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KEEPING QUALITIES OF BUTTER

- VI. Experiments on the Production of Metallic Flavor in Butter and Milk.
- VII. The Microbic Flora of Off-Flavored Butter.

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FOREWORD

The Adams project, "Keeping Qualities of Butter," was first reported on by Savre, Rahn, and Farrand (1) of this section in Part I, General Studies; Technical Bulletin 1, 1908. Rahn, Brown, and Smith (1) presented Part II, The Influence of Salt, and Part III, The Decomposition of Proteins, in Technical Bulletin 2, in 1909. Brown and Peiser (1) presented Part IV, Cream Ripening and its Influence (bacteria in cream, their numbers, and types and their itinerary in the manufacture of butter) in Technical Bulletin 29, in 1916; and Part V, Pasteurization and its Influence (a study of the factors which influence the resistance of lactic acid bacteria to heat) in Technical Bulletin 30, in 1916. Brown, Smith, and Ruehle continued the work in "A Bacteriological and Biochemical Study of Experimental Butters," reported in the Journal of Dairy Science, Vol. III, pp. 375-405, 1920. Mr. Ruehle continued the work from that time until he left to head the department of bacteriology at Idaho University in September, 1926. The annual reports of the Bacteriologist of this Station have made reference to developments in the researches on this project from 1908 until 1927 when it was announced that Mr. Ruehle had undertaken to prepare for publication the final results of his studies on butter. This bulletin, therefore, is a continuation of the previous Parts I to V and is presented under two headings— Parts VI and VII. It has been recommended that the project "Keeping Qualities of Butter," be discontinued as a major project, because of lack of facilities for adequately pursuing the studies aggressively and under suitable conditions, particularly with respect to cold storage. Further work on butter problems will, of necessity, need to be incidental to the studies of market milk. The present contributions by Mr. Ruehle are significant.

-Ward Giltner.

KEEPING QUALITIES OF BUTTER

G. L. A. RUEHLE

VI. EXPERIMENTS ON THE PRODUCTION OF METALLIC FLAVOR IN BUTTER AND MILK

The development of off flavors in dairy products is very intricate and interesting. Moreover, it is very important to the dairy industry that a knowledge of their causes be gained since flavor is perhaps the most important single factor in determining the price and even the salability of dairy products. There are some flavors, disagreeable in themselves, which directly affect the value of the product since they are quite evident to the consumer, while there are other flavors not particularly disagreeable nor even noticeable to the average consumer which are believed by the butter buyer to indicate poor keeping quality. Metallic flavor belongs in this latter class and its presence may reduce the price of the butter several cents per pound.

"By metallic flavor is generally understood a semblance in flavor to the astringent, puckery, and metallic flavor which is characteristic of metallic salts such as are formed by iron, copper, or zinc in acid solutions.

"This flavor defect is not always sharply defined, often being accompanied by other more or less pronounced off-flavors.

"Frequently it borders on oiliness, then again it approaches fishiness and occasionally it appears to be a nuance of tallowy flavor."

The above quotation is from Hunziker (2). According to Guthrie (3) the flavor is that of rusty nails. Evidently there are a number of conditions in dairy products with which flavors are associated. These conditions although due to different causes are hard to distinguish from each other by flavor alone.

In this paper, any flavor to which the terms metallic, astringent, puckery, rusty, brassy, or coppery can be applied will be regarded as metallic. In many cases, the flavor is not so much a flavor as an after taste due to the astringent action of some chemical substance on the membranes of the tongue and mouth. It is perfectly obvious that, theoretically, many substances besides metallic salts possibly could produce this effect. The addition of a small amount of "Difco peptone" to milk produces a bitter, metallic flavor at once. This has been demonstrated repeatedly by the writer. The addition of peptone to butter itself produces the same bitter astringent flavor. Accompanying the bitterness and astringency there is also a distinct "nastiness" similar to the off-flavor in butter usually designated as "unclean."

From the above facts, it can be readily imagined that a number of the off flavors in butter are due to decompositions of protein contained in the butter.

Historical

Very little work has been done on metallic flavor. Most writers on dairying assume that it is due to metallic salts only. A few, however, mention the possibility of bacteria being concerned either directly or indirectly in the production of the flavor.

Boggild (4) of Denmark, writing to Guthrie (5), of Cornell University, made the following statement, "We here in Denmark have for a long time known that the same or a similar taste in butter can be due to rusty utensils, and in some cases to bacteria, and also that the so-called fishy flavor is due

to microorganisms."

Golding and Feilmann (6) in England made a study of some milk which had developed a metallic flavor. They found that contact with copper resulted in the solution of measurable quantities of the copper and they believed that copper salts were largely responsible for the trouble, especially since the replacement of an old, worn, tinned copper cooler resulted in relief from the trouble. They had isolated a large number of microorganisms from samples of the milk, and had been unable to obtain the flavor in inoculated sterile milk; when, however, the role of copper was discovered, it was found that a certain liquefying organism produced the flavor with marked regularity in the presence of copper.

They assumed that the copper salts checked the growth of the lactic acid flora, thus giving a clear field to the metallic flavor organisms, as well as themselves contributing directly to the flavor. The flavor developed about

18 hours after the addition of the copper salt and the organism.

In the discussion following the paper, H. Droop Richmond stated that he had observed similar results and that the flavor occurred most frequently

in pasteurized milk in winter.

Guthrie (7), working on various dairy products, came to the following conclusion, "Direct absorption of metals as well as the presence of members of the *Bact. lactis acidi* group of bacteria and the presence of enzymes may

cause metallic flavor in dairy products."

He states that high acidity is a necessary antecedent of metallic flavor and a high fat content is necessary except in the case of buttermilk. Rosengren (8) after extensive work also found that cream exposed to such metals as copper, lead, and iron acquires the characteristic tastes peculiar to the salts of these metals, especally if exposure is made during high temperature pasteurization, and that these flavors are present in butter made from the cream. Oftentimes the flavors commonly found in butter such as unclean, tallowy, and stale are due to metals in contact with cream or milk.

Experimental Work

The addition of peptone to milk and butter has already been mentioned as a means of producing metallic flavor in dairy products. This work sug-

gested some similar tests of decomposition products of proteins of definite chemical composition. Among the products of the decomposition of casein are the various amino acids which are the component "building stones" of the casein molecule. The following compounds were available in the laboratory: valine, alpha-alanine, glycocoll, leucin, aspartic acid, glutamic acid, histidin hydrochloride, phenyl-alanine, tyrosin, and tryptophane. Dilute solutions of these were made up in water and tasted with the following results:

Table I.—Tastes produced by dilute solutions of amino acids derivable from casein.

Compounds tasted	Taste	After taste
Valine Alpha-alanine Glycocoli Leucin (Sample No. 1) Leucin (Sample No. 2) Aspartic acid Glutamic acid Histidin HCl Phenyl-alanine Tyrosin Tryptophane	Slightly sweet Slightly sweet Unclean Musty (or mousy) Tasteless Sickish Almost tasteless Warm-sweetish Tasteless	Slightly metallic Unclean Musty (or mousy) None Sickish Slightly astringent (metallic Slightly astringent (metallic

Some of these compounds were added to soft, semi-fluid butter of good quality to the extent of 1 per cent. After these samples had been allowed to stand for one hour so that the chemical could go into solution in the moisture of the butter, they were tasted with the following results:

Table II.—Tastes produced by additions of certain amino acids and "peptone" to Butter.

Product added to butter	Taste	After taste
None (control) Valine Glucocoll Alpha-alanine Phenyl-alanine Histidine HCl 'Bacto-peptone''	Sweetish	Astringent (metallic) None None Metallic

A member of the Dairy Husbandry Section was asked to describe the flavors in the butter without being told what to expect. His reactions were as follows:

Valine—slightly bitter after taste

Glycocoll—bitter after taste

Alpha-alanine—sweet, some queer after taste

Phenyl-alanine—bitter after taste

Histidin HCl—curdy taste

"Bacto-peptone"—unclean, bitter after taste, will become fishy

This difference in the descriptions of flavors by different persons is one of the most discouraging features of work of this type. The fact, however, that both tasters recognized an off flavor has some significance from a purely scientific standpoint, even though the practical relationship of these substances to metallic flavor may be thrown in doubt. There is no doubt, however, in the mind of the writer that these after tastes were metallic.

Flavor in Milk by Aerobic Spore Formers

The foregoing results suggest the possibility that certain organisms capable of splitting proteins can cause flavors similar to or identical with the metallic.

Accordingly, aerobic spore bearing organisms were inoculated into three lots of test tubes full of fresh milk. One lot was kept at 6° C. in the ice box, one lot was held at room temperature, and one lot at 37° C. Sterile milk was not used due to the astringent after taste of highly heated milk. After 24 hours, the following tastes were recorded in the tubes held in the ice box:

Table III.—Tastes produced in milk by certain aerobic spore formers after 24 hours in ice box.

Inoculated with	Taste as judged by				
, included with	T	R			
No organism—(control) B. mycoides B. megatherium B. vulgatus B. ramesus B. stamesus B. subtilis (Lab. strain) B. subtilis (Amer. Mus. Nat. Hist. strain)	Like control Woody Bitter—puckery Like control	Sweet			

The cultures held at room temperature and at 37° C. coagulated and were discarded without being tasted.

At the end of 48 hours, the cultures held in the ice box were judged as follows:

Table IV.—Tastes produced in milk by certain aerobic spore formers after 48 hours in ice box.

Inoculated with	Taste as judged by				
Instituted with	T	R			
No organism—(control). B. mycoides. B. megatherium B. vulgatus B. ramosus B. ramosus B. subtilis (Lab.). B. subtilis (A. M. N. H.).	Metallic Metallic Slightly metallic Very metallic Weedy	Slightly astringent Slightly astringent Slightly astringent Slightly astringent			

After 4 days incubation in the ice box the milk was still fluid. The following tastes were recorded:

Table V.—Tastes produced in milk by certain aerobic spore formers after 4 days in ice box.

Inoculated with	Taste as judged by			
inoculated with	T	R		
No organism—control B. mycoides B. megatherium B. vulgatus B. ramosus B. ramosus B. subtilis (Lab.) B. subtilis (A. M. N. H.)	Old, oily, rot metallic Mustard like Slightly metallic Mustard like Old—better than control. Bitter	Old—not metallic Disagreeable—not metallic Metallic Metallic Slightly bitter, very metallic Metallic Metallic, slightly bitter		

The foregoing results indicate rather clearly that certain organisms which are known to split proteins can produce metallic or similar flavors when grown in milk. In section VII, there are recorded numerous other examples of the same kind.

Experiments with liquefying organisms. Previous to the work reported above, an attempt had been made to produce a butter (1) free from such metallic salts as are usually accused of being catalyzers of oxidation processes in butter and also (2) free or nearly free from microbic life. From several low count cows, milk was obtained in well tinned pails, whose interior surfaces had been coated with heated paraffin after they had been sterilized. The cream was separated by standing overnight in a gravity separator treated in the same way as the milk pails. The cream was then pasteurized in a glass bottle at 65-70° C. for 30 minutes. Sterile lactic acid and sterile butter color were added and the cream was then churned by agitation in a sterile glass specimen jar.

This butter when plated gave a count of 20 colonies per gram of butter when fresh. The butter was divided into two portions and packed into sterile glass culture dishes. One portion was sealed with paraffin and the other merely covered with a glass plate. They were stored for seven months in the ice box which maintained a rather constant temperature of 6° C. At the expiration of this period, they were studied again. The sealed samples tasted like old butter but were otherwise all right. The unsealed butter was metallic in taste.

The butters were then both left unsealed and replaced in the ice box for an additional three and one-half months' period. When examined at the close of this period, the formerly unsealed samples were tallowy but not metallic, while the formerly sealed samples were metallic. This suggests that the process is a progressively aerobic one from normal butter flavor through metallic to tallowy flavor. Possibly it is an oxidation hastened by an oxidase secreted by the organism.

The following table (Table VI) gives the colony counts obtained on the butters at various ages:

Table VI.—Colony	counts	on	experimental	butter	at	various	ages.

Container	20 per gram
Sealed	21,600 per gram
Sealed	101,700 per gram 20 per gram (600 molds
Unsealed	9 per gram (130 molds) 47,000 per gram
Unsealed	80,000 per gram
	Sealed Unsealed Sealed Unsealed Sealed

No satisfactory explanation can be offered for the erratic changes in the colony counts on these butters except the one that two distinct floras developed, one succeeding the other by several months as suggested by work reported in section VII. There seems to be little doubt, however, that some growth actually took place. The metallic flavor which developed could not have been due to contact with metals since no metal surfaces had come into contact with the milk or cream at any stage in the process of manufacture or holding.

At any rate, there was isolated from this butter an aerobic spore-forming rod which, when inoculated into either sterile or non-sterile milk, produced a very metallic flavor in two days at room temperature.

The following is a description of this organism: Morphology: Long rods, occurring singly and in pairs the size of the majority being 2.6 by 1.3 microns. The ends are rounded. Spores are central, oval. Rods are actively motile. Gram-positive. Agar stroke: Growth, gray translucent and abundant in 24 hours, filiform at first, later spreading. Elevation, flat. Lustre, dull; consistency, membranous. Gelatine stab: Stratiform lique-faction in 24 hours. Growth best at top. Potato slant: Growth abundant in 24 hours. Dull, yellow, verrucose. Nutrient broth: Membranous growth in 24 hours. Slight clouding; sediment, scant. Fermentation reactions: Acid but no gas from glucose, lactose, saccharose, and glycerol. No growth in closed arm. Litmus milk: Reduced litmus, coagulated and peptonized without a change in reaction. Diastatic action: Strong. Nitrate broth: Reduced to nitrite.

