph Claydon

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Dairy Bacteriology

Approved:

Signature was redacted for privacy.

In charge of Major work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
1939

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INTRODUCTION

Bacteriological changes constitute one of the important factors in the deterioration of butter. Organisms of various types are able to grow and produce conspicuous defects in both the unsalted or salted product. The growth of organisms and the accompanying development of defects are influenced by the temperature at which butter is held. At storage temperatures, such as -23.3°C. (-10°F.), no growth occurs but at temperatures as low as 0°C. (32°F.) some types of organisms are able to multiply and bring about various kinds of deterioration. At still higher temperatures additional microorganisms develop and cause a greater number of defects.

Bacteriological defects in butter that have been common during the past few years are those described as cheesiness. The putrid type of cheesiness is particularly widespread and has caused much concern in the major butter producing countries. In Canada the defect is designated surface taint, in Australia rabbito or a disagreeable aroma, in New Zealand a foetid odor, in Denmark a putrid odor and in the United States a putrid odor, limburger odor, or cheesiness. While much study, in different countries, has been devoted to the cause of the defect the problem is still not satisfactorily

solved.

Because of the serious losses that have resulted to the industry from the putrid type of cheesiness and the necessity for further information on its cause, the work herein reported was undertaken. The investigation deals principally with the bacteriological examination of defective commercial butter and the isolation of the causative organisms. It includes a study of Achromobacter putrefaciens in experimental butter and the effects of various factors on the development of the putrid defect by this species.

GENERAL CONSIDERATIONS

The Problem of Designation

The term cheesiness as applied to butter defects includes a wide range of off flavors and odors which vary from those suggestive of roquefort or of limburger cheese to those suggestive of swiss or of cheddar cheese. The roquefort like defect is probably due to the formation of a methyl ketone, such as methyl n-amyl ketone, from caprylic acid liberated from the butter fat and is readily distinguished from the other types. The limburger defect, often described as putrid or surface taint, is undoubtedly caused by protein breakdown and when typical is distinct from the swiss or cheddar types of cheesiness. The defect suggestive of swiss cheese appears to lie between the limburger and cheddar types and may arise from a combination of protein breakdown and fat hydrolysis. The characteristic cheddar odor is less suggestive of protein breakdown and may be more closely associated with hydrolysis of fat.

There is no sharp dividing line between the different types since, like other bacteriological defects, each undergoes a progressive sequence of changes according to the growth of the causative organism. At some stage of development one type of defect may resemble another. Furthermore,

since organisms capable of causing different types of cheesiness may be present at the same time, various combinations of the limburger, swiss, and cheddar defects may occur in butter. In these combinations one type of defect may predominate and then give way to another, or a blending may result in a defect suggestive of more than one type of cheesiness. Under such conditions a definite description of the defect is difficult.

The putrid type of cheesiness is the most important because of its objectionable characteristics and certain peculiarities in its development. Outbreaks are not confined to plants obviously having unsatisfactory sanitary conditions but often occur spasmodically where adequate precautions apparently are used. The defect develops in butter from cream of high quality as well as in butter from the lower grades. The fact that the defect is often not apparent until the butter is in retail trade channels makes it particularly objectionable.

Reproduction of Bacteriological Defects

Microbiological defects of butter are characterized by the fact that often they can be reproduced by inoculating defective butter into pasteurized cream and churning the cream. Frequently, defects may be carried through four or five successive generations by churning cream inoculated

with the butter just previously made. Such a reproduction is evidence that the defect is caused by microorganisms.

In studying defective butter with the object of isolating the causative organisms there are several factors that affect the situation. The age of the sample is important since the isolation is frequently handicapped by the fact that in old samples only the more hardy types of microorganisms have survived. Unless the original defect is definite, it may be difficult to recognize in experimental butter when reproduced by an isolated organism. The stage of the defect should be considered when attempting to reproduce the condition.

After the isolation of a suspected organism, certain points must be considered in studying its effect on butter. Such factors as temperature and time of storing, salting and working influence the development of defects by bacteria and must be considered in experimental procedures and in interpretation of results.

Most bacteria causing defects in commercial butter grow at comparatively low temperatures, 5° to 10°C. or lower. At such temperatures defects are produced relatively slowly and may be somewhat different than those produced by the organisms at room temperature.

The time of holding butter influences the degree and sometimes the type of defect. As mentioned previously, the progressive changes that occur due to the growth of

the organisms may result in defects that differ from one examination period to the next.

Salt is known to be an inhibiting factor in the growth of bacteria, the effect varying with the concentration. In a lot of butter the concentration is seldom uniform throughout, and organisms may grow at some points and cause a defect even though the general salt content, as indicated by the usual methods of analysis, should be sufficient to prevent growth. The distribution of salt is closely correlated with the size and distribution of the moisture droplets, which in turn are controlled largely by the amount of working the butter receives.

In unsalted butter it has been shown that thorough working is a factor in comtrolling bacterial defects (6, 17, 22). The moisture droplets are increased in number and the proportion infected becomes smaller; they are also reduced in size and the food supply available for the growth of organisms is correspondingly less.

Since some of the above conditions may not be the same in butter made in small lots as in commercial butter, results obtained experimentally should be interpreted carefully.

HISTORICAL

Relationship of Bacterial Flora to the Keeping Quality of Butter

In studying the relationship of bacteria to the quality of graded butter, Sadler and Vollum (28) considered that there was no correlation between bacterial count and grade. Similarly, Grimes (11) found no relationship between flavor score and the microbiological condition of butter.

From an investigation of the factors influencing the keeping quality of salted butter in cold storage, Loftus-Hill, Scharp and Bellair (19) concluded that bacteria had little effect in deterioration. The counts before storage did not correlate with the grade of the butter, neither did the change in grade during storage show any relationship to the bacteria present.

Guthrie, Scheib and Stark (12) stated that the bacterial spoilage of butter under commercial conditions was of minor importance, "--but that the importance of spoilage due to the growth of certain types of bacteria in butter previous to and after removal from commercial storage should not be minimized". They added further that recontamination after pasteurization should not be overlooked as a factor in butter spoilage.

From their work on the keeping quality of butter from sour cream, Flake and Parfitt (9) concluded that large numbers of lipolytic organisms after storage were closely associated with poor keeping quality. The correlation was particularly close with samples developing a putrid defect. High counts of proteolytic organisms apparently did not show as constant a relationship. Later, the same authors (10) studied some causes of deterioration at 15.5° C. of salted butter from sour cream. They observed that the group of samples dropping 2 to 2.5 points in score and also the group dropping 4 points or more, generally contained large numbers of proteolytic organisms. The group dropping 3 to 3.5 points in score, however, generally showed small numbers of proteolytic organisms. The suggestion was made that the organisms causing the deterioration may have died. Butter developing a putrid defect usually had higher proteolytic counts than that developing other off flavors.

Jacobsen (16) observed no correlation between the numbers of lipolytic and proteolytic bacteria and specific flavor defects in butter.

Putrid or Surface Taint Butter

Possible causes of the defect

The putrid defect in butter has been attributed to a variety of organisms and other factors.

Eckles (8) considered Bacterium fluorescens liquefaciens one of the main causative organisms in the putrid butter investigated by him in 1901.

Hunziker (15) stated that abnormally low acidity of the cream brought about by excessive neutralization appeared to favor the limburger flavor defect in butter, when such factors as bacterial contamination were present.

The putrid defect of butter has received considerable study in Canada under the designation surface taint. Sadler and Vollum (28) investigated an outbreak of the defect in western Canada. They stated that newly cut surfaces of the defective butter did not have the putrid odor but soon developed it. These investigators failed to arrive at a definite conclusion concerning the cause of the defect. They found colon types and Bacillus types of bacteria in large numbers, both in the butter and in the wash water used in the plants concerned. Subsequent improvement in sanitation remedied the outbreak of surface taint butter. Experiments with combinations of organisms isolated from defective butter gave some suggestion of producing surface taint when used in neutralized cream. These workers considered that neutralization of the cream might be a partial cause of the defect in the resulting butter.

Brown (4), in reporting on the quality of New South Wales butter, described a disagreeable aroma which was evidently similar to the putrid odor in butter in the

United States. Listing the principal characteristics of the defect, he stated that the flavor was not as bad as the odor. In mildly affected butter the flavor was of a slightly cooked nature. The defect occurred in comparatively high acid butter as well as in low acid butter. It was noted occasionally that when butter was made in different churns with cream from the same lot only some of the churnings developed the disagreeable odor. An interesting observation was that the odor never developed in raw or pasteurized cream or in butter from raw cream. The presence of the slightly heated flavor and the fact that the defect was found only in butter from pasteurized cream suggested that the process of pasteurization contributed to the defect. Bacterial action was considered to be partially responsible, but the defect could not be satisfactorily attributed to this cause alone.

Brown (4) quoted from a report stating that putrefying bacteria were not constantly present in large enough numbers to be the cause of the defect. It was thought that putrescent material was squeezed from cracks in the churn and spread through the butter during working. This material continued to decompose and gave rise to the disagreeable odor in the butter.

In a study of an outbreak of surface taint, Hood and White (14) found large numbers of yeasts, molds, and proteolytic organisms in much of the defective butter.

The acidity and the curd contents of the butter were normal. The water supplies were considered the source of the causative organism. Pure cultures of proteolytic bacteria from the water produced the defect in experimental butter. Surface taint was observed in butter having a salt content of 2.67 per cent. High salt content as a preventive measure in controlling the defect was considered impractical due to the demand for low salt butter.

Shutt (29) pointed out that many surface taint outbreaks occurred in plants using apparently adequate sanitary precautions and churning a high grade of cream. He associated the defect with cream of low acidity. Pseudomonas fluorescens, entering the butter through the wash water, was considered the causative organism.

In describing the decomposed odor occurring in butter, Sutton (31) stated that the defect possessed two striking characteristics, namely: the rapidity with which the defect developed in apparently satisfactory butter, and the apparent lack of correlation between numbers and types of bacteria and the defect.

Derby and Hammer (7) considered that there was little correlation between the general types of bacteria in butter, as shown by beef infusion agar plates, and the occurrence of surface taint. While the defective butter frequently showed large numbers of microorganisms, particularly at the surface, some samples had rather low counts. The flora

obtained from surface taint butter differed little from that of normal butter. The predominant organisms on plates poured with the off flavored butter failed to give a typical surface taint defect when inoculated into cream and the cream churned.