A milk culture of this organism was then used as a starter in making the next experimental butter. The butter was made in a similar manner to the butters made with metallic salts added, "a," "b," "c," and "d" portions corresponding respectively to sweet cream butter, butter made with a lactic starter, butter made with a lactic starter plus a starter of the metallic flavor producing organisms, and a butter made with the latter alone. When fresh, none of these butters possessed a metallic flavor but when 2 months old, "d" was pronounced astringent by all three judges while "c" was pronounced astringent by only one of them; "a," one of the controls, was pronounced astringent by two of the judges; "a," "b" and "c" all were moldy, the molds imparting a spicy flavor to the butter which made it difficult to recognize the metallic flavor if present. The same organism was reisolated from the butter.

An experiment similar to the above with more definite results was then performed with another culture from another source. This organism had been isolated from a case of bitter milk a year before and has since been identified as *Achromobacter liquefaciens* (Eisenberg) Bergey et al. This series of butters was made in exactly the same way as the previous one. The butters when fresh were good except that "d" had a slight metallic taste. After three days of storage and again after two months of storage, butters "c" and "d," the ones inoculated with metallic flavored starters, were pronounced metallic, astringent, or puckery by all three judges. The plate counts on these butters were:

	3 Mo. (harden- ing room)	3 Mo. (ice box)
(a) 22,000 per gram (b) 48,350 per gram (c) 1,359,000 per gram (d) 627,000 per gram	2,500 per gram	

Metallic flavor by B. subtilis group in milk. Since doing the above work on butter, the effect of inoculating other members of the *Bacillus subtilis* group into milk has been tried. The following organisms were available for this work:

1. Bacillus mycoides

2. Bacillus megatherium

3. Bacillus mesentericus vulgatus

4. Bacillus ramosus

- 5. Bacillus subtilis (lab. culture)
- 6. Bacillus subtilis (Am. Mus. Natl. Hist.)

All of these will liquefy casein, the first doing so very slowly. All of them produced metallic flavor when inoculated into milk and placed in the ice box, while control tubes did not. The rate of becoming metallic seemed to be correlated with the speed of liquefaction in litmus milk tubes held at 37° C.

Experiments with iron lactate in butter. The morning milking of certain cows of the college dairy herd was collected in 40 quart cans and separated in a power separator which had previously been washed and steamed. This separator had been used only a few times so that the tin plating was still intact. This cream was pasteurized in a 40-quart can by immersion in a hot water bath, at a temperature of 150°-160° F. for 15 minutes. The next morning the cream was divided into four portions and subjected to the following treatments:

- (x) Churned immediately without further additions. Sweet cream.
- (y) Churned after adding iron lactate at the rate of 200 parts per million of cream.
- (z) Churned after adding one pint of a good lactic starter.
- (w) Churned after adding one pint of good lactic starter, and iron lactate at the rate of 200 parts per million of cream.

The pasteurized cream used in these experiments had a clean, sweet, slightly cooked taste. The creams with iron lactate added had a very metallic, disagreeable taste and the same may be said of the buttermilks and butters from these creams ("y" and "w"). Each lot of butter was then packed into sterile glass topped mason jars and placed in the ice cream hardening room in the dairy building. At the end of the two days in cold storage, some of the butter was removed and scored by the writer and two other judges, Mr. Tweed and Mr. Wyant. The results are given in Table VII.

Table VII.—Iron lactate in butter.

Scores and criticisms of butter when freshly made.

Butter	Ruehle			Tweed		Wyant	
Butter	Score*	Criticisms	Score*	Criticisms	Sccre*	Criticisms	
K—iron free	36	Good clean flavor. Undissolved salt	36	Clean acid (acetic)	36	Good body. Lacking in	
—iron —iron free V—iron	33 38 33	Metallic. Fine, clean. Clean, mild aroma. Me-	32 38	Bitter, rustyClean, acid	$\frac{32}{38}$	Bitter, metallic Clean, mild acid	
		tallic flavor	33	Bitter, rusty flavor	31	Clean, mild aroma. Rusty, metallic flavor.	

^{*}Scores are given on the basis of 45 for perfect flavor.

It was noticeable that the metallic flavored butter was less metallic than when first made. Some of the same butter which had been left at room temperature for the same time was off-flavored but did not taste metallic.

Four months later the butter was again scored by the same judges with the results shown in Table VIII.

Table VIII.—Iron lactate in butter.

Score and criticisms of butter when four months old.

Destina	Ruehle			Tweed	Wyant	
Butter	Score	Criticisms	Score	Criticisms	Secre	Criticisms
X—iron free	35	Very salty, slightly for- eign and puckery	35	Slightly bitter	34	Slightly bitter like al-
Y—iron		Metallic—old . Slightly old . Slightly old .	32 36 33	Metallic salt	31 36 33	monds Rusty Mild, clean, slightly old Slightly rusty

It will be noted that the flavor of metals was so indistinct that it was difficult to recognize it, although the judges were looking for it. The butters with the iron lactate, however, scored distinctly lower than the ones without the iron salt. The bacteriological results showed that the butters contained very low numbers of bacteria. When fresh the counts on the butters were:

x = 800 per gram z = 1900 per gram z = 760 per gram z = 9000 per gram

When four months old the counts were:

x = 900 per gram z = 1700 per gram y = 400 per gram w = 9000 per gram

Experiments on Butter with Copper Lactate Added. This experiment was done in a similar manner to the iron lactate experiment except that 200 parts per million of copper lactate was used instead of iron lactate, the butters, when fresh having the following characteristics:

- (a) Control. Churned sweet. Butter, good flavor. Buttermilk and cream, good clean flavor.
- (b) Copper lactate added. Butter tasted good. Buttermilk and cream were slightly blue in color and had a slight metallic flavor.
- (c) Cream plus starter, plus copper lactate. Good tasting butter. buttermilk and cream slightly blue, with slight metallic flavor.
 - (d) Cream plus starter. Good tasting butter, cream and buttermilk. The results of the scoring at the age of one day appear in Table IX. It

Table IX.-Copper lactate in butter-Scores and criticisms when fresh.

Butter	Ruehle			Tweed	Wyaut		
Butter	Score	Criticisms	Score	Criticisms	Scare	Criticisms	
A (No Cu)	38 34 29 33	Clean, fresh, salty	38 33 30 36	Clean, fresh, salty	38 35 34 38	Good, salty Slightly astringent Slightly astringent.* slightly bitter Clean flavor	Very

^{*}More so than B.

will be noted that the metallic flavor appeared only faintly in the butters having been made with copper lactate. At the end of 110 days, the butter was scored again with the results as they appear in Table X.

Table X.-Copper lactate in butter-Scores and criticisms when 110 days old.

Butter	Ruehle			Tweed	Wyant		
Butter	Score	Criticisms	Score	Criticisms	Score	Criticisms	
A—(No Cu) B—(Cu)	33	Metallic, fruity salty Tallowy, fishy	33	Woody			
			26	Woody Tallowy, coppery, slight fishy after taste		Bleached	
C-(Cu)	24	Fishy, tallowy Old, milky	20	Very fishy, tallowy Old cream		Bleached	
D—(No Cu)	34	Old, milky	34	Old cream			

It will be noted that the presence of the copper salt induced the development of fishy flavor and tallowiness and that the metallic flavor had almost entirely disappeared. These two butters were both somewhat bleached. These experiments are in accord with the work of Hunziker and Hosman (9) and of Palmer and Combs (10) who found that the addition of salts of iron and copper to butter resulted in the development of tallowiness and in a bleaching effect. Hunziker also states that the presence of copper salts hastens the development of fishy flavor when the conditions for its development are right, namely a high acidity. Rogers (11) has shown by experiments that iron and copper salts hasten the development of fishiness and other off-flavors. Rosengren (12) has also shown that contact of the cream with iron, copper, or lead leads to the development of metallic and other off-flavors. Strangely enough, in the case of the copper, he obtained less metallic taste when the cream was sour than when sweet.

The bacteriological results on these butters, when fresh, were as follows:

(a) 350 per gram

(c) 5,575,000 per gram

(b) 2000 per gram

(d) 15,235,000 per gram

The results of the counts at the age of 117 days were as follows:

(a) 1700 per gram

(c) 400 per gram

(b) 1520 per gram

(d) 2,190,000 per gram

Discussion. The foregoing experiments while limited in extent indicate rather definitely that metallic flavor may be imparted to milk and butter by the presence of iron or copper lactate and by the presence of the products of decomposition of proteins whether these latter are added as such or produced by the presence of bacteria or their enzymes.

As far as our limited experiments go the indications are that the metallic flavor due to metallic salts is likely to be succeeded by tallowy flavor or other undesirable flavors, while the metallic flavor due to bacteria is likely to persist for an indefinite period, if not actually to grow worse. However, it is the opinion of the writer that the presence of iron and copper salts is the more serious cause of trouble, since the flavors succeeding the metallic flavor are much more disagreeable than the metallic flavor itself. It is realized that much more work needs to be done before we have enough data to settle this latter point definitely.

VII. THE MICROBIC FLORA OF OFF-FLAVORED BUTTER

Introduction

It is well known that microorganisms by their growth in milk or cream bring about various fermentations which result in off-flavored products. It is also quite generally accepted that it is very difficult or impossible for the buttermaker to make a first class butter out of raw material which has undergone an undesirable fermentation or other decomposition. However, in recent years, studies on the micro-flora of butter have been neglected. This is probably due to the fact that certain workers have shown that some of the off flavors in butter are due to purely chemical processes. The present writer admits the possibility of a purely chemical change capable of causing off-flavors in butter but he believes that the possible microbial factors should not be neglected. The present investigation was undertaken to discover whether microbial factors do play a part, and this paper is a record of some of the work on this project.

Previous Studies

As far as is known to the writer, no systematic study of the flora present in off-flavored butter has ever been attempted. As a basis for the study of the problem of the spoilage of butter, it was thought advisable to isolate the organisms present in samples of off-flavored butters and then study these organisms for their ability to produce flavors in butter or in milk, and to identify or make a descriptive study of the organisms.

Representative colonies from each of the most abundant types present were fished from the butter and placed on plates made for the purpose of obtaining a count of the living organisms. The plating for this work was usually done on lactose agar. Occasionally, other media were used. These included lactose gelatine, milk powder agar, and litmus milk agar. The last was ordinary agar to which one c. c. of sterile litmus milk was added at the time of plating.

Colonies of *Streptococcus lactis* were frequently not fished at all, even though they were the predominating flora. This was because *Str. lactis* may be considered to be the normal flora of either good or bad flavored butter. Even when fished, the colonies were practically all lost in a short time due to the difficulty of maintaining them on agar. Since these isolations were made, there have been developed media which are well adapted for carrying *Str. lactis* in stock for indefinite periods (13) but at that time no such medium was known to the writer.

A brief statement of the history of each sample is found in Table XI along with other data to be presented hereinafter.

Table XI—Summary of Data Presented in Part VII, showing the History of Samples, Being Produced in Milk

Butter sample No.	Score on flavor	Age when tested and plated	Bacteria colonies per gram	Remarks on colony count	Criticisms of flavor of butter	Organisms isolated
Bm 1	34	7 days	6,530,000	Lactose agar	Feed flavor, salty body, one sample oily	Oid. Bm 1 Yeast Bm 1 Bm 1 a Bm 1 b Bm 1 c Bm 1 d Bm 1 d Bm 1 h Bm 1 j
Bm 2	33	Fresh (4 days)	88,000 230,000 200,000 liquifiers 450, oidia	Lactose agar. Litmus milk agar plates.	Woody, sl. astring mt	Bm 2 b
Bm 3	35 T 32 H	4 days.	Oid, 329,000 5,000	Lactose agar	Stale, unclean, body leaky	Oid. Bm 3 Liquified milk Bm 3a yeast Bm 3b yeast (Liquifies milk). Bm 3d yeast Bm 3e Bm 3f
Bm 4	33 H	4 days	295,000,000 Oid. cols. 300.	Lactose agar	Unclean, oily, (Tweed), old, curdy	Bm 4a
						Bm 4j Bm 4k Bm 4l Bm 4m (Oidium)
Bm 5	33½ H 31 T 31 R	7 days	91,000		Pronounced storage (Hagar); weedy, oily (Tweed), rancid, old (Ruehle)	Bm 5a Bm 5b Bm 5c Bm 5d Bm 5d Bm 5e Bm 5f
Bm 6	33 T	8 days	3,870,000 (27,000 liquifiers)	Lactose agar Litmus milk agar	Old, stale, rancid	Bm 6a Bm 6 Oid Bm 6 Yeast
Bm 7	33 H 30 T 30 R	14 days	3,150,000		Gasoline (Hagar). Old, bitter, oily, like old butter color not true gasoline flavor (Ruehle and Tweed).	Bm 7a Bm 7b Bm 7c Bm 7c Bm 7d Bm 7e Oid, Bm 7

Colony Counts, Laboratory Numbers of Organisms Isolated, and Off-Flavors Capable of by these Microorganisms.

Proportion of organisms isolated to total flora	Flavors produced in milk when grown alone	Flavors produced in milk when grown in association with Str. lactis	Name of organism
100 per gram	Slightly stale.	Sour, oily, astringent	Oospora sp.
100 per gram	Sl. metallic, astringent not soured, bitter.	Sour, metallic or very astringent, bitter	Torulá sp.
10.000 per gram	Sweet, sl. astringent	More astringent, sour	M. cereus Migula
Predominant			
10,000 per gram 10,000 per gram 10,000 per gram 450 per gram	Sweet, like control	Sour, astringent	Oospora sp.
4,000 per gram	Slightly stale	Sour, very astringent	Oospora sp.
1,000 per gram	Sweet; (2nd) bitter, sour, astringent Bitter, putrid, curdled	Sour, astringent; (2nd) oily putrid, sour Bitter, sour, putrid, curdled	Torula sp. Torula sp.
10,000 per gram Predominant	Bitter, putrid, curdled, oily	Bitter, sour, putrid, oily and surdled	Torula sp.
	Ditt.	Bitter, astringent, sour	Torula sp.
*****************	Bitter, astringent	Sour, bitter, astringent	Kurthia zopfii (Kurth) Tre-
	Sweet, sl. astringent. Sl. putrid, astringent, (impure cult.)	Sour, curdled, old cream, oily, astringent	visan Kurthia zopfii (Kurth) Tre-
	Yeasty	Sour, yeasty, astringent	visan Achromobacter sp.
	Nasty, stale	Vinegary, astringent	Torula sp.
		N. I. de	
	No change. Sweet, stale. Sl. astringent.	No change, very sour, etc. (contam- inated)	Flavobacterium sp.
			*
Over 10,000 per gram Over 10,000 per gram Sav. 1,000 per gram 10,000 per gram 1,000 per gram 1,000 per gram	Sweet, sl. astringent. Sweet, astring ut, sl. bitter. Oily, yeasty, sl. astring ut.	Sour, astringent Intensely astringent. Astringent	Kurthia (Trevisan) sp. Torula sp. Microcoecus sp. Torula sp.
Over 10,000 per gram	Bitter, cheesy. Bitter, yeasty. Oily, astringent.	Sour	Oospora sp. Torula sp.
	Pecans Sweet—nutty Cooked taste Peculiar taste Sweet, sl. astringent Sweet, sl. stale	Sour, very astringent	Oospora sp.

Table XI

Butter sample No.	Score on flavor	Age when tested and plated	Bacteria colonies per gram	Remarks on colony count	Criticisms of flavor of butter	Organisms isolated
Bm 8	28 H 23 T 25 R	7 days	2,390,000	Lactose agar	Rank, metallic, short grain, salvy (Hagar); stale, weedy, astringent (Tweed): stale, greasy, sl. metallic, astring. (Fabian)	Bm 8a. Bm 8b. Bm 8c yeast. Bm 8d. Bm 8e.
Bm 9	31	8 days	370,000	Litmus milk agar plates	Alkaline, unclean, pronounced fishiness Tweed—old (not fishy)	Bm 9 Oid. Bm 9a yeast. Bm 9b. Bm 9c yeast. Bm 9d. Also many Str. lactis.
Bm 10		11 days	550,000		Decidedly metallic	Bm 10a Oid Bm 10b yeast Str. lactis not isolated.
Bm 11,.		6 days	87,000		Metallic on 6th day; fishy and tallowy on 7 th day.	Predominant flora Str. lactis not fished Bm 11a. Bm 11b Bm 11c
Bm 12		3 days	12,070,000		Sl. metallic	Str. lactis pred. flora not fished Bm 12a Oid Bm 12b Oid Bm 12c Bm 12d yeast
Bm 13		2 days	2,630,000		Metallie	Mostly St. lactis not isolated Bm 13a Oid Bm 13 b Coc Bm 13c Coc Bm 13d yeast
Bm 14		Several weeks.	54,000		Kerosene-like	Bm 14a
Bm 15		Several weeks.	Surface 390,000 Sub-surface 449,000		Metallic on surface	Bm 15a

(Continued)

Proportion of organisms isolated to total flora	Flavors produced in milk when grown alone	Flavors produced in milk when grown in association with Str. lactis	Name of organism
75,000 per gram	Bitter, peculiar, astringent	Sour, very astringent. Sour, very astringent.	Bacillus terminalis ¹ Torula species B. megaterium ² (DeBary)
Many thousand Many thousand Many thousand Many thousand	Yeasty, astringent Sl. sour, stale Yeasty, sweet, astringent, sl. bitter Astringent, oily, intensely bitter, smells and tastes yeasty	Sl. sour, very astringent. Sour, stringent, sl. bitter. Very astringent, sour. Same, only more intense	Torula sp. M. cereus Migula. Torula sp.
Few. Few.	Sweet, sl. astringent	Same	
10,000 per gramMany thousands	Sweet, sl. stale	Stale, sour	Bacillus sp.
Many thousand Several thousand 10,000 per gram 2,000 per gram	Sweet Sweet, sl. bitter Sweet. Vinegar, yeasty, sour dough	Astringent. Sl. bitter, very astringent. Sweet. Vinegar, yeasty, sour dough.	
	Clean, sweet	Astringent Stale, sl. sour, astringent Stale, astringent	Torula sp.
Predominant	Clean, sweet Clean, sweet Machine-oil like flavor Machine-oil like flavor Peculiar astringent flavor Clean, sweet	Sour, astringent	M. ochraceus (Rosenthal) Achromobacter sp. (Bergey et al.)
Predominant	No change. No change, sl. bitter No change No change	Sour Sour, very astringent. Sour, astringent. Sour	Micrococcus sp. B. terminalis Migula Bacillus sp. Micrococcus sp. (colon)

^{1.} Some variation in size from Bergey's classification. 2. Variation, nitrates reduced.

Table XI

Butter sample No.	Score on flavor	Age when tested and plated	Bacteria colonies per gram	Remarks on colony count	Criticisms of flavor of butter	Organisms isolated
Bm 16		Few weeks	2,400,000	Milk powder agar	Metallic (J. W.) odor of decaying animal matter, nasty, ammoniacal	Bm 16a
Bm 17		Few weeks	3,700,000	Milk powder agar	Akaline, metallic sl. ammoniacal odor similar to Bm 16.	Bm 17a Bm 17b Bm 17c Bm 17d Bm 17e Bm 17 yeast Bm 17 Oid
Bm 18	38	Few weeks	18,500,000 plate 58,600,000	Milk powder agar Microscopie	Sl. metallie	Bm 18-1 Bm 18-2 Bm 18-3 Bm 18-4 Bm 18-5
OB 1	20	2 months	3,080,000 3,450,000	Lactose agar Lactose gelatine.	T R Stale Rancid	OB 1aOB 1bOB 1cOB 1d
OB 2	20	2 months	655,000 545,000	Lactose agar Lactose gel	Moldy Moldy Stale Stale	OB 2a
OB 3	23 T 24 R	2 months	157,000 134,000	Lactose agar Lactose gel	Old Old Moldy Tallowy Tallowy	OB 3a OB 3b OB 3c OB 3d
OB 4	32 T 30 R	2½ months	920,000 790,000	Lactose agar Lactose gel	Sl. old	OB 4a OB 4b
OB 5	21 T 24 R	2½ months	1,030,000 960,000	Lactose agar Lactose gel	Old Old Moldy Raneid Alkaline	OB 5a. OB 5b. OB 5c. OB 5d.
OB 6		2 months	920,000 785,000	Lactose agar Lactose gel	Old Old Tallowy Tallowy Bitter Rancid	OB 6aOB 6bOB 6c
ЭВ 7	25	2 months	285,000 192,000	Lactose agar Lactose gel	Bitter. Very salty	OB 7a. OB 7b. OB 7c. OB 7d. OB 7e.

(Continued)

Proportion of organisms isolated to total flora	Flavors produced in milk when grown alone	Flavors produced in milk when grown in association with Str. lactis	Name of organism
200,000 per gram	Yeasty, astringent, metallic Yeasty, nasty, metallic Yeasty Yeasty, bitter, astr. metallic Sweet	Yeasty, sour, metallic. Yeasty, sharp acid, metal. Intensely yeasty, sour. Acid, yeasty, astringent. Sour, astringent	Microecceus sp. (colen) Microecceus sp. (colen) Torula sp. Microecceus sp. Microecceus sp.
300,000 Several hundred thousand. 100,000 100,000 Yeast 2,000 5,000	Yeasty, alcoholic. Yeasty, alcoholic. Yeasty, alcoholic. Acid, yeasty, alcoholic. Bitter, metallic, astringent. Oily, bitter, astringent. Peculiar acid (48 hrs.).	Yeasty, sour, alcoholic Nasty, yeasty, alcoholic, acid Nasty, yeasty, alcoholic, acid Nasty, yeasty, alcoholic, acid Bitter, metallic, oily, putrid Oily, bitter, astringent, sour	Torula sp. Microececus sp. Torula sp. Torula sp. Torula sp. Oospora sp.
Predominant	No change. No change, better than control.	Sour, astringent	Achromobacter sinosum ³ Flavobacterium diffusum (Frankland) Bergey, et al. Achromobacter butyri (Grimm) Bergey, et al.
Predominant	Yeasty. Sl. yeasty Sl. yeasty Sseet	Sour, yeasty Sl. sour, yeasty Sl. sour	Torula sp. Torula sp.
Evenly divided and abundant in all plates.	Slightly yeasty. Yeasty. Sl. yeasty Yeasty.	Sl. yeasty, sour Sour, yeasty Sour, yeasty Sour, yeasty	Torula sp. Torula sp. Torula sp. Torula sp.
Numerous	Yeasty	Sour, intensely yeasty	Torula sp. Torula sp.
Numerous (very) Numerous	Yeasty. Sweet.	Yeasty, oily, sour Peculiar sweetish taste	Torula sp.
Numerous. Numerous. Numerous. 10,000 per gram.	Yeasty. Yeasty. Yeasty Lost	Yeasty, bitter, astringentSour, yeasty.	Torula sp. Torula sp.
Numerous. Numerous. Numerous.	Yeasty	Yeasty, bitter, astringent. Sour, yeasty. Sour, yeasty	Torula sp. Torula sp.
Numerous. Numerous. Numerous. Numerous. 20,000 per gram.	Yeasty. Yeasty Sl. yeasty Sl. yeasty Yeasty, Sl. yeasty Yeasty, bitter.	Yeasty, sl. sour. Sour, yeasty Sour, yeasty. Sour, yeasty. Yeasty, astringent.	Torula sp. Torula sp. Torula sp.

^{3.} Variant of A. Sinosum (Wright) Bergey et al.

Table XI

Butter sample No.	Score on flavor	Age when tested and plated	Bacteria colonies per gram	Remarks on colony count	Criticisms of flavor of butter	Organisms isolated
OB 8	23 T 25 R		3,900,000	Lactose agar	T R Moldy Moldy Old Old	OB 8a
ОВ 9		$1\frac{1}{2}$ months	560,000 486,000	Lactose agar	Sl. moldy Sl. moldy Bitter Bitter Salt Salt	OB 9a OB 9b
OB 10		1½ months	1,080,000	Lactose agar	Old Old Bitter Bitter Salt Salt	OB 10a
ОВ 11	36	1½ months	2,560,000 2,390,000	Lactose agar	Old. Old. Bitter Bitter Salt Salt.	OB 11a OB 11b
5 X	36 34 41 33	4 months	800 900 1,800 150	Lactose agar	Good clean flavor (R); clean acid (acetic) (T); good body, lacking in flavor (W) Slightly bitter (T); slightly foreign (R); slightly bitter (W) Clean (T); clean (R) Tallowy, old	5 X1
5 Y	32 32 32	3 days 4 months 18 months 4 yr. 1 mo	760 400 1,900 Plates lost	Lactose agar	Bitter, met. rusty. Old, metallic, rusty, metallic salt. Weedy, tallowy, metallic. Tallowy, metallic.	B5 Y1
5 Z	38 36 39 35 38	3 days	1,900 1,700 4,100 150	Lactose agar	Fine, clean, mild acid, clean acid Sl. old, mild, clean sl. flat Tallowy, metallic Cooked, old Sl. tallowy	None
5 W	33 33 33 35	3 days 4 months 4 months 18 months 4 yr. 1 mo	9,000 aerobic 23,000 aerobic 840 850 1,900	Lactose agar	Metallic, bitter, rusty Rusty, metallic clean, mild aroma Sl. old, sl. rusty tang Sl. bitter, old Metallic, salty Tallowy, old	B5 W1
VIa	$\frac{40}{35}$	3 months	22,000 0 (0°C room) 3,000 (ice bəx.) 2,500 1,100		Clean, sweet. Trifte flat. Oily. Sl. old, woody. Sl. old, mild. Sl. moldy, old. Trifte tallowy, flat. Tallowy	VI aa

(Continued)

Proportion of organisms isolated to total flora	Flavors produced in milk when grown alone	Flavors produced in milk when grown in association with Str. lactis	Name of organism
Predominant (Surface cols.) Predominant (Deep cols.)	Yeasty, sl. bitter	Sour, bitter, very astringent	Torula sp.
Predominant(Surface) Predominant(Deep).	Yeasty	Yeasty, sl. sour	Torula sp. Torula sp.
Predominant(Surface) Predominant(Deep)	Yeasty, bitter, astringent	Yeasty, sl. sour	Torula sp. Torula sp.
Predominant (Surface) Predominant (Deep)	Yeasty, bitter, astringent	Yeasty	Torula sp.
70 per gram . Predominant . 20 per gram . Predominant . Predominant .	No change No change in milk No change	Sl. bitter, astringent, sharp, acid Sour	Achromobacter lipolyticum
20 per gram	Bitter and oily. No change. No change.	Sour, weedy, astringent. Sour. Sour, astringent.	See footnote 4.
Predominant	No change (no growth in milk)	Sour	
Predominant.	Astringent, bitter, burnt	Sour, bitter, astringent	
3,500 per gram	Bitter, metallic	Sour, sl. bitter, oily	
ManyFew	Sweet, sl. putrid, unclean	Sour, O. K	

^{4.} Near to A. lipolyticum (Huss) Bergey et al.