From a number of samples of surface taint butter, Derby and Hammer (7) isolated an organism which they named Achromobacter putrefaciens. By plating on beef infusion agar, they were unable to obtain this organism consistently from defective butter and they attempted to develop an enrichment method which would give better results. Since the organism grew well at low temperatures they inoculated surface taint butter into litmus milk, incubated at 5°C. until reduction occurred, and then plated on beef infusion agar. This method resulted in additional isolations of Achromobacter putrefaciens, although it was obtained only in small numbers. The organism was capable of producing a strong surface taint defect in butter at both 21° and 5°C. Several other types of bacteria capable of producing the defect were isolated. Although a variety of enrichment methods were used, a number of samples of surface taint butter failed to yield organisms capable of producing the defect.

Herreid, Macy and Combs (13) investigated the causes of cheesy flavors of the cheddar type in unsalted butter. They obtained mixed cultures from defective butter that

reproduced the cheesy defects when added to the cream used for churning. The cultures would also produce a defect in the butter when inoculated into the water used for washing. Certain of the mixed cultures produced a putrid rather than cheesy odor in butter at some stage of the holding period.

According to Flake and Parfitt (9), putrid flavors in butter were associated with pH values of 6.75 to 7.25 as well as with low salt contents.

Bacterium fluorescens liquefaciens, among other organisms, was associated with putrid flavors in butter by Sproule and Hamilton (30). Low acidity and low salt content were also suggested as conditions favoring the defect. Water supplies were considered a source of contamination.

Cullity and Griffin (6) cited the work of Loftus-Hill, Scharp and Searle in which organisms capable of producing rabbito were isolated from factory water supplies, churns, and raw and pasteurized cream. The water supplies were considered to be their natural habitat. The organisms were similar to those isolated by Derby and Hammer (7) from surface taint butter. Loftus-Hill, Scharp and Searle considered that poor texture in butter favored the production of the putrid condition.

The rabbito defect in Australian butter was discussed by Cullity and Griffin (6). They stated that a sweetish flavor suggestive of condensed milk was sometimes noted in the defective butter. After the butter was held for some

time at comparatively high temperatures, this flavor became very objectionable but the putrid odor seemed to disappear. The authors reported a number of investigations made at plants experiencing outbreaks of rabbito. The examinations were thorough and in each case the cause was traced eventually to the factory water. New water sources eliminated the trouble. During the investigations, many types of bacteria, particularly proteolytic types, were used in experimental churnings. These organisms failed to reproduce the typical defect. Other organisms were isolated from water supplies and, on sterile butter plates, gave an odor resembling surface taint. The cultures were short lived which suggested that the organisms causing rabbito might have the same characteristic. Apparently, Achromobacter putrefaciens was isolated from two water supplies. The authors stated that there appeared to be some variation in the types of these organisms. An instance was described in which the defect developed after faulty working of the butter.

Cullity and Griffin (6) concluded tentatively that the causative organism was probably water borne and that foci of contamination were built up in churns and equipment from initial contamination from the water. Thorough working and high salt content of the butter, as well as high acidity in the cream, were factors that retarded the development of the defect. The authors remarked that the whole story was not

known.

In Canada an organism that apparently is Achromobacter putrefaciens has been isolated from surface taint butter as well as from water supplies of plants experiencing trouble with the defect (33).

Water supplies as a source of organisms causing the putrid defect

Various investigators have considered that the organisms causing cheesy or putrid butter come from contaminated wash water. Early workers demonstrated that butter washed with water having a high bacterial count, or containing particular types of bacteria, was usually of poor keeping quality.

Milich (23), in studying the effect of bacteria in wash water, found that butter washed with water of low bacterial count kept better than that washed with water of a higher count.

Bacterium fluorescens liquefaciens was found by Busch (5) in 13 per cent of the water samples examined. The ability of the organism to hydrolyze fat and its influence on the keeping quality of butter were noted. General caution was advised regarding the wash water used in butter plants.

Virtanen (32) considered that the greatest bacterial spoilage in butter was due to water types, such as Bacterium fluorescens and Bacterium punctatum. He stated that these

organisms could withstand high temperatures but were susceptible to salt and acid, Virtanen suggested that enzymes produced in the cream might not be destroyed by pasteurization and hence could cause defects in butter, even though the bacteria themselves were not present. Gelatin liquefying bacteria were considered the cause of most bacteriological defects in butter.

Among the conclusions reached by Rumment (27) on the relationship of wash water to the keeping quality of butter were: sweet cream butter held more bacteria than sour cream butter during washing; butter retained fewer bacteria from the wash water when the consistency was firm and the lumps large; Bacterium fluorescens liquefaciens was one of the more common water types affecting butter; the fluorescent bacteria generally did not multiply in butter at a pH of 4.2 to 4.3.

Sadler and Vollum (28), Shutt (29), Hood and White (14), Herreid, Macy and Combs (13) and Cullity and Griffin (6) have all considered that the organisms causing surface taint and cheesiness frequently come from contaminated wash water; a number of instances have been cited in which a change of water supply was a remedy for putrid butter outbreaks.

The churn as a source of organisms causing the putrid defect

For a number of years it has been recognized that the

churn is a significant source of microbiological contamination of butter.

In studying the microorganisms in churns, Olson and Hammer (24) isolated a number of organisms that produced cheesiness in experimental butter. They found with unsalted butter that rancidity was the most common defect in butter from contaminated churns, while cheesiness was the most common defect in butter from clean churns.

The report of Cullity and Griffin (6) showed that churns and other equipment were an immediate source of organisms causing the putrid defect. The water supplies, however, were considered to be the primary source of these organisms.

Distribution and Development of Bacteria in Butter

Rahn, Brown and Smith (26) made successive bacteriological examinations of samples of butter and found that daily platings did not always show the same types of bacteria. A certain species might be found in reasonably large numbers on the first day, not appear in the next several examinations, and then be observed on final examination. The explanation offered was that there were only a few places in the butter where such organisms occurred and perhaps multiplied. These organisms were regarded as too rare to be of significance. The authors also suggested that in other places the salt might not

reach all the smallest buttermilk drops even after careful working. Organisms would develop readily in these places.

In a study of the distribution and growth of bacteria in butter, Rahn and Boysen (25) pointed out that there were more than 100 moisture droplets to every bacterial cell present. The amount of moisture infected depended on the number of bacteria and the amount of working of the butter. In overworked butter more moisture was made sterile. They suggested that acid might diffuse from one droplet to the next and hence allow more bacterial growth in the first droplet. Working butter as much as possible assisted in preventing deterioration by bacteria.

Knudsen and Jensen (17) stated that butter worked considerably and having a fine distribution of water kept better than butter worked little and having a coarse distribution of water. Also, such butter, when unsalted, kept better than salted butter with a less thorough distribution of water. They offered the explanation that the salt drew the water and made coarser droplets and that organisms not inhibited by salt could grow. Knudsen (18) also showed that large moisture droplets favored mold growth in butter in a manner similar to bacterial growth.

Long and Hammer (21) demonstrated by the use of the Burri smear culture technic that the distribution of bacteria in butter was very irregular, both in numbers and types. They further showed that types not obtained by

the usual plating method sometimes could be obtained by smearing small portions (approximately 1/20,000 gram) of the butter on agar.

The same workers (22) found that, in unsalted butter, the growth of various organisms and the production of defects by them were influenced by the extent of working, there being less growth and deterioration with increased working. The results were attributed to the finer dispersion of moisture obtained and to the corresponding decrease in food supply available for microorganisms in infected droplets.

METHODS

Samples Studied

The samples of defective butter studied came from plants in several states of the middle west. The original sources of a number of samples were unknown since they were obtained from marketing organizations. Some of the samples were 4 or 5 days of age but the majority was from several weeks to several months old. The samples showed the putrid defect to varying degrees, some being very marked and typical while others were mild or questionable; a few samples definitely suggested other types of defects. Most of the butter was salted.

Methods of Bacteriological Examination

In most cases the bacteriological examination was begun as soon as the butter sample was received. When this was impossible the sample was held at about 5°C. and examined as soon as convenient.

Butter was prepared for plating by placing a portion in a sterile petri plate, warming gently over a small flame and stirring with a warmed, sterile pipette. Care was taken to avoid settling of the serum. Dilution blanks were warmed

in a 45°C. incubator.

Beef infusion agar having a pH of 6.8 to 7.0 was used in all bacteriological examinations. Various other media and modifications were used from time to time in comparative studies but were discarded as inferior to beef infusion agar. For the purpose of demonstrating lipolytic and proteolytic characteristics of the bacterial flora, fat emulsion and sterile skim milk were added to the media before plating. The fat emulsion was prepared according to the method of Long and Hammer (20). Five per cent of both fat emulsion and skim milk were used. Plates were incubated at room temperature for 5 days; frequently duplicate sets were held at 5° or 10°C. for 10 to 14 days. Examinations were made often during incubation.

Methods for pH of Serum and Acid Number on Fat

When the original butter samples were large enough, pH values were determined on the butter serum. The serum was obtained by melting the sample and then centrifuging. The pH determinations were made with a quinhydrone electrode. Acid numbers on fat were determined on 10 gram samples according to the method of Breazeale and Bird (3), and expressed as milliliters of 0.1 normal KOH.

Churning Methods

For making the experimental churnings sweet cream of

good flavor was thoroughly pasteurized at 85° to 90°C. for about 30 minutes. After being cooled the cream was divided among a number of quart mason jars in each of which a pint of cream could be churned. Defective butter, cultures of organisms, or water samples were then inoculated into the cream and the jars placed at about 10°C. overnight for incubation. When inoculating with butter, 5 to 10 grams were used; the cream was warmed and the butter well mixed with it. After cooling again it was placed at the incubation temperature. The cultures or water samples were added directly in varying amounts.

The churning was done in an experimental churn having compartments for holding the mason jars. The devise was connected with a motor and agitation was obtained by rotation of the shaft. The butter from each jar was washed, worked with hand paddles in enamel bowls and divided into two portions. These portions were placed in small glass jars (covered) and stored at 21°C. and about 5°C.

All equipment, wash water, and salt (where used) were sterilized by autoclaving.

When it was desirable to have more than two samples of butter from one churning, a one gallon Dazey churn was used. This churn was sterilized by filling with a chlorine solution and allowing it to stand overnight. It was rinsed with sterile water before using.

Examinations of experimental butter held at 21°C. were

made daily. Samples held at 5°C. were examined at intervals of about 3 to 4 days.

RESULTS

Preliminary Studies

Examination of defective butter by plating original samples

In the early part of the work attempts were made to relate the putrid defect to the dominant types of organisms appearing on beef infusion agar plates poured with commercial defective butter. Various samples of typically putrid butter of different ages were plated and the plates incubated at room temperature. Colonies representing the main types were picked into litmus milk. After development the cultures were inoculated into pasteurized cream and the cream churned. The unsalted butter was held at 21° and 5°C. and examined frequently for the appearance of a defect similar to the original. No positive results were obtained, the defects which developed being indefinite and not suggestive of the typical putrid condition.

Attention was next directed to the isolation of organisms which might be expected to cause a noticeable change in butter. With the aid of skim milk and fat emulsion added to the agar, numerous proteolytic and lipolytic types were picked and, after development, added to cream for churning. Various combinations of the cultures

were also used in attempts to reproduce the original defect. Many of the organisms appearing on the plates, when used in pure culture or combinations, produced defects in butter and these varied widely. None of the odors produced, however, were of the characteristic putrid type, and hence the organisms could not be considered the cause of the original defect.

The examination of the original butter indicated that the flora, as shown by beef infusion agar plates, was not related to the defect and that other methods of isolating the causative organism would need to be applied.

Development of enrichment methods

Since the putrid defect develops commercially at comparatively low temperatures, it was thought that enrichment methods at about 10°C. would increase the relative numbers of the causative organisms. Accordingly, the following scheme was adopted: Pasteurized cream was inoculated with defective butter and incubated overnight at about 10°C. It was then churned, the resulting unsalted butter stored at 21° and 5°C. and examined frequently for the development of a defect similar to the original. When the butter became putrid it was plated and colonies picked in the manner used with the original commercial samples.

In a number of cases the defective experimental butter was again inoculated into cream which was then held overnight

at about 10°C. and churned. The butter was stored as before and the same bacteriological examination was applied. In other cases the original butter was inoculated into litmus milk which was incubated at 10°C. for 4 days or at 5°C. for 2 weeks. These cultures were then used in making experimental butter which was examined and plated as usual.

The purpose of the above procedures was to increase the numbers of organisms capable of growing at low temperatures and also to assist in recognizing that certain organisms were not the cause of the defect. For example, if the flavor defects of the original butter and the succeeding experimental samples were similar while the floras shown on plates differed significantly, organisms not common to the various floras probably could be eliminated as the cause of the defect.

From the plates poured with the experimental butter, colonies of different types were picked into litmus milk. Particular attention was given to those types that appeared consistently in samples that developed the putrid defect through successive churnings. From the colonies picked, certain cultures were selected for inoculation into cream which was subsequently churned. Some cultures were selected on the basis of their ability to cause noticeable changes in litmus milk and also on the frequency with which they occurred in the butter. In the early stages of the work it was believed that cultures might fail to produce an

objectionable change in litmus milk and yet cause strong flavor and odor defects in butter. Consequently, cultures producing little change in litmus milk were also investigated.

It was considered that organisms bringing about little change in litmus milk might produce off odors in other media. Accordingly, several different liquid media were tried. These consisted of cream, casein medium (2), casein medium plus peptone, and each of the two latter media adjusted to a slightly alkaline reaction. Litmus milk was used as a basis for comparison. No consistent advantage was obtained from the use of these media.

A number of different solid media were also used for plating the butter in an effort to obtain growth of organisms not appearing on ordinary beef infusion agar plates. These consisted of casein agar medium (2), casein agar medium plus peptone, beef infusion agar plus butterfat and casein, and beef infusion agar with a pH of approximately 7.5. The results with these media on different butter samples showed no consistent advantage over beef infusion agar and several were quite inferior.

In the attempts to use enrichment methods, no success was achieved in isolating, from defective butter, organisms capable of producing a putrid odor characteristic of the original butter when they were inoculated into cream and the cream churned.

Application of the Burri Technic to the Examination of Defective Butter

In applying the Burri smear culture technic to the examination of butter, Long and Hammer (21) found that the method sometimes demonstrated types of organisms not evident on plates poured in the usual manner. They believe that organisms were better able to initiate growth when clumps of cells were seeded on the agar, as with the Burri method, and that organisms present in small numbers were not diluted out when portions of butter containing them were smeared on agar.

The procedure (21) consisted of picking tiny portions of butter (about 1/20,000 gram) with a platinum needle and the aid of a 6X binocular and smearing them thoroughly over the surface of a dry beef infusion agar slant. Usually 10 to 25 portions were picked from different points in the butter and smeared on separate agar slants. The slants were incubated at room temperature for 4 days and then examined for numbers and types of organisms present.

Modification of the method as applied to the problem

Because of the failure of previous efforts to isolate the causative organism from putrid butter, the above procedure, with slight modifications, was applied to the examination of defective samples. In place of the agar slants, petri plates were poured with beef infusion agar plus fat emulsion and skim milk and allowed to solidify.

Attempts were made to avoid the formation of water droplets on the agar, and if such occurred the plates were placed at 37°C. until dry. The plates were marked into six sectors and each sector smeared with a tiny portion of butter. Usually three plates were smeared from each sample of butter. These were incubated at room temperature for 5 days and were examined daily for the appearance of different types of organisms. The use of plates in place of slants was of advantage in that colonies were easier to examine and pick, while the addition of fat emulsion and skim milk to the agar made possible the detection of lipolytic and proteolytic colonies.

The effectiveness of the smear technic in demonstrating types of organisms not obtained by the usual plating procedure was shown by examinations of samples of butter having various defects. A number of typical examples are outlined below.

General types of organisms obtained from defective butter by plating and smearing

EXAMPLE NO. 1. The usual plating method gave largely orange and white Micrococcus colonies with a small number of moist, dark colonies.

On smearing the butter the same three types of colonies were apparent. In addition, a number of small dark colonies, many brownish white, proteolytic colonies, and also a number of colonies rather similar

to the Ach. putrefaciens type, but not proteolytic, were present; these three additional types were quite conspicuous.

EXAMPLE NO. 2. The types appearing on poured plates were entirely orange and white Micrococcus colonies.

The smear method demonstrated the same Micrococcus types and in addition a number of dark, proteolytic colonies.

EXAMPLE NO. 3. On poured plates moist, white colonies that were both lipolytic and slightly proteolytic constituted practically the whole flora of the butter.

Plates smeared with butter showed the same type to be most common. However, other types were readily apparent. These included a small transparent colony and a rather white, transparent, non-proteolytic colony.

EXAMPLE NO. 4. The plating method demonstrated the following types: mainly white proteolytic colonies suggestive of Pseudomonas fragi; a few Bacillus colonies; a number of slightly fluorescent colonies.

The smear method gave types as follows: many colonies similar to Pseudomonas fragi; a number of strongly fluorescent colonies; some small transparent types and a few Bacillus colonies. In addition, colonies resembling those of Ach. putrefaciens were readily observed.

With very few exceptions, results similar to the above were obtained when samples of butter were examined by the two methods.

The smear technic rather regularly demonstrated types of organisms that did not appear on poured plates. Frequently, an organism that was absent on the poured plates was obtained in almost pure culture on one or more smeared sections. The superiority of the smear method for isolating the various species of organisms from butter was recognized and it was accordingly used in attempts to isolate the organisms responsible for the putrid defect in commercial butter.

Effectiveness of the Burri Technic in Isolating *Achromobacter putrefaciens* from Commercial Putrid Butter

Because of the effectiveness of the Burri method in demonstrating in butter, organisms that were not found by plating, the method was used in conjunction with the plating procedure in attempting to isolate the causative organism from putrid butter. The examinations involved commercial putrid butter and also butter made by churning cream inoculated with an original sample. In many cases experimental butter was inoculated into cream and the cream churned so that additional experimental churnings were available for study. Occasionally, other modifications in enrichment procedure were applied. In testing isolated

organisms for their action on butter they were inoculated into cream and the cream churned; the butter was held at 21° and 5°C. and examined frequently. Data involving a number of examinations and leading to the detection of the causative organism are presented in the following examples.

Sample A.

The sample was obtained from a car of butter and represented an ordinary commercial churning. The putrid defect was so distinct that the butter was practically unsalable and resulted in a significant loss to the producing plant. The sample was relatively fresh and mildly salted. Since the Burri procedure was not applied until the later stages of the examination, some of the data comparing the Burri and plating methods are not as complete as with later samples.

The original butter was plated in the usual manner. The bacterial counts and the colony types on the plates were as follows:

Bacterial counts		Remarks on main colony types
Total	125,000 per ml.	The dominant types were white proteolytic and non-proteolytic colonies and pale orange, proteolytic colonies. Neither these nor less common types reproduced the original defect experimentally.
Proteolytic	35,000 " "	
Lipolytic	1,000 " "	

At the time of plating the original butter a portion was inoculated into pasteurized cream, held overnight at

about 10°C., and then churned. Portions of butter from this churning were held at 21° and 5°C. and examined frequently.

The sample held at 21°C. became putrid in 2 days. The bacterial counts and general colony types shown by plating were as follows:

Bacterial counts		Remarks on main colony types
Total	31,000,000 per ml.	Generally the same types were present as with the original butter. A few other types were present in small numbers. None produced a putrid defect in experimental butter.
Proteolytic	2,800,000 " "	
Lipolytic	4,500,000 " "	

The sample held at 5°C. was putrid in 7 days. Plating showed the following bacterial counts and colony types:

Bacterial counts		Remarks on main colony types
Total	6,200,000 per ml.	Large, moist, lipolytic colonies constituted the main type; they did not reproduce the original defect in experimental butter.
Proteolytic	1,000 " "	
Lipolytic	3,500,000 " "	

When the first experimental churning was made a small portion of the original butter was inoculated into litmus milk. After incubating for 5 days at 10°C. it was added to cream, and the cream churned; the butter was divided and stored at 21° and 5°C.

The sample held at 21°C. became putrid in 1 day. Plating showed the following bacterial counts and general colony types:

Bacterial counts		Remarks on main colony types
Total	4,700,000 per ml.	The majority of the colonies were white non-proteolytic and non-lipolytic. A putrid defect was not obtained in experimental butter with these types.
Proteolytic	65,000 " "	
Lipolytic	30,000 " "	

The 5°C. sample developed a putrid defect in 7 days. The bacterial counts and colony types obtained on plates were as follows:

Bacterial counts		Remarks on main colony types
Total	25,000,000 per ml.	Practically the whole flora was made up of lipolytic, non-proteolytic colonies which did not produce a putrid condition in experimental butter.
Proteolytic	20,000 " "	
Lipolytic	17,000,000 " "	

At this point the smear method was first applied in attempting to isolate the causative organism. Tiny portions of the 5°C. sample were smeared on agar plates which were examined frequently for the appearance of various colony types.

The colonies that developed included those found on the poured plates and two types not previously noted. One of these was a small, white, non-proteolytic colony and the other was typical of Ach. putrefaciens. Both of the types were obtained in relatively large numbers, with several smeared sections showing almost pure cultures of

one or the other. When tested, the Ach. putrefaciens type produced a putrid defect similar to that of the original butter while the other type did not.