Table XI

Butter sample No.	Score on flavor	Age when tested and plated	Bacteria colonies per gram	Remarks on colony count	Criticisms of flavor of butter	Organisms isolated
VI b	40 37 36½	3 days	48,000		Clean, fresh Old, rusty Oily	VI b
	34	18 months	6,000 (0°C room) 1,350 (ice box) 5,900 100		Sl. old, rusty Sl. old, mild Sl. moldy, old Clean, sweet	VIba VI b8 VI b9 VI b10 None None
VI c	33 31	3 days	1,359,000		Bitter, astringent. Old, bitter, astringent.	VI ccVI c.
	32 39	18 months	2,500 (0° room) 91,500 (ice box)		Stale cream Sl. metallic. Sl. astringent, fruity. Sl. stringent struity. Sl. metallic. Tallowy, old, unclean	VI ca VI cb. VI c8. VI c9.
		4 yr. 2 mo		************	Old, sl. alkaline	VI c10VI c11. VI c13
VI d:	$\begin{array}{c} 32 \dots \\ 27 \dots \\ 34 \dots \end{array}$	3 months			Astringent Sl. bitter and astringent Metallic Sl. metallic, sl. old Sl. astringent	VI ddVI dVI dVI dsVI daVI daVI daVI dbVI db
	32	***********			Sl. metallie	VI de
	37 30	18 months 4 yr. 2 mo	1,900 400		Tallowy, stale	VI d8 VI d10 VI d11
VII a	37 28	2 days 3 months 1 yr. 4 mo	6,400 43,000 121,000 218,000	Lactose agar (0°C. room) (Ice box)	Good, clean, light flavor	VII aa
		4 years	,		Greasy, tallowy, curdy	VII a5 VII a10
VII b	35 36 38 34 29	3 months			High acid, sl. met Sl. old cream Clean Old cream, sl. bit. Sl. moldy, spicy	VII ba
	35 30				Clean, mild Old, moldy Old tallowy, metallic, bitter salt.	VII b5 VII b6 VIII b7
	39 40	4 years	33		Sl. old, tallowy	VII b10

(Continued)

Proportion of organisms isolated to total flora	Flavors produced in milk when grown alone	Flavors produced in milk when grown in association with Str. lactis	Name of organism
Many	Bitter, sw. curdling	Sour, unclean, oily, bitter, astringent.	
	No change	Sour, bitter, metallic	
	Sweet, astringent, weedy	Sour, astringent	
Many	No change	Sl. sour and astringent	
Predominant	Bitter, oily	Intensely bitter	
	No change	Sharply acid	
D. J			
Predominant	Sl. bitter	Bitter, sour, astringent	Pseudomnas fluorescens
			Migula ⁵
Many	Sweet, astringent	Sour, sl. bitter, astringent:	
	Sl. sour	V	
Predominant	Sweet.	Very sour	
	Sweet	Sour, astringent	
Sev. thousand	Oily, sl. metallic. Sweet.	Sour, weedy, astringent	
	No change	Sharply—acid	Microeoccus sp.
	No change	Sour	Micrococcus sp.
	No change	Sour	Micrococcus sp.
Many	Weedy, oily, astringent	Weedy, oily, sour	Microeoccus saccatus ⁶
many	Bitter, sw., curdling	Sour, bitter, unclean, astringent	Achromobacter sp.7
Many	Ditton mando silo		Bacillus sp.
30,000	Bitter, weedy, oily	Bitter, weedy, oily, sour	Pseudomonas fluorescens Migula
80,000	Bitter, astringent	Bitter, sour (not curdled)	Pseudomonas fluorescens
	Bitter (not curdled)	Sour, bitter, salty	Migula Pseudomonas fluorescens
			Migula
	No change in milk	Sour	
Predominant	Metallic	Sour, weedy, astringent	
	Slightly bitter No change	Sharply acid	Micrococcus sp.
	No change	Sharply acid	Micrococcus sp.
Predominant			Achromobacter sp.
redominant	Intensely bitter	Bittor oily gove estringent	Achromobacter sp.
0,000 per gram	Bitter, weedy	Bitter, oily, sour, astringent Sour, astringent	Achromobacter sp. Achromobacter sp.
	Very bitter	Greasy, sour, bitter, vomitus-like smell.	
Predominant	Bitter, putrid (putrid odor)	Sharply acid	Flavobacterium sp.
Predominant			
Predominant	No change—no change	Sour like control	
		Sour, like control.	
Thousands per gram Thousands per gram Hundred per gram	No change	Oily, very sour	
Predominant	No change	Sour	Flavobacterium sp.

Alkali producer.
 Near to Micrococcus saccatus migula.
 Near to Achromobacter nebulosum and Achromobacter geniculatum.

Table XI

Butter sample No.	Score on flavor	Age when tested and plated	Bacteria colonies per gram	Remarks on colony count	Criticisms of flavor of butter	Organisms isolated
VII c	37	2 days	169,000	Lactose agar	Clean	VII ca
	37				Clean	VII cb
	35		*5::**************		Old cream	
	$\begin{array}{c} 29 \dots \\ 32 \dots \end{array}$	3 months	500 (cold room) 500 (ice box)		Sl. moldy	VII c4
	29		31,500,000		Old, moldy. Sl. astringent.	************
	36 38	1 yr. 3 mo	31,500,000		Metallic. Tallowy, old, metallic	
	35	4 years	9,000		Metallic, tallowy, old	VII c10
		-				VII e11
						VII 011
VII d	36	2 days	11 000	Lactora agar	Old cream	VII da
	37	2 days	11,000	Dactose agai	Clean	
	35				Old cream	VII db
_	29		400 (cold room)		Astringent	
_	$\frac{29}{27}$				Rusty, sl. astringent	VII d4
	36	1 yr. 3 mos	74,310,000		Old, astringent. Tallowy, old, bitter.	
	37 30	A voore	74,310,000		Metallic. Strongly metallic, old	VII dio
	00	x yours	0,000		Swangij meterite, old.	VII d10 VII dII
VIII a	38	1 day	350		Clean, fresh, salty	
	33	45 days	1,700 4 mos.) 1,300 (4½ mos)		Metallic, salty fruity, woody	VIII a1
			1,300 (4½ mos)			VIII a 2 VIII a3
	34	1 year	740,000		High salt, old, fruity	
	33 40	9 vr 0 mog	740,000		High salt, old, musty. Too salty	VIII a5
4	10	2 yr. 5 mos	000		200 Series	VIII a5 VII a10
VIII b.	34		2,000 1,520 (4 mos.)		Woody, milky	
	33	1 day	2,000		Coppery	
	26	45 days			Sl. astringent	VIII bi
-			1,520 (4 mos.)		Tallowy, fishy	VIII b2
			240 (4½ mos.)		Tallowy, coppery	VIII b3
	30	1 year	03 000		Sl. fishy after taste. Old, unclean, fishy.	VIII b4
	39	1 year	33,000		Fishy, oily, old	VIII b5
		3 yr. 9 mo	190		Metallic, tallowy	VIII b10
		5 yr. 5 mo	150		incomine, only	VIII b11
VIII c	29	1 day	5,575,000	Only str. lactis		
				on plates	Sl. bitter	
	30				Metallic, coppery More astrin. than VIII b—sl. bitter	
	24	45 days		(1)		YUYY 4
	20		400	(4 mos.) (4½ mos.)	Fishy, tallowyVery tallowy.	VIII c1
	28	1 year	33,000		Fishy	VIII c4
-	25	3 yrs 9 mo	33,000 770		Very Fishy, oily	VIII c10
					Intensety tanowy, metanic, si. hshy	1111 010
/III d	33	1 day	15,235,000	Only str. lactis	Cooked flavor	
	36				Heavy salt. Clean	
	38 34	45 days			Clean Old cream	VIII d1
	J	22 400,011111	2,190,000	(4 mos.)	Old, milky	VIII d2
_	39	1 year	590,000 7,280,000	(4½ mos.)	Stale	VIII d3
	04,				Stale	VIII d4
	- 1	3 yr. 9 mo	650	Visible mold in butter and on		- X- X-
1	1					VIII d10

(Concluded)

Proportion of organisms isolated to total flora	Flavors produced in milk when grown alone	Flavors produced in milk when grown in association with Str. lactis	Name of organism
Predominant	No change	Sl. weedy, sour, very astringent	Bacillus sp.
Predominant	No change. No change. Bitter	Sour, astringent. Sharp acid. Sour, astringent.	
Only kinds present in equal numbers	No change Bitter (vomitus smell).	Sour	Achromobacter sp. Eberthella sp.
Predominant			
	No change in flavor No change	Sour, sl. astringent	Micrococcus sp.
Predominant	No change	Over astringent	
ManyPredominant	No change	Sour. Sour, astringent.	Achremobaeter sp. Achremobaeter sp.
Predominant	Like control No change No change	Sour, astringent Sour Sour	
Predominant Many (probably same as above)	Oily, bitter, astringent	Oily, bitter, astringentSI. sour, bitter, astringent.	Bacillus sp.
Predominant	No change Bitter, putrid Sw. curdled, very bitter, oily, astringent. No change. No change.	Sour Intensely bitter, yeasty Putrid, intensely bitter Sour. Sour	Bacillus megatherium ⁸
,			
Predominant			
Predominant	Like control	Curdled, sour, abnormal	Bacillus sp.
Predominant	No change	Acid, sl. bitter	
PredominantPredominantFew	, 1	-	
Only kind on plates			_
Predominant	No change		

^{8.} Near to Bacillus megatherium DeBary.

Table XII—Comparisons of flavors produced by associate organism when introduced alone and in association with Str. lactis in milk, cream and butter.

Organism	Alone or with Str. lactis	Taste of cream before churning	Taste of butter when fresh	Taste of butter when aged	Taste of milk
01/1	Mana	M. a. W.	Y	No. 11.	
BM 1a	Alone With	Metallie	Very metallic, greasy	Metallic, putrid, oily	Slightly metallic, astringent, bitter (not soured
3M 1a	1 7	Sharp, acid, metallic	Metallic, rancid	Metallic, putrid, oily	Sour, very metallic, bitter
Oidrum BM1		Metallie	Stale, metallic	Salvy, bitter, old, woody	Slightly stale
idium BM1	1.1	Sour, nasty	Stale, metallic	Salvy, metallic, old, woody	Sour, oily, astringent
idium BM2		Sweet	Good	Old, woody, salvy	Sweet, no change
idium BM2	With	Sour	Good	Old, greasy, salvy	Sour, astringent
idium BM3	Alone	Slightly metallic	Slightly metallic	Old, woody	Slightly stale
idium BM3	With	Sharp, acid, astringent	Metallic	Old, woody, greasy	Sour, very astringent
M 3a	Alone	Slightly astringent	Good	Rancid, oily	Bitter, sour, astringent
M 3a	With	Sour and astringent	Good	Rancid, oily	Oily, putrid, sour
M 3c	Alone	Oily, astringent	Vomitus	Salvy, greasy, rancid	Bitter, putrid, (curdled)
M 3e	With	Sour, putrid	Vomitus, putrid	Same, metallic (intensely) sour	Sour, bitter, putrid, (curdled)
M 4g	Alone			Good	Nasty, stale
M 4g	With			Old, stale	Vinegary, astringent
M 4g	Alone	Stale	Good	Old cream	Nasty, stale
M 4g	With	Sour, slightly stale	Good	Rancid	Vinegary, astringent
M 4d	Alone	Stale, putrid	Tallowy, stale	Old	Slightly putrid, astringent
M 4d	With	Sour, putrid, astringent	Putrid	Old, tallowy	Sour, (curdled) old cream, oily, astringent
M 5e	Alone	Oily, slightly greasy	Tallowy, metallic	Sour, oily, cheesy	Oily, yeasty, slightly astringent
M 5e	With	Oily, sour, yeasty, astringent	Tallowy, metallic	Good	Astringent
M 5e	Alone	Sweet	Astringent	Stale	Oily, greasy, slightly astringent
M 5e	With	Bitter	Oily	Stale	Astringent
M 8a	Alone			Woody, old	Bitter, astringent, peculiar
M 8a	With			Slightly woody	Sour, very astringent
M 8c	Alone			Woody	Nasty sweetish taste, astringent
M 8e	With			Good	Sour, very astringent
idium BM7	Alone	Slightly bitter	Greasy	Greasy, oily, stale	Sweet, slightly stale
idium BM7	With	Normal	Normal	Greasy, flat	Sour, very astringent
M 9d	Alone			Bitter, putrid, oily	Astringent, oily, very bitter, yeasty
M 9d	With			Bitter, putrid, oily	Astringent, oily, very bitter, yeasty and metall
M 9d	Alone	Oily and alcoholic	Good.	Bitter, oily, kerosene-like, salvy	Astringent, oily, very bitter, yeasty
M 9d	With	Sour (curdled) alcoholic	Slightly bitter	Same	Same, only more intensely.
M 12d	Alone	sour (our died) trionione,	onguery breed	Old. stale	Vinegary, yeasty, sour dough
M 12d	With			Good	Vinegary, yeasty, sour dough
M 12d	Alone	Stale	Good	Tallowy	Vinegary, yeasty, sour dough
M 12d	With	Stale, slightly sour	Yeasty	Tallowy metallic	Vinegary, yeasty, sour dough
M 13d	Alone		T Gasty	Good	Stale
M 13d	With			Old, stale	Stale, astringent
M 13d	Alone	Slightly bitter and flat	Slightly stale	Old cream	Stale, astringent
M 13d	With	Sl. bitter and flat, sl. sour	Slightly stale	Old cream	Stale Stale and astringent
M 15a	Alone	Good.	Good	Good	No change
M 15a	With	Sour, astringent	Good	Flat, tasteless	Sour

BM 15b	Alone	Stale, slightly astringent	Good	Tallowy	Slightly bitter
BM 15b	With	Sour, astringent	Metallic	Tallowy, metallic	Sour, very astringent
BM 15e	Alone	Good	Good	Old cream	No change
BM 15c	With	Sour, slightly astringent	Good	Old cream	Sour, astringent
				Tallowy	
				Sour, vomitus	
				Flat	
BM 16d	With			Sour dough	Acid, yeasty, astringent
BM 16d	Alone	Sweet	Salvy	Yeasty, bitter	Yeasty, bitter, metallic, astringent
BM 16d	With	Bitter	Tallowy	Slightly woody	Acid, yeasty, astringent
BM 17e	Alone			Oily	Bitter, metallic, astringent
BM 17e	With			Oily, old, stale	Bitter, metallic, oily, putrid
BM 17e	Alone	Putrid	Bitter	Bitter, oily, salvy (white)	Bitter, metallic, astringent
BM 17e	With	Putrid, sour (curdled)	Bitter	Same and sour burning (sensation)	Bitter, metallic, oily, putrid

It will be observed from examination of Table XI, that in every case a number of microorganisms were isolated and were assigned a laboratory number. Some of these were undoubtedly of little importance since they were present in small numbers. An attempt was made to determine which organism was the predominant type of those present and able to grow aerobically on laboratory media. Microscopic examinations of the butter were always made to determine the different morphological types present, and, in nearly every case, each of these types was found on the plates.