From an examination of the plates poured with the original butter and with subsequent experimental samples, it was evident that the numbers and types of organisms shown by this method bore no relation to the defect since a great variation in both numbers and types resulted in no change in the development of the putrid odor. The situation suggested that organisms other than those appearing on the usual plates were concerned with the defect. This was supported by the fact that as the bacteriological examination progressed many cultures and combinations of cultures obtained from poured plates were used to inoculate cream for experimental churnings, without reproducing the original defect. With the application of the smear method to the experimental butter, additional bacterial types were immediately made evident. Of these Ach. putrefaciens was present in considerable numbers and was the only type isolated that was capable of producing the putrid defect.

Sample B.

The shipment of butter from which this sample was obtained was of high quality when sold to the wholesaler. Shortly afterwards, however, it developed the putrid defect and was returned. The sample, which was lightly salted,

was relatively fresh when received at the laboratory.

The original butter, when plated, showed the following bacterial counts and general colony types:

Bacterial counts		Remarks on main colony types
Total	9,200,000 per ml.	Most of the colonies were white micrococci which produced no defect in experimental butter.
Proteolytic	300,000 " "	

The smear method showed the same colony types as the plating method and in addition a number of fluorescent, proteolytic colonies.

Cream was inoculated with a portion of the original butter, held overnight at 10°C., and churned. The butter was divided and stored at 21° and 5°C.

The 21°C. sample developed the putrid odor in 2 days. The bacterial counts and general colony types obtained on poured plates were as follows:

Bacterial counts		Remarks on main colony types
Total	105,000,000 per ml.	The dominant types were micrococci and grey, proteolytic colonies. A few fluorescent colonies were present. None produced a typical putrid defect in experimental butter.
Proteolytic	35,000,000 " "	

The smear method showed a large number of the fluorescent, proteolytic colonies in addition to the above types. There were also a number of colonies of the Ach.putrefaciens type. Only the latter type produced a putrid defect in butter.

The sample held at 5°C. became putrid in 4 days and when examined by plating showed the following bacterial counts and colony types:

Bacterial counts		Remarks on main colony types
Total	4,500,000 per ml.	The same general flora was obtained as with the 21°C. sample.
Proteolytic	1,800,000 " "	
Lipolytic	1,100,000 " "	

Smearing gave fewer fluorescent colonies than were obtained from the 21°C. sample. Most colonies were dark and proteolytic and a number were typical of Ach. putrefaciens. When tested, only the Ach. putrefaciens type reproduced the original defect.

The early use of the smear technic in the examination of the sample resulted in Ach. putrefaciens being isolated sooner than was the case with sample A.

Sample C.

This sample, which was mildly salted, was from a commercial churning that had developed the putrid defect. It had been held at room temperature to test the keeping quality. On the development of the defect it was sent to the laboratory for examination.

The numbers of organisms and the colony types obtained by plating were as follows:

Bacterial counts		Remarks on main colony types
Total	6,800,000 per ml.	All the colonies appeared to be Micrococcus or Streptococcus types which failed to produce a defect in experimental butter.
Proteolytic	1,000 " "	
Lipolytic	1,000 " "	

The smear method showed the same types as the poured plates and in addition a number of dark colonies. None reproduced the putrid odor in butter.

At the time of smearing and plating, the original butter was inoculated into cream, which was held at 10°C. overnight and churned. As before, the butter was stored at 21° and 5°C.

In 2 days the sample held at 21°C. became putrid. The results of the examination by plating were as below:

Bacterial counts		Remarks on main colony types
Total	9,100,000 per ml.	Most colonies were white, moist and lipolytic. Neither this type, nor the less common types studied reproduce the defect in experimental butter.
Proteolytic	35,000 " "	
Lipolytic	1,400,000 " "	

In addition to the types evident on plating, the smearing method showed a number of colonies typical of Ach. putrefaciens as well as some less typical. Only the typical Ach. putrefaciens type reproduced the original defect.

The sample stored at 5°C. developed the putrid defect in 7 days. On plating, the following bacterial counts and

colony types were obtained:

Bacterial counts		Remarks on main colony types
Total	9,700,000 per ml.	The types were the same as with the 21°C. sample.
Proteolytic	300,000 " "	
Lipolytic	2,800,000 " "	

Smeared plates from the 5°C. sample gave the same types as smeared plates from the 21°C. sample, but showed a greater number of Ach. putrefaciens colonies.

Since the Ach. putrefaciens cultures obtained during the above examination were the only ones that reproduced the putrid condition they were considered the cause of the original defect.

General considerations in the isolation of Ach. putrefaciens

The results presented above are typical of the majority of those obtained on samples of putrid butter. The smear technic was effective in demonstrating Ach. putrefaciens in various samples of putrid butter from which it otherwise could not be isolated. While colonies of the organism were often present in small numbers and scattered among other types, they sometimes formed a heavy growth on certain of the smeared sectors of the plates.

Figure 1 shows the development of Ach. putrefaciens on a plate smeared with putrid butter and, for comparison, a plate poured in the usual manner with the sample. The Ach. putrefaciens colonies are very evident on several of the

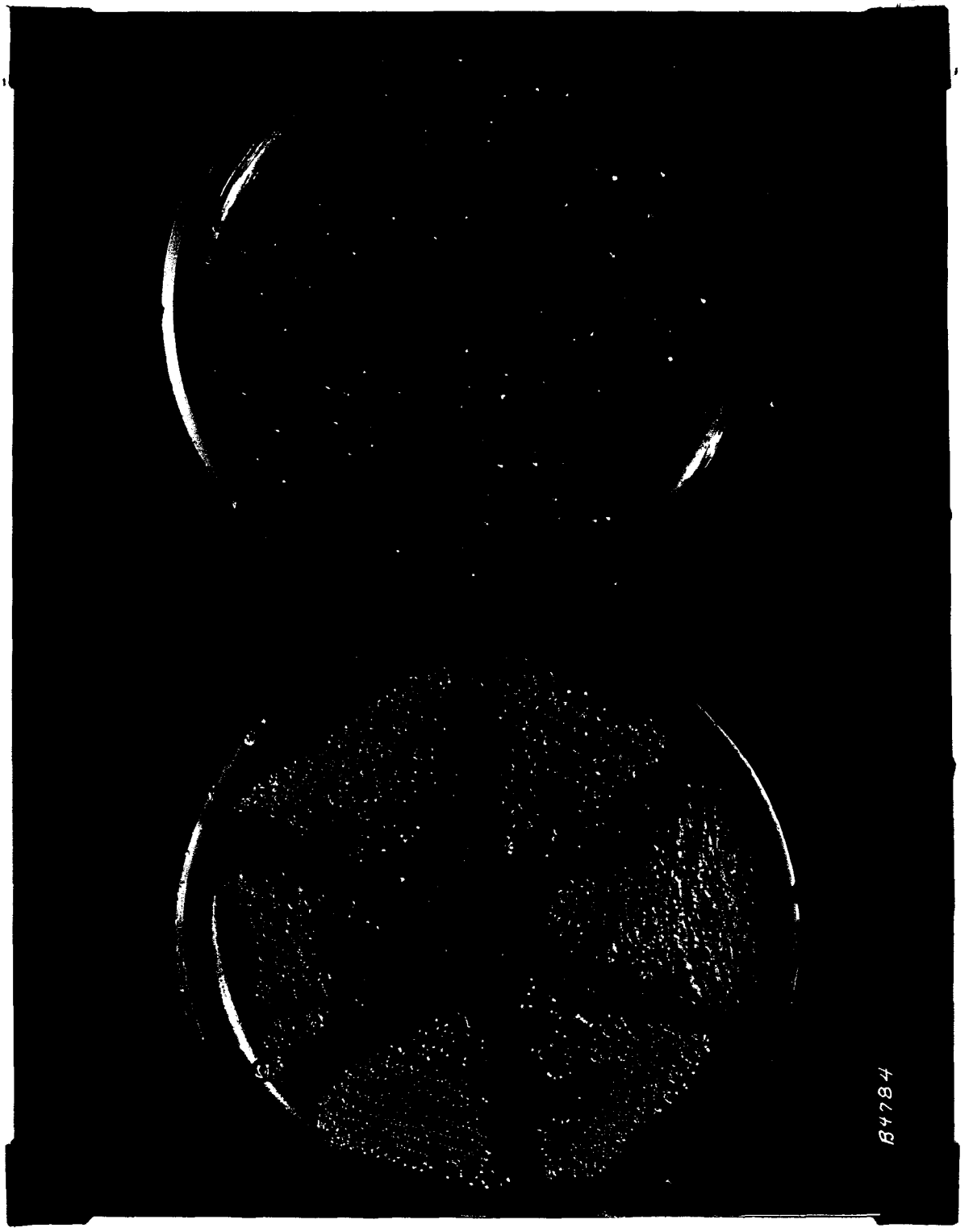

Figure 1. A smeared plate (lower) and a poured plate from the same sample of putrid butter. Several colonies of the Ach. putrefaciens type are indicated.

sections. The other colonies are micrococci. On the poured plates no Ach. putrefaciens colonies were present and micrococci constituted the whole flora.

From the reports of examinations of samples A, B, and C it will be noted that, even with the use of the smearing method, Ach. putrefaciens was not isolated from the original butter. In only one of the samples subsequently examined was the organism obtained from the original sample. This may have been due to the age of the butter and the possibility that many of the organisms had died before examination was begun. Later studies seemed to bear out this suggestion. Had the butter been examined when the defect first developed, Ach. putrefaciens might have been isolated from the original.

The reports also show that Ach. putrefaciens was frequently obtained from butter made from cream inoculated with the original sample. Apparently, the process stimulated the growth of the organisms and resulted in the presence of greater numbers in the experimental butter. This may have been due partially to the incubation of the inoculated cream at low temperatures before churning. In some of the subsequent examinations where Ach. putrefaciens could not be obtained from the first experimental butter, it was found when a second generation churning was prepared.

In general, Ach. putrefaciens was obtained in larger numbers from plates smeared with the experimental butter

held at 5°C. than from plates smeared with the butter held at 21°C. This was more evident with a number of the samples studied later, from some of which Ach. putrefaciens could not be obtained when the experimental butter was held at 21°C. The lower temperatures undoubtedly inhibited some types of organisms that grew well at 21°C. and allowed Ach. putrefaciens to develop more readily and be detected.

In a number of cases smeared plates from putrid experimental samples were incubated at 5°C. Occasionally these plates showed a greater percentage of Ach. putrefaciens colonies than did plates incubated at room temperature, and in one or two cases they were the only plates from a sample on which the organism was found.

With some experimental butter samples, smears were made on successive days after the development of the defect. In several instances Ach. putrefaciens was obtained on the second or third set of smears (made on the second or third day after the defect developed) when it was not observed on the first set of smears. While the various samples examined were not consistent in this respect, the procedure occasionally resulted in the isolation of the organism when it was not obtained otherwise.