No molds were selected except colonies of Oospora, which was such a frequent and conspicuous contaminating genus that it was listed and studied whenever it was present. Though the media used were not those most suited for growing yeasts and torulae, it is believed that whenever they were present they were isolated and studied. This belief is based on the microscopic study. It will be observed that yeast-like organisms were among the most constant types present in these butters, although usually present in smaller numbers than the bacteria.

An attempt was made to identify our cultures by comparing our descriptions with those in Bergey's Manual (14) but only a few were capable of being identified. This was due in part to the fact that the study was made before Bergey's Manual was published and hence data are often lacking, and in part to the fact that Bergey's Manual contains too few descriptions. Some of the organisms mentioned in the table died before they could be studied for identification, so the organisms finally studied represent only the hardier species. Organisms of the *Str. lactis* group were not kept since these were regarded as normal butter flora common to both good flavored and off flavored butter.

The detailed descriptions of the organisms which were subjected to pure culture study will be found in the appendix to this bulletin.

Off-flavors Produced in Milk and Butter by the Flora of Off-flavored Butters

No study of the flora of off-flavored butters is complete since many of the organisms found may have little or no effect on the flavor of the butter. The organisms isolated from the off-flavored butters were inoculated into flasks of nearly sterile, in some cases sterile, whole milk and were incubated at room temperature for 48 to 72 hours. The flasks were inoculated with each organism. One of these flasks was also inoculated with a pure culture of Str. lactis 24 hours after the associate organism so the latter always had a chance to develop somewhat before it was subjected to competition with the acid forming Str. lactis. In every case, the milk was studied microscopically by the use of stained smears to see if growth of the desired organisms had taken place. The milk used for these tests was obtained in a fresh condition by milking cows into a sterile flask. Only the milk from cows which produced milk with a low bacterial content was used in these experiments. The reason for using raw milk was that during sterilization a certain astringency develops as well as a cooked taste. These properties make it difficult to detect certain off-flavors and to differentiate between the metallic flavor and the astringency due to heating.

In 23 instances, similar studies were made by inoculating the organisms into nearly sterile or pasteurized sweet cream and, after a short incubation, churning the cream into butter. It must be freely admitted that the results

were not always identical by the two methods (inoculation in milk and inoculation into cream made into butter) but it was observed that, if the organisms produced an off-flavor in one dairy product, it also did in the other. In many cases the off-flavors were identical or very similar indeed, tending to suggest the common opinion that some of these flavors are forerunners of others (see Hunziker's definition of metallic flavor on p. 3). The results on the cream and butter inoculated with the associate organisms and associate organisms plus *Str. lactis* are presented in Table XII, which gives not only these data but also a comparison of these results with those obtained by inoculating into nearly sterile raw milk.

The remainder of the results of inoculating in milk are presented in the 9th and 10th columns of Table XI, already shown on page 15, part VII.

DISCUSSIONS AND CONCLUSIONS

It seems to the writer that this work has proved that microorganisms can cause off-flavors in butter. It may seem strange to some that this should need proof but the opinion has been expressed freely that organisms do not cause off-flavors in butter except in instances where the cream is already off-flavored before the butter is made. A comparison of columns three and

four in Table XII should dispose of this idea.

Another current idea which is not supported by our experiments is that metallic, kerosene, woody, weedy, and other similar flavors are due only to the substances suggested by the name of the flavor. This is disproved not only by the present work but also by the work of European workers, notably Weigman (15) who showed that cowy, barny, turnipy, and soapy odors and flavors are sometimes due to bacteria. Besides these, certain other interesting flavors due to microorganisms, as shown by the present work, are pecan flavor, nutty flavor, fruity, and vomitus. The last is evidently closely related to kerosene and machine oil flavors, because these latter flavors are often followed by a strong vomitus flavor (or odor) when the organism has grown in the milk for a longer period of time. That is, young milk cultures have a flavor suggestive of kerosene or machine oil, while old cultures have a flavor suggestive of vomitus.

A striking fact brought out by this study is that there is a distinct associative action between organisms in the production of flavors and odors. The only organisms tested together in this study were the organisms isolated from off-flavored butters and a pure culture of *Str. lactis*. Some of the more striking cases of such action are grouped together in Table XIII.

It will be noted that frequently the presence of *Str. lactis* intensifies the flavor but occasionally the flavor is lessened by its presence or even changed entirely. The bitter flavor produced by certain torulae may be entirely changed to astringent (or metallic) flavor by the presence of *Str. lactis*, although the bitterness is sometimes intensified.

It would have been interesting to have tried various other combinations

of organisms together but time did not permit.

In conclusion, the writer believes he has demonstrated that the inoculation into fresh whole milk or butter of the organisms, isolated from off-flavored butters, is likely to produce off-flavors either identical with those originally present or closely similar to them.

Table XIII-Cases of Associative Action.

Organism	Type of organism	Flavor when grown alone	Flavor when grown with Str. lactis
VI a	Rods. Cocci in tetrads Mycelial torula. Mycelial torula. Oospora. Oospora. Oospora. Large and medium rods. Large and medium rods. Large torula. Torula. Short rods. Medium rods. Short rods. Rods.	Slightly stale Slightly stale Moldy, bitter Bitter Sweet Slightly bitter, astringent Metallie Bitter, metallie Slightly putrid, sweet	Vinegary, astringent Sour, yeasty, astringent Very astringent, not bitter Sour, oily, putrid Sour, intensely astringent, slightly bitter Sour, oily, astringent Sour, very astringent Sour, very astringent Sour, very astringent Sour, oily, astringent Sour, ownitus, astringent Sour, vomitus, astringent

^{*}Other butter organisms not included in this study.

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APPENDIX

DESCRIPTION OF MICROBES ISOLATED

Lab. Designation: Oidium B M 1. Morphology: Branched filaments, 2 to 3 microns wide, producing cylindrical oospores 1.5 to 2.5 microns in diameter, 6 to 8 microns in length. Grampositive. Agar colonies: Large, round with filamentous edge. Gelatine colonies; similar to agar colonies, slight saucer liquefaction. Agar slant: Moderate, filiform, opaque, white growth in 24 hours. Later growth became rhizoid. Gelatine stab; uniform, filiform growth; slight liquefaction in 15 days. Potato: Abundant, filiform to rhizoid, raised, dull white growth: No change in medium. Litmus milks; no change. Broth: White membranous, surface growth; no clouding; scant, flocculent sediment. Nitrates not reduced. Dunham's solution: Ammonia produced. Indol not produced. No diastatic action. Acid in glucose and glycerol. No action on lactose, mannite or sucrose. Aerobic. Oospora species.

Lab. Designation: B M 1a. Morphology: Yeast-like organism reproducing by budding; mature cells 3.5 to 5.0 by 1.5 to 3 microns. No ascospores observed either on ordinary culture media or on gypsum blocks. Gram-positive. Agar colonies: Large, white, rapidly growing, circular. Edge undulate, saucer liquefaction in 2 days, completely liquefied in 7 days. Agar slant: Abundant, filliorm, white, opaque, butyrous. Gelatine stab: Similar to agar slant. Crateriform to stratform liquefaction. Potato: White, filliorm growth, which later became red. Medium grayed. No diastatic action on medium. Litmus milk: Akaline in 4 days; partial peptonization without coagination. Broth: Membranous surface growth. Strong clouding. Granular heavy sediment. Nitrates not reduced. Dunham's solution: Indol not produced; ammonia produced; no nitrates produced. No diastatic action in starch agar plates. Slight acidity in glucose and glycerol. None in lactose, sucrose, or mannite. Aerobic. Torula species.

Lab. Designation: Oidium B M 2. Morphology: Branched filaments, producing cylindrical cospores 6 by 2.5 microns. Diameter of filaments 1.5 to 2 microns. Gram-positive. Agar colonies: Ci

Abundant, filiform, convex, dull, white. No change in medium, 2nd day growth is spreading and medium slightly grayed. Litmus milk: No change. Broth: Membranous surface growth; slight clouding, scanty flocculent sediment. Nitrates not reduced. Dunham's solution: Ammonia produced; indol not produced. No diastatic action. No action on glucose, lactose, sucrose, glycerol or mannite. Aerobic. Oospora species.

Lab. Designation: B M 3a. Morphology: Yeast-like organism reproducing by budding; mature cells 3 to 4.5 microns by 1.2 to 3 microns. Some mycelial cells produced on agar. No ascospores observed either on ordinary culture media or on gypsum blocks. Gram-positive when young and vigorous. Agar colonies: Rapidly growing, round, white, flat surface colony. Filamentous, subsurface colony. Saucer liquefaction complete in 7 days. Agar slant: Abundant, filiform, flat, dull, rugose, opaque, white, butyrous. Odor absent. Gelatine stab: Uniform, filiform, white growth, producing crateriform, liquefaction in 3 days, which was not complete in 17 days. Peptonization started in 4 days. Broth: Membranous surface growth. Scant clouding which cleared up on second day, leaving a scant sediment. Nitrates reduced in 7 days. Dunham's solution: Ammonia positive; indol negative. No diastatic action. Acid in glycerol; glucose, lactose, sucrose and mannite negative. Aerobic. Torula species.

Lab. Designation: B M 3b. Morphology: Yeast-like organisms reproducing by budding. Mature cells 4 to 6 microns by 1.5 to 2 microns, some cells being mycelial. No ascospores produced on ordinary laboratory media or on gypsum blocks. Gram-positive. Agar colonies: Rapid, round, rough, raised, undulate, finely granular to filamentous surface colonies. Subsurface colonies are filamentous. Gelatine colonies: Similar to agar colonies. Liquefaction in 7 days. Agar slant: Abundant, filiform, flat, dull, rugose, white, butyrous. Gelatine stab: Uniform, filiform, white. Produces crateriform liquefaction almost complete in 7 days the growth is convex, contoured

Torula species.

Lab. Designation: B M 3c. Morphology: Yeast-like organisms, reproducing by budding. Mature cells 3.5 to 4 by 1.5 to 2 microns. No ascospores observed on ordinary laboratory media or on gypsum blocks. Gram-positive. Agar colonies: Rapidly growing, circular, rough, convex, Mature cells 3.5 to 4 by 1.5 to 2 microns. No ascospores observed on ordinary laboratory media or on gypsum blocks. Gram-positive. Agar colonies: Rapidly growing, circular, rough, convex, undulate, white, coarsely granular surface colonies. Filamentous subsurface colonies. Gelatine colonies: Rapidly growing, circular, flat, undulate, white, coarsely granular surface colonies. Filamentous subsurface colonies. Liquefaction complete in 7 days. Agar slant: Abundant, filiform, flat, dull, contoured, opaque, white, butyrous. Later, spreading, raised, smooth, Gelatine stab: Uniform, filiform, white. Liquefaction, crateriform almost complete in 7 days. Potato: Abundant, spreading, flat, dull, smooth, white. Medium grayed. Later, raised, contoured, creamy. Slight starch decomposition. Litmus milk: Alkaline with peptonization of casein. Broth: Membranous surface growth, strong clouding, abundant viscid sediment. Later clouding disappears and sediment increases. Surface membrane persists. Nitrates not reduced. Dunham's solution: Ammonia produced; indol not produced. No diastatic action. No action on glycerol, mannite, sucrose, lactose and glucose. Aerobic. Torula species.

surface growth, strong clouding, abundant viscid sediment. Later clouding disappears and sediment increases. Surface membrane persists. Nitrates not reduced. Dunham's solution: Ammonia produced; indol not produced, No diastatic action. No action on glycerol, mannite, sucrose, lactose and glucose. Aerobic. Torula species.

Lab. Designation: B M 3 D. Morphology: Yeast-like cells, reproducing by budding. Mature cells 3 to 4 5 by 1.5 to 2 microns. No accospores observed on ordinary laboratory media or on gypsum blocks. Gram-positive. Agar colonies: Rapidly growing, large, round, radiate, raised, filamentous, white surface colonies. Filamentous subsurface colonies. Gelatine colonies: Rapidly growing, round, flat, undulate and filamentous edge; white surface colonies. Filamentous subsurface colonies. Liquefaction complete in 7 days. Agar slant: Abundant, filiform, flat, dull, ugose, opaque, white, butyrous. Gelatine stab: Uniform, filiform; heavy surface growth. Liquefaction crateriform. Potato: Abundant, filiform, flat, dull, smooth, white. Medium grayed. Later spreading, glistening, raised, contoured. No starch decomposed. Litmus milk: Alkaline: no peptonization or coagulation. Broth: Membranous surface growth. No clouding, Abundant, compact, flaky sediment. Nitrates reduced in 7 days. Dunham's solution: Ammonia produced and mannite. Aerobic. Torula species.