It was frequently noted that Ach. putrefaciens was obtained in larger numbers from the interior of a sample of experimental putrid butter than from the surface. While this seldom applied to butter that was only a few days old,

it was often true of samples that were held a week or more at 21°C. Furthermore, in the case where the organism was isolated from the original butter, it was obtained from the interior but not from the surface.

Suggested procedure for isolation

From the foregoing observations it is apparent that the isolation of Ach. putrefaciens from putrid butter containing the organism is often difficult. Various procedures facilitate the isolation but they were not successful in all cases and several samples of putrid butter failed to yield the organism. More information is required concerning the growth characteristics of Ach. putrefaciens in order to develop a more effective method of isolation. The following procedure is suggested as being relatively successful and can be used in whole or in part.

The original butter is smeared on a number of plates (about 6), some of which are incubated at room temperature and some at 5°C. The butter is also inoculated into thoroughly pasteurized cream which is held overnight at 10°C. and churned. The resulting butter is divided and held at 21° and 5°C. After development of the defect these experimental samples are smeared in a similar manner to the original and are used to inoculate cream for the production of second generation samples; these are also smeared as soon as the defect develops. Even a third

successive churning may be advisable in case the organism has not been isolated. At the time of examining the original butter a portion is inoculated into litmus milk and held at about 5°C. until reduction occurs. This reduced culture is then smeared and the plates incubated at room temperature and at 5°C. It is also used to inoculate cream for churning and the resulting butter is held and smeared when the defect appears. All smeared plates held at room temperature are examined daily for Ach. putrefaciens colonies; the plates held at 5°C. are examined when the colonies are reasonably well developed. The purpose of the whole procedure is to obtain sufficient enrichment of Ach. putrefaciens to allow its appearance on some of the plates.

Number of samples yielding Ach. putrefaciens

The procedure suggested above was used in more or less detail on various samples of commercial putrid butter. Some of these were not typically putrid and when inoculated into cream and the cream churned usually produced rancidity or the cheddar type of cheesiness in the butter. The samples were of different ages and represented churnings made from sour cream as well as from sweet cream. They came from plants in widely separated parts of the United States. Each sample did not necessarily represent a separate source since a number of samples were obtained at intervals from the same plants and represented churnings made at

different periods. While some of the plants apparently produced only an occasional churning of the defective butter, others experienced outbreaks of considerable duration. There were undoubtedly variations in the conditions under which the butter was produced. While some plants were attempting to use adequate sanitary precautions, it is probable that conditions were less desirable in the others.

Fifty-eight samples of typical commercial putrid butter were examined. Of these 41 or 70.7 per cent yielded Ach. putrefaciens, 6 or 10.3 per cent contained a type of organism that was similar to Ach. putrefaciens, but different in certain characteristics, and 3 or 5.2 per cent yielded a different type of organism that was capable of producing a putrid defect somewhat like the original. From the remaining 8 or 13.8 per cent, the causative organism could not be obtained.

The 41 samples from which typical Ach. putrefaciens was obtained varied in age but were generally less than 1 month old with many of the samples being less then 2 weeks old. All produced a putrid odor in experimental butter when inoculated into cream and the cream churned. The defect was occasionally slight in the first churning but usually became more marked if a second churning were made using the previous experimental butter as inoculating material.

From one sample possessing the defect to a very marked degree, Ach. putrefaciens was obtained from the original butter; the age of this butter was not known. Most of the definitely putrid samples yielded the organism in comparatively large numbers from the first experimental churning. The samples showing the defect to a lesser degree usually did not give Ach. putrefaciens till a later stage of the examination procedure was reached and then in small numbers.

Defective samples from the same source were rather consistent in yielding Ach. putrefaciens. For example, 12 out of 15 samples from one plant, 3 out of 4 samples from a second, and 3 out of 4 samples from a third yielded the organism.

The 6 samples from which Ach. putrefaciens was not obtained, but from which a similar type of organism was isolated, did not differ greatly from the others. In general, the defect was less marked in both the original butter and in the butter made from cream into which the original sample had been inoculated. The organisms were obtained at various stages of the examination procedure and usually in comparatively large numbers. There was no evident connection between this apparent variant form of Ach. putrefaciens and the source of the original butter, since the organism was found in samples from different sources.

The 3 samples from which organisms different than Ach. putrefaciens were obtained were distinctly putrid. However, when they were inoculated into cream and the cream churned, the resulting butter was not typical of the defect and failed to show the characteristic stages of change. The same general type of defect was produced by the organisms isolated from the samples.

The 8 samples from which the causative organisms could not be obtained were very definitely putrid. While organisms were isolated that were capable of causing off odors in butter they did not reproduce the original defect and hence were not considered the causative type.

It appeared that Ach. putrefaciens was a common cause of the putrid defect in the butter studied. While it could not be obtained from all the samples, it was isolated from most of the butter that was fresh enough to reproduce the defect in experimental churnings.

Examinations of Churns and Water Supplies for Achromobacter putrefaciens

Since Ach. putrefaciens is easily destroyed by heat, its presence in butter suggests that the pasteurized cream has been recontaminated by the equipment or that the butter is contaminated by the water used for washing. An attempt was therefore made to determine the prevalence of the organism in churns and in water supplies of commercial plants.

Examination of churns

In making the examinations, different parts of a churn were cultured with sterile cotton swabs moistened in sterile water. Swabs were made on doors, rolls, and ends of churns in 10 different plants. The swabs were placed in sterile test tubes and returned to the laboratory the same day. On arrival, each swab was placed in a tube of litmus milk and incubated at 10°C. until a noticeable change occurred. The cultures were then inoculated into cream, the cream churned, and the butter held at 21° and at 5°C.

In most cases the butter developed off odors in 2 to 4 days at 21°C. and in about 10 days at 5°C. While many of the odors were disagreeable none were of the typical putrid type. Attempts to isolate Ach. putrefaciens by smearing the butter were unsuccessful, although one of the more putrid samples yielded an organism capable of reproducing a somewhat similar defect.

The results indicated that Ach. putrefaciens was not a type commonly present in the churns in the commercial plants studied, although the difficulties involved in isolating the organism might account for the failure to obtain it.

Examination of water supplies

Examinations were made on samples taken from water supplies of three commercial plants.

Sample 1 was from the supply used for washing butter

in a plant experiencing difficulty with the putrid defect. The sample was taken at the pump after 10 minutes operation. In examining the water as a possible cause of the putrid defect, a portion was used for washing a sample of butter made from uninoculated cream. Another portion (about 50 ml.) was inoculated into cream (about 400 ml.) and the cream churned. With each procedure the butter was held at 21°C. and at 10°C.

Butter washed with the water became putrid in 2 days at 21°C. and in 6 days at 10°C. The sample made from cream into which the water had been inoculated developed an off odor in 3 days at 21°C. and in 8 days at 10°C.; this odor was not typically putrid. On smearing the butter, Ach. putrefaciens was obtained from that washed with the water, but not from the other.

At the time of churning, the water was smeared on agar slants. A comparatively large number of colonies was obtained, some of which were suggestive of Ach. putrefaciens. When these colonies were picked into litmus milk there was a rapid reduction characteristic of Ach. putrefaciens. The cultures, when inoculated into cream and the cream churned, rapidly produced the putrid defect.

Similar examinations were made on water samples obtained from two other plants experiencing occasional defective churnings. While butter washed with the water developed an objectionable odor somewhat resembling the

putrid defect, Ach. putrefaciens could not be obtained either from the water or the butter. Some of the organisms isolated caused strong off odors in butter but these were not of the typical putrid type.

The examinations indicated that contaminated water supplies from commercial plants were capable of causing defects when used for washing butter. While only one of the three samples yielded Ach. putrefaciens, the data suggest that the water supply may be a source of the organism.

Observations on Achromobacter putrefaciens

Relation to original description

The Ach. putrefaciens cultures isolated from putrid butter generally conformed to the description given by Derby and Hammer (7). They agreed in morphology, cultural characteristics, and most biochemical characteristics, although slight variations occurred in reaction changes in some sugar bouillons. Almost all cultures produced acid from maltose and sucrose and the variability occurred in the action on glucose, galactose, lactose, and levulose. However, as suggested by Derby and Hammer, the rapid production of ammonia is an interfering factor in the sugar fermentations and may explain the differences in reactions. It was noted that strains isolated from samples of butter from the same source generally produced similar reactions

in sugar media.

Some variation was also noted in growth temperatures with a number of cultures growing at 37°C. However, as the tests were made several months after isolation, the temperature relationships may have been influenced. The thermal resistence of the organism was low and freshly isolated cultures were destroyed by heating at 60°C. for 2 minutes.

One of the Ach. putrefaciens cultures obtained was slow in growing both on agar and in litmus milk. Two days at 21°C. were generally required for the development of colonies on agar and from 1 to 2 days were required for the complete reduction of litmus milk. The strain was probably similar to the slow culture obtained by Derby and Hammer (7) since acid was similarly produced from glucose. Subsequent study indicated that the strain required heavy inoculations for the initiation of growth on artificial media. The same characteristic, to a lesser degree, was found to be true of Ach. putrefaciens generally.

Variant forms

In addition to the cultures considered as typical Ach. putrefaciens, several others were obtained that, while differing in a number of respects, were sufficiently similar to Ach. putrefaciens to be regarded as a possible variant form. This type produced a putrid defect in butter, but to a milder degree and at a slower rate than Ach. putrefaciens.

It possessed the same morphology and produced the same sort of colony, although growth was less abundant.

The principal difference was in the action in litmus milk. Normal reduction did not occur, but after 6 to 8 days at room temperature the culture was partially proteolyzed and possessed the characteristic amber color of typical Ach. putrefaciens cultures of the same age. In general, the type was more fastidious in growth requirements than typical Ach. putrefaciens and, occasionally, transfers of the organism to litmus milk or bouillons failed to develop. Heavy inoculations were required for growth on agar or in liquid media. A significant point was that this variant form was usually isolated from butter showing the putrid defect to a comparatively mild degree and from which typical Ach. putrefaciens was seldom obtained.

In general, the observations made suggest the possibility of variant forms of Ach. putrefaciens being the cause of some outbreaks of putrid butter.

Growth on artificial media

The ineffectiveness of the plating method in demonstrating the presence of Ach. putrefaciens in putrid butter, from which it could be obtained by the smear technic, suggested some peculiarity in the growth of the organism. Since development was abundant after once established, it appeared that the peculiarity lay in the difficulty of

initiating growth on artificial media. When heavy inoculations were obtained, as was more likely with the smear method, the organisms seemed better able to develop colonies.

Plate counts on milk cultures of Ach. putrefaciens further emphasized the peculiarity by showing little correlation between the different dilutions. The plates from one or more dilutions of a series were sometimes crowded while those from the next higher dilution had very few or no colonies although on a comparative basis they should have had considerable numbers.