Lab. Designation: B M 4a. Morphology: Yeast-like cells, reproduced by budding. Mature cells 4 to 5 by 1 3 to 2.5 microns. Ascospores not observed on ordinary laboratory media or on gypsum blocks. Gram-positive. Agar colonies: Rapidly growing, round, rough, raised. undulate, filamentous, white surface colonies, filamentous subsurface colonies. Gelatine colonies: Rapidly growing, round, flat, undulate, white surface colonies, producing a spreading liquefaction. Potato: Abundant, filiform, flat, tudll, white; medium grayed. Litmus milk: Alkaline, no coagulation: complete peptonization. Broth: Membranous surface growth, slight clouding and scant sediment. Later, m Torula species.

Lab. Designation: B M 4d. Morphology: Short rods, occurring singly and in pairs and chains. Size, 1 to 1.8 by 0.5 to 0.8 microns; ends rounded. Motile by means of 1 polar flagellum.

No spores or capsules. Gram-positive. Agar colonies: Slowly growing, round, smooth, convex elevation, edge entire, amorphous internal structure. Gelatine colonies: Rapidly growing, round, and and the state of the

termined. Gram-positive. Agar colonies: Rapidly developing, circular, radiate, umbonate, lobate, coarsely granular. Deep colonies lens-shaped. Agar slant: Abundant filiform to echimulate, convex, glistening, smooth becoming rugose, opaque, gray, butyrous. Gelatine stab: Growth best at top, filiform; no liquefaction. Potato: Abundant, echimulate, convex, dull, opaque, contoured becoming rugose, brown. Litmus milk: Alkaline and reduced in 10 days. No coagulation or peptonization. Broth: Membranous surface growth, moderate clouding, moderate amount of viscid sediment. Nitrates reduced to nitrité. Dunham's solution: Ammonia strongly positive, indol negative. No action on glucose, lactose, sucrose, glycerol or mannite. Aerobic. Kurthia (Travisca) species (Trevisan) species.

Circuisan) species.

Lab. Designation: B M 5c. Morphology: Oval to elliptical, yeast-like organisms, multiplying by budding. Size of mature cells 3.4 to 4.5 microns wide by 5.2 to 9 microns long. No ascospores observed. Agar colonies: Rapidly developing, circular, smooth, raised, entire, coarsely granular. Agar slant: Abundant, filiform becoming echinulate, convex, dull, contoured, becoming rugose, opaque, gray, butyrous, with fecal odor. Gelatine stab: Uniform, filiform to beaded, with crateriform liquefaction. Potato: Abundant, filiform becoming spreading, raised, dull, rugose, opaque, prownish-gray. Litmus milk: Strongly alkaline and peptonized. No coagulation or reduction. Broth: Membranous surface growth, slight clouding with some flakes adhering to walls of tube. Very abundant granular sediment. Nitrates not reduced to nitrite. Dunham's solution: Ammonia strongly positive, indol negative. No action on glucose, lactose, sucrose, glycerol and mannite. Aerobic. Torula species.

Lab. Designation: B M 5d. Morphology: Spheres occurring singly and in pairs and irregular.

Lab. Designation: B M 5d. Morphology: Spheres, occurring singly and in pairs and irregular clusters 0.4 to 0.8 microns; size of majority, 0.5 micron. Non-motile, no flagella, no spores or capsules. Gram-positive. Agar colonies: Slowly developing, circular, smooth, convex, entire, amorphous. At first colorless, later yellow. Gelatine colonies: Slowly developing, circular, raised, entire, amorphous. Cup liquefaction in 7 days. Agar slant: Growth at first scanty, abundant in 7 days. At first filiform, later spreading. At first colorless, later yellow, smooth, glistening, butyrous. Gelatine stab: Uniform, filiform, white, stratiform liquefaction beginning on 2nd day. One-third complete in 15 days. Potato: In 7 days, heavy growth, raised, filiform, glistening,

bright orange color. Medium grayed. Litmus milk: Alkaline in 7 days. No coagulation. Peptonization in 7 days. Broth: Surface ring; strong, persistent clouding. No sediment in 7 days. Nitrite produced in 7 days. No gas. Dunham's solution: Ammonia produced; indol not produced. No diastatic action on potato or starch agar plate. No action on glucose, lactose, sucrose glycerol

Normaniation in 7 days. Broth: Surface ring; strong, persistent clouding. No scaling in 7 days. No gas. Dunham's solution: Ammonia produced; indol not produced. No diastatic action on potato or starch agar plate. No action on glucose, lactose, sucrose glycerol or mannite. Aerobic. Micrococcus species.

Lab. Designation: B M 5e. Morphology: Yeast-like cells, reproducing by budding; mature cells, 2 microns. Ascospores not produced on ordinary laboratory media or on gypsum blocks. Grampositive. Agar colonies: Slowly developing, circular, smooth, convex, entire, amorphous. Gelatine colonies: Circular, flat, entire, amorphous. No liquefaction. Agar slant; Moderate, filiform, falt, glistening, smooth, white. Gelatine stab: Uniform, filiform, glistening, white. Medium grayed in 7 days. Litmus milk: No change. Broth: Surface ring in 7 days, no clouding. Viscid sediment in 2 days. Nitrates not reduced. Dunham's solution: No ammonia; no indol. No diastatic action on starch agar plates. Slight action on potato. Acid in glucose and sucrose. No action on lactose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: Oidium B M 6. Morphology: Branched filaments, 1.5 to 2 microns wide, producing cylindrical oospores 1.5 to 2 by 6 to 8 microns in size. Gram-positive. Agar colonies: Rapidly developing, circular, mycelioid, surface rough, concentrically ringed, convex, entire, filamentous internal structure. Gelatine colonies: Same appearance as agar colonies. Agar slant: Abundant, filiform (later spreading with filamentous edge), opaque, dull, white. Consistency membranous. Gelatine stab: Best growth at top, filiform. In 15 days, slight (½) liquefaction, appearing as a saucer-like depression. Potato: Similar to agar stroke. Dirty white color. Medium grayed. Litmus milk: No change. Broth: Heavy membranous surface growth, no clouding. Heavy viscid sediment. Putrid odor. Nitrite positive in 7 days. Dunham's solution: Ammonia produced; indol negative. No diastatic action. Acid in glucose and glycerol. None in lactose, su

in 7 days. Dunham's solution: No ammonia produced; no indol produced. No diastatic action. Slight acid production in glucose and glycerol. No change in lactose, sucrose and mannite. Aerobic. Torula species.

Lab. Designation: Oidium B M 7. Morphology: Branched filaments 2 to 4 microns wide, producing cylindrical oospores 4.5 to 6, by 2 to 3.5 microns. Gram-positive. Agar colonies: Rapidly developing, circular, mycelioid, rough, radiate, convex, entire filamentous. Gelatine colonies: Rapidly developing, circular, mycelioid, flat, filamentous. No liquefaction in 2 days. Agar slant: Moderate, filiform to rhizoid, raised, dull, filamentous, opaque, white. No change in medium. Gelatine stab: Uniform, filiform, on second day growth distinctly best at top. Crateriform liquefaction in 15 days. Very slight. Potato: Moderate, filiform to rhizoid, convex, dull, filamentous, white. Medium grayed. Litmus milk: No change. Broth: Membranous surface growth, very slight clouding, scant, flocculent sediment. Nitrates not reduced. Dunham's solution: Ammonia present, indol absent. No diastatic action. No acid or gas from glucose, lactose, glycerol, mannite or sucrose. Aerobic. Oospora species.

Lab. Designation: B M 8c. Morphology: Yeast-like organisms reproducing by budding. Mature cells 1.5 by 3.5 microns. No ascospores observed either on ordinary culture media or on gypsum blocks. Gram-positive. Agar colonies: Slowly developing, circular, smooth, convex, entire, amorphous. Gelatine colonies: Slowly developing, circular, smooth, convex, entire, amorphous. Gelatine stab: Heavy uniform growth, filiform. No liquefaction in 15 days. Potato: Scant to moderate, filiform, convex, glistening, smooth, opaque, white, butyrous. Putrid odor. Gelatine stab: Heavy uniform growth, filiform. No liquefaction in 15 days. Potato: Scant to moderate, filiform, convex, glistening, white, smooth. Medium grayed. Litmus milk: Good growth but no change. Broth: Slight surface ring in 7 days, slight clouding with abundant viscid sediment. Nitrate reduced t

mannite. Aerobic. Torula species.

Lab. Designation: B M 9a. Morphology: Yeast-like organism, reproducing by budding. Mature cells 2.3 by 2.5 to 3.5 microns. No ascospores produced either on ordinary culture media or on gypsum blocks. Gram-positive. Agar colonies: Slowly developing, circular, smooth, convex, entire, amorphous. Gelatine colonies: Slowly developing, circular, raised, entire, amorphous. No liquefaction. Agar slant: Moderate, filiform, filiform, no liquefaction. Potato: Moderate, filiform, raised, white, dull, medium grayed. Litmus milk: No change. Broth: Surface ring, slight clouding, scant viscid sediment. Nitrates not reduced. Dunham's solution: No indol or ammonia produced. No diastatic action. Acid but no gas produced in glucose, lactose and glycerol. No action on mannite or sucrose. Aerobic. Torula species.

Acid but no gas produced in glucose, lactose and glycerol. No action on mannite or sucrose. Acrobic. Torula species.

Lab. Designation: B M 9c. Morphology: Yeast-like organisms, reproducing by budding. Mature cells 3.5 to 5 by 2 to 3 microns. No ascospores observed either on ordinary culture media or on gypsum blocks. Gram-positive. Agar colonies: Slowly developing, circular, convex, entire, amorphous. Gelatine colonies: Slowly developing, circular, flat, entire, amorphous. No lique-faction. Agar slant: Scanty, filiform, convex, glistening, white, smooth, butyrous. Decided putrid odor. Gelatine stab: Uniform, filiform; no liquefaction in 15 days. Potato: Abundant, filiform, convex, slightly contoured, dull, white. Medium grayed. Putrid odor. Litmus milk: Good growth but no change in medium. Broth: White surface ring, moderate granular clouding, heavy, compact sediment. Putrid odor. Nitrates slightly reduced to nitrite. Dunham's solution: Ammonia and indol absent. No diastatic action. Acid, but no gas, from glucose and sucrose. No action on lactose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: B M 11b. Morphology: Rods appearing singly and in pairs 1.5 to 2.5 by 0.4 to 0.7 microns, with rounded ends and central elliptical endospores with thin walls. Motile by means of one polar flagellum. Gram-negative. Agar colonies: Rapidly developing, circular, smooth, convex, entire, amorphous. Gelatine colonies: Liquefied in 2 days. Agar slant: Moderate, fili-

form, raised, glistening, smooth, opaque, white to cream-color. Gelatine stab: Uniform, filiform; liquefaction stratiform in 7 days, complete in 15 days. Potato: Moderate, filiform, yellow to brown. No change in medium. Litmus milk: Alkaline, completely peptonized in 7 days with no production. Rooth: No surface growth, strong clouding, viscid sediment. Nitrates

iquefaction stratiform in 7 days, complete in 15 days. Potato: Moderate, filiform, yellow to brown. No change in medium. Litmus milk: Alkaline, completely peptonized in 7 days with no coagulation or reduction. Broth: No surface growth, strong clouding, viscid sediment. Nitrates not reduced. Dunham's solution: Ammonia present, indol absent. No diastatic action. No acid or gas from glucose, lactose, sucrose, glycerol or mannite. Aerobic. Bacillus species.

Lab. Designation: B M 12d. Morphology: Rods occurring singly and in chains, 0.2 to 0.3 by 1 to 2 microns, with rounded ends and polar, spherical endospores 1/4 micron in diameter. Non-motile. Gram-positive. Agar colonies: Slowly developing, circular, convex, entire, amorphous. Gelatine colonies: Slowly developing, circular, convex, entire, amorphous. Cup liquefaction on 3rd day, changing to saucer liquefaction on 7rh day. Agar slant: Scanty, filiform, fliat, glistening, smooth, opaque, white. Gelatine stab: Uniform, filiform: liquefaction napiform. Potato: Abundant growth, flat, glistening, rugose, cream-colored. Medium grayed. Litmus milk: No change. Broth: Moderate clouding, later clearing with a slight flocculent sediment. Nitrates not reduced. Dunham's solution: Ammonia present, indol absent. No diastatic action. Acid, but no gas from glucose, no change in lactose, sucrose, glycerol or mannite. Aerobic.

Lab. Designation: B M 13b. Morphology: Yeast-like cells, multiplying by budding. Mature cells 1 to 3 by 2 to 5 microns. Ascospores not observed on ordinary culture media, or on gypsum blocks. Gram-negative. Agar colonies: Slowly developing, circular, smooth, flat, entire, amorphous. Gelatine colonies: Slowly developing, circular, flat. Cup liquefaction. Agar slant: Moderate, filiform, raised, glistening, white, opaque. Gelatine stab: Growth best at top, filiform, or liquefaction in 7 days. Potato: Moderate, filiform, raised, dull, smooth, white, later changing to medium brown. Litmus milk: No change. Broth: Surface repliced, heavy clouding, scanty, flaky se

absent. Diastatic action: Strong both on starch agar plates and potato media. Acid, but no gas in glucose, glycerol, sucrose and mannite. No action on lactose. Aerobic.