Observations on the growth of Ach. putrefaciens in litmus milk further indicated that the organism was slow in establishing itself. When very small colonies were picked into the medium, several days were often required for reduction. With one of the more fastidious strains, reduction would not occur at all unless large amounts of inoculum were used. However, once growth was well established regular loop transfers brought about rapid reduction in succeeding cultures.

Attempts were made to obtain more satisfactory growth of Ach. putrefaciens by using different media or modifications of media. Tomato juice, liver infusion, and tryptone-glucose agars were tried but were found to be generally inferior to beef infusion agar. Variation of the reaction of a medium was tried by adding different amounts of lactic acid or sodium hydroxide to cover a pH range of approximately 5.0

to 8.5. While the organisms were able to grow over a wide pH range, development was best when the reaction was approximately neutral. Sterile butter serum and sterile butter fat, alone or in combination, were added to beef infusion agar but did not improve the growth of Ach. putrefaciens.

Various substances were added to beef infusion agar in an attempt to alter the oxidation-reduction potential of the medium and thereby facilitate growth. The materials included reduced iron, sodium thiosulfate, cysteine, and potassium permanganate, each of which was sterilized and used separately in the agar. The reduced iron was placed in petri plates or added to the agar before pouring, using about 0.5 gram per plate. The sodium thiosulfate, cysteine, and potassium permanganate were added to the agar before pouring in amounts from 0.1 to 1.0 per cent, from 0.05 to 0.1 per cent and from 0.01 to 0.05 per cent, respectively; they were also used in agar blocks according to the method of Allyn and Baldwin (1).

In general, the results were disappointing. While some of the modifications, particularly the addition of sodium thiosulfate, resulted in more abundant development of Ach. putrefaciens they did not alter the irregular results obtained on plating cultures in various dilutions. Futhermore, materials that increased the growth of Ach. putrefaciens likewise stimulated other types and hence

were of no advantage in the isolation of the organism from defective butter.

Effects of Various Factors on the Production of the Putrid Defect in Experimental Butter by Achromobacter putrefaciens

Since Ach. putrefaciens appears to be a common cause of the putrid defect in commercial butter, further information regarding its action in butter was desired. Accordingly, studies were conducted on the effects of various factors on the production of the putrid defect by the organism.

Time required for production of the putrid defect in unsalted butter at 21° and 5° C.

Litmus milk cultures of various strains of Ach. putrefaciens were added to separate lots of cream used for churning. The unsalted butter was stored at 21° and 5°C.

Table 1 shows that the production of the defect was extremely rapid. While some variation occurred in the time required, in most cases the defect was evident in 1 day at 21°C. and in 7 days or less at 5°C. The putrid defect passed through different stages of development. At 21°C. the butter had a distinctly putrid odor in 1 or 2 days which greatly decreased during the next 5 or 6 days becoming somewhat suggestive of swiss cheese. At 5°C. the changes were of the same general type but were slower in development.

Production of the putrid defect with varying amounts of inoculum added to the cream

Since the usual plating method gave unsatisfactory

Table 1.

Time required for production of the putrid defect by Ach. putrefaciens in experimental unsalted butter at 21° and 5°C.

Culture no.	Days at 21°C.	Days at 5°C.
1	1	2
2	1	6
3	1	6
4	1	6
5	1	6
6	1	6
7	1	5
8	1	6
9	1	7
10	1	7
11	1	9*
12	1	6
13	1	7
14	1	4
15	1	7
16	1	7
17	1	7
18	1	7
19	2	7
20	2	9*

Table 1. (continued)

Culture no.	Days at 21°C.	Days at 5°C.
21	1	9*
22	2	7
23	1	9*
24	2	9
25	1	10*
26	1	11*
27	2	6
28	2	11*

* The samples were not examined earlier.

results in attempts to count the numbers of Ach. putrefaciens organisms, it was not possible to determine, by plating, the minimum number of organisms in the cream which would cause the putrid defect in the butter. Nevertheless, general observations concerning the numbers required were made by controlling the amount of inoculum used and determining approximate numbers by smearing loops of inoculated cream on agar plates.

In the first trial different amounts of a litmus milk culture of Ach. putrefaciens, varying from 0.01 ml. to 1.0 ml., were added to five lots of cream of about 400 ml. each. After inoculation, 1 loop* of each lot was smeared on agar. The numbers of colonies obtained per loop varied from none on the plate smeared with the cream having the smallest inoculation to 200 on the plates smeared with the cream having the heaviest inoculation. The inoculated cream was held 20 hours at 10°C., again smeared on agar, and churned. The numbers of organisms present in the cream at the time of churning had increased greatly and the smeared plates from each lot were crowded with colonies. The putrid odor developed in all five samples of the unsalted butter in less than 24 hours at 21°C. It was very intense with little or no variation between the different samples.

The results indicated that, with a 20 hour holding

* The loop used for smearing was not standardized since the work was on a comparative basis.

period at 10° C. before churning, a small initial contamination of the cream was apparently as effective as a larger one in producing the putrid defect in unsalted butter.

The experiment was repeated with the holding period after inoculation shortened to 2 hours and the temperature lowered to 5° C. to avoid the increase in the numbers of organisms before churning. The number of colonies obtained by smearing 1 loop of each lot of cream (after the holding) varied from 2 with the lot having 0.01 ml. of added culture to 60 with the lot having 1.0 ml. of added culture. The unsalted butter from the cream having the largest inoculation developed the putrid defect in 1 day at 21° C. while the samples from the lots of cream receiving the lower inoculations developed the defect in 2 days.

It is probable that the numbers of organisms in the different lots of cream were greater than the method of examination indicated. Nevertheless, the trials suggest that the putrid defect might develop in butter made from cream containing such low numbers of Ach. putrefaciens organisms that they would be very difficult to detect.

Production of the putrid defect by washing with contaminated water

Since wash water has been suggested as the source of organisms causing putrid butter outbreaks, trials were conducted to determine whether Ach. putrefaciens present

in the wash water would produce the defect in butter.

Samples of butter were churned from 400 ml. lots of cream and each washed with 400 ml. of water containing different amounts of a litmus milk culture of Ach. putrefaciens. A general indication of the numbers of organisms in the water was obtained by smearing 1 loop from each portion.

Trial 1 contained five lots of unsalted butter. The amount of inoculum added to the different lots of wash water varied from 0.01 ml. to 1.0 ml. The numbers of colonies obtained by smearing 1 loop of the inoculated water ranged from 5 with the lot receiving the smallest inoculation to 50 with the lot receiving the largest inoculation. The butter was stored at 21° C. The putrid defect developed in 1 day in the sample washed with water having 1.0 ml. of added culture and in 2 days in the remaining samples.

Since the defect developed so rapidly in the first trial, the experiment was repeated using smaller inoculations. Trial 2 included three samples of unsalted butter. The amounts of inoculum added to the water used to wash the different lots of butter were 0.001 ml., 0.002 ml., and 0.02 ml. respectively. On smearing, 1 colony was obtained from a loop of each lot of water. The defect developed in 2 days in all these samples of butter at 21° C.

The trials demonstrated that wash water contaminated

with Ach. putrefaciens was capable of producing the putrid defect in the unsalted butter. Evidently sufficient numbers of organisms were retained in the butter during washing to produce spoilage under favorable conditions. The numbers of organisms present in the different lots of water were probably larger than the method of examination indicated. However, the data suggest that butter might develop the putrid defect when washed with water in which the number of Ach. putrefaciens organisms was low enough to make detection difficult.

Effect of pH of the cream

The putrid defect in butter has often been attributed to the neutralization of the cream to a relatively high pH. Accordingly, pH values were determined on the sera of a number of samples of commercial putrid butter. Acid numbers were also obtained on most of the samples.

The results are presented in table 2. The pH values range from 5.8 to 6.8 and indicate that the defect is not necessarily confined to butter with a high pH value but develops over the same range as most bacteriological defects. In none of the instances do the pH values indicate over-neutralization. The acid numbers range from 0.85 to 6.25 and do not seem to indicate a relationship to the defect.

Since considerable variation was noted in the pH

Table 3.

pH values of serum and acid numbers of fat from samples of commercial putrid butter.

Sample no.	pH value	Acid number
1	6.8	1.35
2	6.2	1.18
3	6.4	1.60
4	6.1	3.45
5	6.1	6.25
6	6.4	4.45
7	6.6	1.40
8	6.6	1.70
9	5.8	0.85
10	6.3	1.00
11	6.5	
12	5.8	4.60
13	6.4	4.35
14	6.0	2.60
15	6.4	

values of commercial putrid butter, several experiments were carried out to determine the effect of pH of the cream on production of the defect by Ach. putrefaciens. In each trial, after removing about 400 ml. of cream to be churned as a control sample, a lot of cream was inoculated with a litmus milk culture of Ach. putrefaciens and divided into five portions of about 400 ml. each. Each of the five portions was then adjusted to a different pH value with sodium bicarbonate or lactic acid.

Table 3 shows the results obtained on the development of the defect in the unsalted butter at 21° and 5°C. The putrid defect developed over a wide pH range as determined on the cream from which the butter was churned. In trial 1, where the pH range was from 6.5 to 7.2, the defect appeared to a marked degree in all samples of butter after 1 day at 21°C. and after 7 days at 5°C. In trial 2 where the pH range was from 5.2 to 7.2, the three lots of cream having pH values of 6.7, 7.1, and 7.2 developed the putrid odor to a very marked extent while the samples from the two lots of cream having pH values of 5.2 and 6.0 were only slightly defective after 1 day. With trial 3 the pH range in the cream was from 4.5 to 7.8. The samples from the four lots of cream having pH values of 6.3, 7.1, 7.4, and 7.8 respectively became strongly putrid in 1 day at 21°C. and in 7 days at 5°C. However, the sample from cream having a pH value of 4.5 failed to show the defect after 7 days at 21°C.

Table 3.

Effect of pH of the cream into which Ach. putrefaciens was inoculated on development of the putrid defect in unsalted butter.

Trial no.	Sample no.	pH of cream	Degree of defect	
			1 day at 21°C.	7 days at 5°C.
1	*control	6.5	none	none
	1	6.5	marked	moderate
	2	6.7	extreme	moderate
	3	6.9	marked	marked
	4	7.0	extreme	marked
	5	7.2	extreme	marked
2	*control	6.0	none	none
	1	5.2	very slight	very slight
	2	6.0	slight	slight
	3	6.7	extreme	marked
	4	7.1	extreme	marked
	5	7.2	extreme	marked
3	*control		none	none
	**1	4.5	none	none
	2	6.3	marked	moderate
	3	7.1	extreme	marked
	4	7.4	extreme	marked
	5	7.8	extreme	marked

* Control = cream not inoculated.
** Sample failed to show a defect after 7 days at 21°C. or 20 days at 5°C.

or 20 days at 5°C.

It was noted on subsequent examination of the butter that the samples showing a slight defect at one day (made from cream with the lower pH values) were usually as strongly putrid after several days as those which had previously been more noticeable. Furthermore, as all samples became older they tended to assume a swiss cheese odor, the degree of which varied considerably between samples of different pH values but seemed to bear no definite correlation to these values.

The data show that Ach. putrefaciens is capable of producing the putrid defect in unsalted butter made from cream having pH values below that commonly used in commercial churning as well as in the higher range. Since the defect developed strongly at pH values such as were obtained from original defective samples, further evidence is obtained for considering Ach. putrefaciens as a probable cause of the original defect.

Effect of salt and of working

Salt is known to have an inhibitory effect on the development of bacterial defects in butter. Accordingly, it was of interest to determine the salt content of a number of the commercial samples of typically putrid butter.

The results are presented in table 4. Although the

Table 4.

Salt content of samples of commercial putrid butter

Sample no.	Per cent salt
F1	1.08
F2	1.20
F3	1.27
F4	1.18
F5	1.62
Sa	2.41
S 38	1.76
S 41	1.40
S1	2.21
S2	2.33
S3	2.33

salt content of some of the samples was low, several containing only slightly more than 1 per cent, four of the samples had a salt content of over 2 per cent which indicates that ordinary salting did not necessarily prevent the development of the defect.

To investigate the effect of salt, and also of working, on the development of the putrid defect by Ach. putrefaciens three experimental trials were made. In each trial cream was inoculated with a litmus milk culture of Ach. putrefaciens and then churned in a one gallon Dazey churn. The butter was divided into seven portions; 2 per cent salt was added to three portions, 1 per cent salt was added to three other portions, and one portion was unsalted. The three samples in each of the salted sets were worked to different degrees designated as slight, moderate, and thorough. The unsalted sample was worked moderately. The butter was held at 21°C.

The results obtained are presented in table 5. In each case the unsalted butter, which was moderately worked, became putrid in 1 day. The production of the defect in the salted samples was dependent on the amount of working as well as on the amount of salt. Of the samples having 1 per cent salt, those worked little became putrid in 1 or 2 days; those worked moderately were slightly or questionably defective at 2 days, while those worked thoroughly failed to develop the defect after 6 days. Of the samples having 2 per cent

Table 5.

Effect of salt and of working on development of the putrid defect in butter made from cream inoculated with *Ach. putrefaciens*.

Samples held at 21°C.

Trial no.	Amount of salt*	Degree of working	Days to show defect	Degree of defect
1	none	moderate	1	extreme
	1 per cent	little	2	extreme
		moderate	2	slight
		thorough		none at 6 days
	2 per cent	little	2	extreme
		moderate		none at 6 days
		thorough		none at 6 days
2	none	moderate	1	extreme
	1 per cent	little	1	definite
		moderate	2	questionable
		thorough		none at 6 days
	2 per cent	little	2	definite
		moderate	2	slight
		thorough		none at 6 days
3	none	moderate	1	slight
	1 per cent	little	1	slight
		moderate	2	slight
		thorough		none at 6 days
	2 per cent	little	2	definite
		moderate		none at 6 days
		thorough		none at 6 days

* The per cent of salt represents the amount added rather than the amount on analysis.

salt those worked little were putrid in 2 days; only one of the samples worked moderately became putrid; none of the thoroughly worked samples developed the defect in 6 days.

The results of the different trials were fairly consistent and demonstrated that the amount of working that the butter receives is an important factor in controlling the defect. Salt, while tending to inhibit the defect, was not completely effective unless combined with thorough working.

Effect of butter cultures

Since the use of butter cultures aids in controlling many bacterial defects of butter, two trials were conducted to determine its effect on the development of the putrid defect. The samples of butter in each trial were churned from approximately 400 ml. of cream. The butter culture was added to the cream the night before churning, and the cream was held at 10°C. Five hundred ml. of water were used for washing the butter. The samples were unsalted and held at 21°C.

In trial 1 Ach. putrefaciens (24 hr. culture in litmus milk) was added to the cream immediately prior to the addition of the butter culture. The amount of Ach. putrefaciens culture added to each lot of cream was 0.5 ml. while the amount of butter culture used varied from 5.0 per cent to 12.0 per cent. The butter was washed with sterile water.

In trial 2 Ach. putrefaciens was not added to the cream with the butter culture but was inoculated into the water used for washing the butter. The amount of butter culture used in each lot of cream was 10.0 per cent while the amounts of Ach. putrefaciens culture used in the water for the different samples of butter varied from 0.01 ml. to 1.0 ml.

The results are presented in table 6. In trial 1, where Ach. putrefaciens was added to the cream, none of the samples with butter culture developed the putrid defect in 6 days while the sample with no butter culture became markedly putrid. Five per cent of culture inhibited the defect as effectively as 12.0 per cent.

In trial 2, where Ach. putrefaciens was added to the wash water, the samples with butter culture showed no defect in 6 days while the sample having no butter culture developed a strong defect. The butter culture inhibited the defect as effectively when the wash water contained 1.0 ml. of Ach. putrefaciens culture as when it contained only 0.01 ml.

The results indicated that butter culture had an inhibiting effect on the development of the putrid defect in unsalted butter when Ach. putrefaciens gained entrance to the butter either from the cream or from the wash water. The use of butter culture, as suggested by Derby and Hammer (7), would appear to be an important measure in attempting

Table 6.

Effect of adding butter culture to the cream on the development of the putrid defect in the butter when Ach. putrefaciens was inoculated into the cream or into the wash water.

Butter unsalted and stored at 21°C.

Trial no.	Sample no.	Per cent butter culture added	Ml. of Ach. putrefaciens culture added	Degree of defect after 6 days
1	1	0.0	0.5	marked
Ach. putrefaciens added to 400 ml. portions of cream	2	5.0	0.5	none
	3	8.0	0.5	none
	4	10.0	0.5	none
	5	12.0	0.5	none
	6	0.0	0.0	none
2	1	0.0	0.5	marked
Ach. putrefaciens added to 500 ml. portions of wash water	2	10.0	0.01	none
	3	10.0	0.1	none
	4	10.0	0.5	none
	5	10.0	1.0	none
	6	10.0	0.0	none

to control the putrid defect under commercial conditions.

Effect of adding calcium propionate to the cream

Since calcium propionate has an inhibitory effect on the growth of certain microorganisms, several trials were conducted to determine its influence on the development, by Ach. putrefaciens, of the putrid defect in unsalted butter at 21° and 5°C.

In trials 1 and 2, after inoculating with litmus milk cultures of Ach. putrefaciens, the cream was held 16 hours at 10°C. before churning. In trials 3 and 4 no holding period was used and the Ach. putrefaciens and calcium propionate were added at the same time. The calcium propionate was prepared by making an 8 per cent solution in water; it was added to the cream in amounts varying from 0.02 per cent to 1.0 per cent.

The results are presented in table 7. Calcium propionate in amounts less than 0.5 per cent (calculated on the basis of the amount of cream) failed to completely inhibit the putrid odor in the butter, while amounts of 0.5 per cent or more effectively controlled the defect. In those cases where the defect was prevented a slight but distinct odor of propionic acid was detected. This odor was not unpleasant.

The results indicate that calcium propionate added to the cream inhibited the development of the putrid defect

Table 7.

Effect of adding calcium propionate to the cream into which Ach. butrefaciens was inoculated on development of the putrid defect in unsalted butter.

Trial no.	Sample no.	Per cent calcium propionate added	Days to show defect at 21°C.	Days to show defect at 5°C.	Degree of defect
1	1	0.0	2	7	extreme
	2	0.5			none *
	3	1.0			none
2	1	0.0	1	8	marked
	2	0.02	1	8	slight
	3	0.1	2	12	definite
	4	0.5			none
3	1	0.0	2	9	extreme
	2	0.02	2	9	extreme
	3	0.05	2	9	extreme
	4	0.1	2	9	slight
	5	0.5			none
4	1	0.0	2	9	extreme
	2	0.1	2	9	extreme
	3	0.5			none
	4	0.8			none

* None = no defect developed during the examination period.

in the butter. However, relatively large amounts were required to definitely prevent the condition.

Effect of age of the butter on the number of organisms present

The difficulty of isolating Ach. putrefaciens from most samples of putrid butter and the failure to obtain it from others suggest that the organisms die comparatively rapidly after causing the defect. Accordingly, several experiments were conducted to study the effect of age of the butter on the number of organisms present.

Portions of cream were inoculated with varying amounts of a litmus milk culture of Ach. putrefaciens, churned in the usual manner, and stored at 21°C. When the putrid defect developed in the butter, and at subsequent intervals, samples were examined for the approximate numbers of organisms present. The modified Burri method was used in preference to the plating procedure because of the extreme inconsistency of the latter.

Table 8 presents the data on the unsalted butter in which all samples were worked to a moderate degree. At 21°C. there appeared to be a definite tendency for the organisms to die as the storage period increased. While a few irregularities occurred, in all cases the counts at the last examination were much smaller than at the first. In trials 1 and 2 the numbers of colonies obtained on

Table 8.

Effect of age of the butter made from cream into which *Ach. putrefaciens* was inoculated on the number of organisms present.

Butter unsalted and stored at 21°C.

Sample no.	Degree of defect at 2 days	Approximate number of colonies per smeared plate after butter held: 1 day	3 days	8 days	15 days	25 days
		Trial 1				
1	extreme	450	275	275	175	
2	extreme	450	300	225	65	
3	extreme	1000	600	450	300	
4	definite	300	3	22	18	
5	definite	900	300	250	75	
		Trial 2				
1	definite	120	180	100		0
2	extreme	250	225	50		3
3	extreme	1500	400	400		4
4	extreme	1500	800	900		50
5	extreme	2000	1600			0
		Trial 3				
1	definite		75	1		
2	definite		11	0		

smeared plates at 1 day were often high, ranging from 120 to 2000 per plate, and the defect produced was very marked. In trial 3 much smaller amounts of culture were used and although the defect was not correspondingly lessened, the numbers of colonies obtained on plates were considerably lower; sample 1 showed 75 colonies and sample 2 showed 11 colonies at 3 days. It is evident that the numbers of colonies obtained at the end of the examination period bore a relationship to the number obtained on the first examination. The samples showing the lowest numbers of colonies on the first examination generally showed the lowest numbers at the end of the period. This is particularly evident in trial 3 where sample 1 gave 1 colony and sample 2 gave none after 8 days. In two other instances (samples 1 and 5, trial 2) no colonies could be obtained at 25 days, though the original count was high in the case of sample 5.