Lab. Designation: B M 13d. Morphology: Oval, yeast-like organisms, multiplying by budding, 4 to 6 microns wide by 7 to 8 microns long. No ascospores observed. Agar colonies: Rapidly developing, circular, smooth, convex, entire, coarsely granular. Deep colonies lens-shaped. Agar slant: Moderate, filiform, flat becoming convex, glistening, smooth, opaque, gray, slimy, with stale odor. Gelatine stab: Growth best at top, filiform to beaded. No liquefaction. Potato: Abundant, filiform, convex, glistening, becoming dull, contoured, opaque, gray, becoming brown. Litmus milk: Strongly alkaline and peptonized in 10 days. No coagulation or reduction. Broth: Surface ring, moderate clouding, granular sediment. Nitrates not reduced to nitrite. Dunham's solution: Ammonia strongly positive, indol negative. No action on glucose, lactose, sucrose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: B M 15a. Morphology: Spheres occurring in irregular clusters .7 micron in diameter. No spores, no motility. Gram-positive. Agar colonies: Slowly developing, circular, smooth, flat, entire, amorphous. Gelatine colonies: Slowly developing, circular, flat, entire, amorphous; liquefaction spreading. Agar slant: Abundant, filiform, raised, glistening, yellow. No odor. Gelatine stab: Uniform, filiform; liquefaction complete in 7 days. Potato: Scant, filiform, yellow, medium grayed in 7 days. Litmus milk: No change. Broth: Surface ring, moderate clouding, viscid sediment, odor absent. Nitrates not reduced. Dunham's solution: Ammonia present, indol absent. No diastatic action. No acid or gas from glucose, lactose, glycerol, mannite and sucrose. Aerobic. Micrococcus species.

Lab. Designation: B M 15c. Morphology: Rods, occurring in pairs and-chains, 1.5 by 0.3 microns. Ends rounded, polar elongated endospores 0.3 by 0.5 micron, and thin walls. Non-motile for a p

glycerol. Aerobic. Bacillus species.

Lab, Designation: B M 15d. Morphology: Spheres in pairs and irregular clusters 0.3 micron in diameter. No endospores, no motility. Gram-negative. Agar colonies: Slowly developing, circular, smooth, convex, entire, amorphous. Gelatine colonies: Slowly developing, circular, convex, entire, amorphous; liquefaction spreading. Agar slant: Abundant, filiform, raised, glistening, smooth, opaque, yellow. Gelatine stab: Uniform, filiform. Liquefaction complete in 7 days. Potato: Scanty, filiform, yellow, medium grayed. Litmus milk: No change. Broth: Moderate clouding, abundant flaky, viscid sediment. Nitrates not reduced. Dunham's solution: Ammonia present, indol absent. No diastatic action. No action on glucose, lactose, sucrose, glycerol or mannite. Aerobic. Micrococcus species. Aerobic. Micrococcus species.

mannite. Aerobic. Micrococcus species.

Lab. Designation: B M 15d. 2. Morphology: Spheres occurring singly and in pairs. Size of the majority 0.8 micron. Gram-negative. Agar colonies: Rapidly developing, circular, smooth, convex, entire, coarsely granular. Deep colonies lens-shaped. Agar slant: Abundant, filiform, convex, glistening, smooth, opaque, slimy, gray, becoming yellow. Gelatine stab: Growth best at top, filiform, slight crateriform liquefaction. Potato: Scanty, filiform, convex, glistening, smooth, translucent, pale yellow. Litmus milk: Alkaline, strongly peptonized and reduced with no coagulation. Broth: Surface ring, moderate clouding, abundant viscid sediment. Nitrates not reduced to nitrite. Dunham's solution: Ammonia positive, indol negative. Acid, but no gas from glucose. No action on lactose, sucrose, glycerol or mannite. Aerobic. Micrococcus (Cohn) species. species.

Lab. Designation: B M 16a. Morphology: Spheres occurring in irregular clusters .3 micron in diameter. Gram-negative. Agar colonies: Circular, smooth, flat, entire, amorphous. Gelatine colonies: Circular, flat, entire, no liquefaction. Agar slant: Moderate, filiform, raised, glistening, contoured, opaque, white. Gelatine stab: Slight crateriform liquefaction in 7 days. Potato: Scant, white, flat, medium unchanged. Litmus milk: No change. Broth: No surface growth. Strong clouding and heavy sediment. Nitrates not reduced. Dunham's solution: Ammonia present, indol absent. No diastatic action. Acid from glucose, lactose, sucrose, glycerol and mannite.

Micrococcus species.

Strong clouding and heavy sediment. Nitrates not reduced. Dunham's solution: Ammonia present, indol absent. No diastatic action. Acid from glucose, lactose, sucrose, glycerol and mannite. Acrobic. Micrococcus species.

Lab. Designation: B M 16b. Morphology: Spheres occurring singly and in pairs .25 micron in diameter. Gram-negative. Agar colonies: Slowly developing, circular, smooth, flat, entire, amorphous. Gelatine colonies: Slowly developing, irregular, flat, with spreading liquefaction. Agar slant: Moderate, filiform, stratiform liquefaction observed in 7 days. Plotato: Moderate, raised, white. No change in medium. Litmus milk: Coagulated in 7 days. Slight reduction but no peptonization or change in reaction. Broth: No surface growth, heavy clouding, viscid sediment. Nitrates not reduced. Dunham's solution: Ammonia positive, indol negative. No diastatic action. Acid in glucose, lactose, sucrose and glycerol. No action on mannite. Aerobic. Micrococcus species.

Lab. Designation: B M 16c. Morphology: Yeast-like cells multiplying by budding. Mature cells 1.25 by 1.75 microns. Ascospores not observed in ordinary culture media nor on gypsum blocks. Gram-negative. Agar colonies: Rapidly developing, punctiform to circular, smooth, raised, entire, amorphous. Gelatine colonies: Slowly developing, punctiform to circular, smooth, raised, entire, amorphous. Ro liquefaction. Agar slant: Scanty, filiform, raised, glistening, smooth, opaque, white. Gelatine stab: Growth best at top, filiform, no liquefaction. Potato: Growth raised, glistening, filiform, white. Medium unchanged. Litmus milk: No change. Broth: No surface growth, no clouding, abundant viscid sediment. Nitrates reduced in 7 days. No gas. Dunham's solution: Ammonia positive, indol negative. No diastatic action. Acid on glucose, lactose and sucrose. No action on glycerol or mannite. Aerobic. Torula species.

Lab. Designation: B M 16d. Morphology: Spheres occurring in irregular clusters 0.3 micron in diameter. Gram-negative. Agar colonies: Rapidly developing, ci

positive, indoi negative. No diastate action. Actio in glacose, factose, sucrose, su colonies: Slowly developing, punctiform to circular to mycelloid in form. Surface rough, elevation flat, edge filamentous. Internal structure amorphous in central portions, filamentous on edge. Gelatine colonies: Slowly developing, punctifirm to mycelloid, flat, entire; no liquefaction. Agar slant: Moderate, filiform, raised, dull, contoured, opaque, white. Gelatine stab: Best at top, filiform, no liquefaction. Potato: Abundant, raised, filiform, white. Medium unchanged. Litmus milk: Slight reduction of litmus in 2 days. Strongly coagulated in 7 days. No peptonization or rection in 7 days. Broth: Membranous surface growth. Slight clouding. No sediment. Nitrates not reduced. Dunham's solution: Ammonia strongly positive, indol negative. No diastatic action in 7 days. Acid in glucose, lactose, sucrose, and glycerol. No action on mannite. Aerobic. Opspora species.

in 7 days. Acid in glucose, lactose, sucrose, and glycerol. No action on mannite. Aerodic Oospora species.

Lab. Designation: Yeast B M 17. Morphology: Yeast-like cells reproducing by budding. Mature cells from 3 by 2 microns. Ascospores not observed on ordinary media or on gypsum blocks. Gram-positive. Agar colonies: Rapidly developing, punctiform to filamentous, rough, raised, filamentous, coarsely granular. Gelatine colonies: Rapidly developing, circular, raised, undulate to filamentous. Internal structure filamentous liquefaction at first cup, later saucer. Agar slant: Abundant, filiform, raised, dull, contoured, opaque, white. Gelatine stab: Best at top, filiform, crateriform liquefaction. Potato: Heavy, raised, glistening to dull, contoured, grayish. Medium grayed. Litmus milk: Litmus reduced in 2 days. Strong peptonization in 7 days. No coagulation or change in reaction. Broth: Membranous surface growth, strong clouding, heavy sediment. Nitrates not reduced. Dunham's solution: Ammonia strongly positive, indo negative. No diastatic action in 7 days. No change in glucose, lactose, sucrose, glycerol or mannite. Aerobic. Torula species. Torula species.

Torula species.

Lab. Designation: B M 17a. Morphology: Yeast-like cells reproducing by budding. Mature cells 2 by 1.5 microns. Ascospores not observed on ordinary media or gypsum blocks. Gramnegative. Agar colonies: Slowly developing, circular, smooth, flat, entire, amorphous. Gelatine colonies: Slowly developing, punctiform to circular, raised, entire, amorphous; no liquefaction. Agar slant: Scanty, filiform, raised, glistening, contoured, opaque, white. Gelatine stab: Uniform, filiform. No liquefaction. Potato: White, filiform growth. No change in medium. Litmus milk: No change. Broth: No surface growth, no clouding. Abundant viscid sediment. Nitrates not reduced. Dunham's solution: Animonia present, indol absent. No diastatic action. Acid in glucose, lactose, sucrose, glycerol and mannite. Aerobic. Torula species.

Lab. Designation: B. M 17c. Morphology: Spheres occurring in pairs and irregular groups 0.3 micron in diameter. Gram-negative. Agar colonies: Rapidly developing, circular, smooth, raised, entire, amorphous. Gelatine colonies: Slowly developing, punctiform, raised, entire, amorphous best at top, filiform; no liquefaction in 7 days. Potato: Growth scanty, filiform, white; no change in medium. Litmus milk: No change. Broth: No surface growth no clouding, abundant viscid sediment. Nitrate reduced to nitrite in 7 days. No gas. Dunham's

solution: Ammonia positive, nitrate positive, indol negative. No diastatic action. Acid in glucose, lactose and sucrose. No action on glycerol and mannite. Aerobic. Micrococcus species.

Lab. Designation: B M 17d. Morphology: Yeast-like cells multiplying by budding. Mature cells 1.25 by 2.5 microns. No ascopores observed on gypsum blocks or on ordinary laboratory media. Gram-negative. Agar colonies: Slowly developing, round, smooth, flat, entire, amorphous. Gelatine colonies: Slowly developing, circular, raised, entire, amorphous; no liquefaction. Agar slant: Scanty, filiform, raised, glistening, contoured, opaque, white. Gelatine stab: Growth best at top, filiform; no liquefaction. Potato: Growth filiform and white; no change in medium. Litmus milk: Coagulated in 7 days; no peptonization, reduction or change in reaction. Broth: No surface growth, no clouding, abundant sediment. Nitrates not reduced. Dunham's solution: Ammonia present, indol absent. No diastatic action. Acid in glucose, lactose, sucrose, glycerol and mannite. Aerobic. Torula species. Aerobic. Torula species.

mannite. Aerobic. Torula species.

Lab. Designation: B M 17e. Morphology: Yeast-like cells, multiplying by budding, mature cells 2 by 2.5 microns. Ascospores not observed on ordinary culture media or on gypsum blocks. Gram-positive. Agar colonies: Rapidly developing, punctiform to circular, to irregular, smooth, flat, undulate, amorphous. Gelatine colonies: Slowly developing, circular, flat, undulate, showing saucer liquefaction. Agar slant: Abundant surface growth, filiform, raised, dull, contoured, opaque, white. Gelatine stab: Uniform, filiform; liquefaction started to form in 7 days. Potato: Growth abundant, brown; no change in medium. Litmus milk: Slight reduction in 2 days. No coagulation. Strong peptonization and reduction in 7 days. Reaction neutral. Broth: Membranous surface growth. Slight clouding. Scant viscid sediment. Nitrates not reduced. Dunham's solution: Ammonia strongly positive, indol negative. Diastatic action: Starch reduced in potato medium. No action on starch agar plates in 3 days. No acton on glucose, lactose, sucrose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: O B 1a. Morphology: Yeast-like organisms, multiplying by budding. Mature cells 4.5 microns. Ascospores not observed on gypsum blocks nor on ordinary laboratory

or mannite. Aerobic. Torula species.

Lab. Designation: O B la. Morphology: Yeast-like organisms, multiplying by budding. Mature cells 4.5 microns. Ascospores not observed on gypsum blocks nor on ordinary laboratory media. Gram-positive. Agar colonies: Moderate, round, smooth, convex, entire, coarsely granular. Gelatine colonies: Slowly developing, circular, flat, entire, amorphous. No liquefaction. Agar slant: Moderate, filiform, convex, glistening, smooth, opaque, white, slimy; putrid odor. Gelatine stab: Growth best at top, filiform, gray. Medium unchanged. No liquefaction. Potato: Growth moderate, filiform, white. Medium unchanged. Litmus milk: No change. Broth: Surface ring, slight clouding, granular sediment. Nitrates not reduced. Dunham's solution: Ammonia absent, indol negative. No diastatic action. Acid but no gas from glucose, lactose and sucrose. No action on glycerol or mannite. Aerobic. Torula species.