In trial 3 a portion of butter from the same churning as sample 1 was frozen immediately after churning and held in this condition for 3 days. At the end of this time it was placed at 21°C. and in 3 days developed a slight but definitely putrid odor. On smearing the sample no Ach. putrefaciens colonies could be obtained. A subsequent examination at 8 days likewise failed to reveal any colonies.

Table 9 presents the data on samples that were salted and worked to varying degrees. It again appeared that, at

Table 9.

Effect of age of the butter made from cream into which Ach. putrefaciens was inoculated on the number of organisms present.

Butter salted, worked to varying degrees and stored at 21°C.

Sample no.	Per cent salt	Extent of working	Degree of defect at 2 days*	Approximate number of colonies per smeared plate after butter held 1 day	4 days	10 days	20 days
			Trial 1				
1	0.0	moderate	extreme	90	275	200	20
2	1.0	little	definite	45	90	90	4
3	1.0	moderate	slight	10	25	0	0
4	1.0	thorough	none	0	14	0	0
5	2.0	little	definite	15	100	20	0
6	2.0	moderate	none	0	4	2	0
7	2.0	thorough	none	0	0	0	0
			Trial 2				
1	0.0	moderate	extreme	120	270	150	3
2	1.0	little	definite	120	180	170	80
3	1.0	moderate	question-able	25	180	150	30
4	1.0	thorough	none	12	3	50	0
5	2.0	little	definite	18	20	25	0
6	2.0	moderate	slight	18	18	0	0
7	2.0	thorough	none	0	0	0	0

*Samples showing no defect at 2 days kept well for the duration of the examination period.

21°C., the organisms died comparatively rapidly. Evidently fewer organisms were present originally than with the samples reported in table 8 since at 1 day the numbers of colonies obtained on smeared plate ranged from none to 120; in many instances no colonies could be obtained after 20 days. The numbers of colonies obtained on smearing generally increased during the first few days and then decreased. This situation was not observed in the unsalted set of samples.

It is of interest to note that in those cases where the defect was present to a slight degree colonies could be obtained at 1 and 4 days but not after 10 or 20 days. An exception was sample 3 in trial 2 where the defect was considered questionable. This sample showed 30 colonies per plate after 20 days.

The relationship between the amount of salt, the extent of working the butter, and the development of the defect to the numbers of colonies obtained on smearing is of interest. As pointed out in a previous experiment the degree of working affected the development of the defect. It may be seen from table 9 that the degree of working definitely affected the development of the organisms.

In general the samples containing 2 per cent salt showed fewer colonies than the samples containing 1 per cent salt. In both cases an increase in the amount of working decreased the number of colonies obtained. The samples

containing 2 per cent salt and receiving a thorough working did not develop the defect and at no time showed any colonies on smeared plates.

In samples 4 and 6 (trial 1, table 9 and in sample 4, trial 2, table 9) while no defect developed in the butter, Ach. putrefaciens organisms were obtained at certain examinations.

The experiment indicated that, with butter containing Ach. putrefaciens, colonies of the organism became more difficult to obtain as the butter aged. The amount of inoculum that had been added to the cream represented a contamination undoubtedly much higher than would be obtained under plant conditions. Even with a large initial inoculation, Ach. putrefaciens in some instances was not regained from the defective butter after 20 days, and in one instance (sample 2, trial 3, table 8) not after 3 days. Accordingly, it is not unusual that there should be much difficulty in isolating the organism from commercial defective butter which has been held for some time, particularly at 21°C. or above.

DISCUSSION

The failure to find organisms capable of reproducing the original defect when commercial putrid butter was plated on beef infusion agar is in accordance with the observations of Derby and Hammer (7) and emphasizes the difficulty of isolating the causative organisms by the plating procedure.

The effectiveness of the Burri smear technic in demonstrating organisms not obtained by plating butter suggests its use in studying the putrid defect. Probably it would be of value in studying other bacteriological defects of butter and various other products, when difficulty is encountered in isolating the causative organisms. Even with the Burri smear method the isolation is not simple since the number of organisms involved appears to be small and many may have died by the time the bacteriological examination is begun. Some enrichment procedure to increase the relative number of the causative organisms should be used in conjunction with the Burri method. The application of such methods to the study of commercial putrid butter resulted in various samples yielding Ach. putrefaciens.

The isolation of Ach. putrefaciens from 70.7 per cent of the typically putrid samples studied indicates that it is a common type in butter showing the defect. Its isolation from a number of defective samples from the same source, for example, from 12 of 15 typically putrid samples received at intervals from one plant, further suggests its relationship to the defect.

The fact that Ach. putrefaciens was the only organism isolated from most of the typically putrid butter that could reproduce the putrid odor in butter and bring about the changes characteristic of the condition commercially is additional evidence on the relationship of the organism to the defect.

The inability of Ach. putrefaciens to initiate growth readily on artificial media may partially account for the difficulty of isolating the organism from putrid butter and hence the difficulty of regularly associating it with the defect. Furthermore, the presence of an apparent variant form of Ach. putrefaciens which is even more fastidious in growth requirements complicates the situation since this type often fails to develop when picked into litmus milk.

One of the principal objections to accepting Ach. putrefaciens as a cause of the putrid defect has been the fact that it is often obtained in very small numbers. However, experimentally Ach. putrefaciens was capable of

producing a distinct putrid defect in the butter when added to cream in such small amounts that reisolation was difficult. Because of the difficulty of growing Ach. putrefaciens, this does not necessarily prove that the organism actually was present in very small numbers in the cream, but it at least suggests that a similar situation might exist when plating commercial butter and the organisms not be discovered. The fact that Ach. putrefaciens produced a definite putrid odor in butter when added to the wash water in such small amounts that reisolation was difficult is also significant. As well as showing that contaminated wash water may be responsible for defective butter, it indicates that the water may contain sufficient Ach. putrefaciens organisms to cause a putrid defect even though their presence may not be readily demonstrated.

While relatively low pH values in the cream did not prevent the development of the defect in the unsalted butter by Ach. putrefaciens, the use of butter culture in the cream prevented the development of the defect. Apparently the presence of the butter culture organisms, rather than a relatively low pH of the cream, was the controlling factor. However, the use of butter culture is not a completely effective control measure under all conditions, since some of the commercial putrid samples were made from cream containing butter culture.

The presence of the putrid defect in butter containing over 2 per cent salt and the ability of Ach. putrefaciens to produce the putrid defect in poorly worked butter when 2 per cent salt was added is in accordance with the report of Hood and White (14) who noted that the putrid condition may occur in butter having comparatively high salt content.

Since Ach. putrefaciens in experimental putrid butter became increasingly difficult to regain as the butter aged, the same situation would be expected with commercial butter containing the organism. The greater difficulty of isolating Ach. putrefaciens from old commercial samples than from those that were comparatively fresh may be partially explained on this basis.

The isolation of Ach. putrefaciens from the water supply of a plant having difficulty with the defect is an indication of one of the probable sources of the organism and is in agreement with the observations of other workers. The failure to obtain Ach. putrefaciens from a number of churns in plants not experiencing difficulty with the defect may indicate that the organism is not a common type under normal conditions. However, the difficulties associated with the isolation of the organisms must be considered in interpreting the results.

SUMMARY AND CONCLUSIONS

1. The predominating organisms that developed on beef infusion agar plates poured with commercial putrid butter did not reproduce the defect when they were inoculated into cream and the cream churned.

2. The use of the Burri smear technic in the examination of samples of butter having various defects demonstrated types of organisms not detected by the usual plating procedure.

3. By means of the Burri smear technic and certain enrichment procedures, Ach. putrefaciens was obtained from various samples of commercial putrid butter. With few exceptions it was the only organism obtained that was capable of reproducing the typical defect.

4. Ach. putrefaciens was most easily isolated from commercial putrid butter by inoculating the butter into thoroughly pasteurized cream, churning the cream, and smearing portions of the resulting butter on agar after the defect had developed. Inoculation of defective experimental butter into cream for the production of second or third generation samples occasionally resulted in isolation of the organism when it had not been obtained previously.

5. Incubation of the inoculated cream, the experimental butter, and the smeared plates at 5° to 10°C., rather than at 21°C., further facilitated the isolation; certain other modifications were also of assistance.

6. Ach. putrefaciens did not readily initiate growth in artificial media, which probably accounts, in part, for the difficulty of isolating it from putrid butter; for the same reason the numbers of the organisms in butter and other materials could not be satisfactorily counted by cultural methods.

7. Of 58 commercial samples having the typical putrid defect, 41 (70.7 per cent) yielded Ach. putrefaciens and 6 (10.3 per cent) yielded an apparent variant of the organism. Three (5.2 per cent) yielded other types of organisms capable of producing objectionable odors which were not typical of the putrid defect. From 8 (13.8 per cent) the causative organism could not be isolated.

8. The age of the butter seemed to bear a relationship to the ease with which Ach. putrefaciens was isolated since fresh samples yielded the organism more readily than older samples.

9. Ach. putrefaciens was isolated from the water supply of a plant having difficulty with the putrid defect.

10. When inoculated into the cream used for churning, Ach. putrefaciens produced a typical defect in unsalted butter in 1 day at 21°C. and usually in 7 days at 5°C. The defect passed through the same stages as those occurring in commercial butter showing the typical defect.

11. Ach. putrefaciens added to the cream in such small amounts that reisolation was difficult caused a putrid defect in the unsalted butter.

12. Ach. putrefaciens added to the wash water in such small amounts that reisolation was difficult caused a putrid defect in the unsalted butter.

13. The putrid defect was observed in samples of commercial butter with pH values varying from 5.8 to 6.8.

14. When added to lots of cream adjusted to pH values varying from 5.2 to 7.8, Ach. putrefaciens produced the putrid defect in the unsalted butter; when the pH of the cream was 4.5 no defect developed.

15. The salt content of a number of samples of commercial putrid butter ranged from 1.08 per cent to 2.41 per cent.

16. Although salt tended to prevent the development of the defect produced by Ach. putrefaciens, it was not entirely effective unless the butter was thoroughly worked.

17. Five per cent butter culture added to the cream had an inhibitory effect on the production of the putrid defect in unsalted butter by Ach. putrefaciens.

18. Calcium propionate added to cream at the rate of 0.5 per cent, or more, had an inhibitory effect on the production of the putrid defect in unsalted butter by Ach. putrefaciens. Smaller amounts of calcium propionate were ineffective.

19. In experimental butter Ach. putrefaciens decreased in numbers as the butter aged and became increasingly difficult to regain.

20. Because of its characteristics, action in experimental butter, and presence in much commercial putrid butter, Ach. putrefaciens was considered an important cause of the putrid type of cheesiness in commercial butter.

ACKNOWLEDGMENT

Acknowledgment is gratefully made to Dr. B. W. Hammer for his advice in planning and carrying out the work herein reported and in the preparation of the manuscript.

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