Lab. Designation: O B 1b. Morphology: Yeast-like organisms, multiplying by budding. Mature cells 3.1 to 4.6 microns. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, coarsely granular. Agar slant: Moderate, filiform, convex, glistening, smooth, opaque, white, slimy. Gelatine stab: Growth best at top, filiform, medium unchanged. No liquefaction. Cider: Ringed surface growth. Sediment adhering to walls of tube, and abundant on the bottom. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: O B lc. Morphology: Spheres occurring in pairs and irregular clusters 0.8 to 1 micron in diameter. Gram-positive. Agar colonies: Rapidly developing, round to irregular, smooth, convex, undulate, finely granular. Gelatine colonies: Irregular, flat, undulate exhibiting cup liquefaction. Agar slant: Abundant, filiform to spreading, raised, glistening, smooth, later contoured, slimy, cream-colored and possessing a fecal odor. Gelatine stab: Growth best at top and filliform and light brown. Medium unchanged. Liqu

gas. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: O B 2b. Morphology: Yeast-like organisms, multiplying by budding. Mature cells 0.7 to 1.2 microns. Agar colonies: Moderate, round, smooth, convex, entire, finely granular. Subsurface colonies lens-shaped. Agar slant: Moderate, filiform, flat, glistening, smooth, opaque, white, slimy. Gelatine stab: Growth best at top, filiform, gray; liquefaction crateriform. Cider: No surface growth, slight clouding. Gray, scant sediment, viscid on agitation. Glucose broth: No surface cell growth, strong clouding, abundant viscid sediment. Gray chromogenesis. Acid, but no gas from glucose, lactose, sucrose, maltose, and glycerol. No action on mannite. Aerobic. Torula species.

Lab. Designation: O B 2c. Morphology: Yeast-like organisms, multiplying by budding. Mature cells 1 micron. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, finely granular. Subsurface colonies lens-shaped or cunciform. Agar slant: Abundant, filiform, convex, glistening, smooth, opaque, white, slimy. Gelatine stab: Growth hest at top, filiform, gray; liquefaction stratiform. Cider: No surface growth. Fluid turbid, scanty sediment, viscid on agitation. Glucose broth: No surface growth. Abundant turbidity. Moderate viscid sediment, growth grayish white. Acid, but no gas from glucose, lactose, sucrose, maltose and glycerol. No action on mannite. Aerobic. Torula species.

Lab. Designation: O B 2d. Morphology: Yeast-like organisms multiplying by budding. Mature cells .8 to 1 micron. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, finely granular. Agar slant: Abundant, filiform, flat, glistening, smooth, opaque, white, slimy. Gelatine stab: Growth best at top, filiform, gray. No liquefaction. Cider: No surface growth, slight clouding, scant viscid, gray sediment. Glucose broth: No surface growth, strong turbidity, abundant, gray, viscid sediment. No

Mature cells 0.8 to 1 micron. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, finely granular. Agar slant: Moderate, filiform, raised, glistening, smooth, opaque, white, slimy, with a decided yeasty odor. Gelatine stab: Growth best at top. Filiform, gray; liquefaction stratiform. Cider: No surface growth, fluid turbid, scanty gray sediment viscid on agitation. Glucose broth: Slight surface ring, strongly turbid, moderate whitish gray sediment, viscid on agitation. Acid but no gas in glucose, lactose, sucrose, maltose, glycerol and mannite. Aerobic. Torula species.

Lab. Designation: O B 3b. Morphology: Yeast-like organisms, multiplying by budding. Mature cells 0.8 to 1 micron. No ascospores observed on gypsum blocks. Agar colonies: Rapidly developing, round, smooth, convex, entire, finely granular. Subsurface colonies lens-shaped or cuneiform. Agar slant: Growth best at top, filiform, gray; liquefaction stratiform. Cider: No surface growth, fluid turbid, gray, scanty sediment viscid on agitation. Glucose broth: Marked or gas on glucose, lactose, sucrose, maltose, glycerol and mannite. Aeorbic. Torula species.

Lab. Designation: O B 4a. Morphology: Yeast-like organisms, multiplying by budding. Mature cells 3 5 to 4.8 microns. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, finely granular. Agar slant: Moderate, filiform, gray; no liquefaction. Cider: Surface ring, slight clouding, with flakes adhering to walls of tube. Abundant gray, granular to flaky sediment, viscid on agitation. Glucose broth: Decided surface ring, no clouding, abundant grayish white sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

Lab. Deesignation: O B 5a. Morphology: Yeast-like organisms, multiplying by budding. Mature cells 4 4 to 7 microns. No ascospores observed on gypsum blocks. Agar colonies: Moderate, filiform, convex, glistening, smooth, opaque, white, slimy. Odor putrid. Gelatine

Agar slant: Moderate, filiform, convex, glistening, smooth, opaque, white, slmy. Odor putrid. Gelatine stab: Growth best at top, filiform, gray; no liquefaction. Cider: Surface ring. No clouding, yeasty odor, abundant granular gray sediment. Glucose broth: Surface ring, no clouding, abundant gray, granular sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: O B 5b. Morphology: Yeast-like organisms multiplying by budding. Mature cells 2.8 to 4.6 microns. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, opaque, white, coriaceous. Gelatine stab: Growth best at top, filiform, convex, glistening, smooth, opaque, white, coriaceous. Gelatine stab: Growth best at top, filiform, pray; no liquefaction. Cider: Surface ring, no clouding but flakes adhering to tube. Moderate amount of gray granular sediment. Glucose broth: Marked surface ring, no clouding but with flakes adhering to wall; abundant grayish white, flaky sediment. No acid or gas on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: O B 5c. Morphology: Spheres occurring in irregular clusters 0.7 too, 9 micron in diameter. No spores observed. Agar colonies: Moderate, round, smooth, raised, convex, entire, finely granular clusters of the colonies lens-shaped. Agar slant: Moderate, filiform, raised, glistening, smooth, opaque, slimy, white. Odor slightly pungent. Gelatine stab: Growth best at top, filiform, white; liquefaction infundibuliform. Glucose broth: No surafce growth, strong granular clouding, abundant gray viscid sediment. Acid but no gas from glucose, lactose, sucrose, and glycerol. No action on maltose or mannite. Aerobic. Species?

Lab. Designation: O B 6a. Morphology: Spheres occurring in irregular clusters 0.7 to 0.9 micron in diameter. No spores observed. Agar colonies: Moderate, round, smooth, opaque, slimy, slident stab: Growth best at top, filiform, gray; no liquefaction of convex, glistening,

Agar colonies: Moderate, round, smooth, entire, finely granular. Agar slant: Moderate, echinulate, convex, glistening, smooth, opaque, white, coriaccous. Gletine stab: Uniform, filiform, white; no liquefaction. Cider: Surface ring, moderate clouding, abundant white, granular sediment. Glucose broth: Surface ring, no clouding but flakes adhering to walls. Abundant cream-colored flaky sediment. No action on glucose, lactose, sucrose, maltose, glycerol and mannite. Aerobic. Tornla species.

flaky sediment. No action on glucose, lactose, sucrose, maltose, glycerol and mannite. Aerobic. Torula species.

Lab. Designation: O B 7b. Morphology: Spherical organisms occurring in irregular clusters. Average size .9 micron in diameter. No spores observed. Agar colonies: Moderate, round, smooth, convex, entire, finely granular. Subsurface colonies lens-shaped. Agar slant: Abundant, filiform, convex, glistening, smooth, opaque, pale yellow, slimy, musty odor. Gelatine stab: Growth best at top, filiform, yellow: no liquefaction. Cider: No surface growth, slight clouding with flakes adhering to walls. Scant gray, viscid sediment. Glucose broth: Hairy surface growth with flocculent streamers hanging from surface. Considerable turbidity. Abundant gray, viscid sediment. Acid but no gas from glucose. No action on lactose, sucrose, maltose, glycerol or mannite. Aerobic. Species?

Lab. Designation: O B 7c. Morphology: Yeast-like organisms, multiplying by budding. Mature cells 2 to 2.3 microns in diameter. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, finely granular. Agar slant: Moderate, filiform, convex, glistening, smooth, slimy, white. Odor slightly pungent. Gelatine stab: Growth best at top, filiform, white; no liquefaction in 30 days. Cider: Surface ring. Moderate clouding, abundant white, flaky sediment, viscid on agitation. Glucose broth: Surface ring, moderate clouding, gray flaky sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: O B 7c. Morphology: Elliptical, yeast-like organisms, multiplying by budding. Mature cells, ranging from 3 to 4 microns in width to 4.5 to 9 microns in length. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, coarsely granular 1.5 mm. in diameter; subsurface colonies round to lens-shaped 0.5 mm. in diameter. Agar slant: Moderate, filiform, convex, glistening, smooth, later contoured, opaque, white, slimy. Gelatine stab: Growth best at top, filiform, white; liquefaction stratiform. Begins in 3 days, complete in 30 days. Cider: No surface growth, slight clouding, scanty, flaky white sediment. Glucose broth: Surface ring, strong turbidity, abundant, gray viscid sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: O B 8a. Morphology: Yeast-like organisms, multiplying by budding. Mature cells 3.8 to 4.6 microns in diameter. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, coarsely granular. Subsurface colonies lens-shaped. Agar slant: Moderate, filiform, convex, dull, contoured, opaque, white, slimy. Gelatine stab: Growth best at top, filiform at first, later beaded, gray; no liquefaction. Cider: Surface ring. No clouding but flakes adhering to walls of tube. Abundant, gr

flaky sediment. Noic. Torula species

gray, flaky sediment. No acid or gas from gracose, factore, success, success, success. Aerobic. Torula species.

Lab. Designation: O B 8b. Morphology: Yeast-like organisms, multiplying by budding. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth to radiate, convex, entire, finely granular, 2.5 microns in diameter. Subsurface colonies lens-shaped to cunciform 1 mm. by 0.5 mm. Agar slant: Moderate, filiform, raised, glistening, smooth, later rugose, opaque, white, coriaceous. Odor slightly fecal. Gelatine stab: Growth best at top, filiform, white; no liquefaction in 30 days. Cider: Surface ring, strong clouding, abundant granular white sediment. Glucose broth: Surface ring, no clouding but flakes adhering to walls of tube. Abundant cream-colored flaky sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

colored flaky sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannuc. Aerobic. Torula species.

Lab. Designation: O B 9a. Morphology: Oval yeast-like organisms, multiplying by budding. Mature cells 2.2 to 3.3 microns in diameter. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, coarsely granular. Subsurface colonies lensshaped. Agar slant: Moderate, filiform, convex, glistening, smooth, opaque, white, slimy. Gelatine stab: Growth best at top, filiform, gray; no liquefaction. Cider: Surface ring, no clouding but flakes adhering to walls of tubes, abundant, gray, granular sediment. Glucose broth: Surface ring. No clouding but with flakes adhering to walls of tube. Abundant flaky, grayish white sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species

Lab. Designation: O B 9b. Morphology: y: Yeast-like organisms multiplying by budding. Agar colonies: Moderate, round, smooth, convex, No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, finely granular. Subsurface colonies lens-shaped. Agar slant: Moderate, filiform, raised, glistening, rugose, white, brittle; having a fecal odor. Gelatine stab: Growth best at top, filiform, glistening, rugose, white, brittle; having a fecal odor. Gelatine stab: Growth best at top, fillform, white; no liquefaction in 30 days. Cider: Surface ring; strong clouding; white granular, scanty sediment. Glucose broth: Surface ring, no clouding but with flakes adhering to walls of tube; abundant, flaky, cream-colored sediment. No action on glucose, lactose, sucrose, maltose, glycerol and mannite. Aerobic. Torula species.

Lab. Designation: O B 10a. Morphology: Yeast-like organisms, multiplying by budding.

and mannite. Aerobic. Torula species.

Lab. Designation: O B 10a. Morphology: Yeast-like organisms, multiplying by budding. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, finely granular. Subsurface colonies are lens-shaped. Agar slant: Moderate, filliorm, raised, glistening, rugose, white, chalky. Gelatine stab: Growth best at top, filliorm, white; no liquefaction in 30 days. Cider: Surface ring, strong clouding; scanty white sediment. Glucose broth: Flocculent surface ring, no clouding but with flakes adhering to walls of tube; abundant, flaky cream-colored sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite.

flaky cream-colored sediment. No action on glucose, lactose, multiplying by budding. Mature cells 3.5 to 4.2 microns in diameter. No ascospores observed on gypsum blocks. Agar colonies. Moderate, round, smooth, convex, entire, coarsely granular. Subsurface colonies lens-shaped. Agar slant: Moderate, filiform, convex, glistening, smooth, opaque, white, slimy. Gelatine stab: Growth best at top, filiform, gray; no liquefaction in 30 days. Cider: Surface ring, no clouding; abundant, gray, granular sediment. Glucose broth: Surface ring, no clouding; abundant graying white flaky sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite.

Growth best at top, milorin, gray, no aqueractor in the state of the convex, granular sediment. Glucose broth: Surface ring, no clouding; abundant graying white fllaky sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

Lab, Designation: O B 10c. Morphology: Oval yeast-like organisms, multiplying by budding, 3.5 to 4.6 microns in diameter. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, coarsely granular. Agar slant: Moderate, echinulate, convex, dull, contoured, opaque, white, slimy. Gelatine stab: Growth best at top, filiform, gray; no liquefaction. Cider: Surface ring, no clouding but with flakes adhering to walls of tube; abundant, gray, granular sediment. Glucose broth: Very marked surface ring, no clouding but with flakes adhering to walls of tube; abundant flaky, graying white sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: O B 11a. Morphology: Oval, yeast-like organisms multiplying by budding. Mature cells 2.8 to 4.5 microns in diameter. No ascospores observed on gypsum blocks. Agar colonies: Moderate, round, smooth, convex, entire, coarsely granular. Subsurface colonies lens-shaped. Agar slant: Moderate, filiform, convex, glistening, smooth, opaque, white, slimy. Gelatine stab: Growth best at top, filiform, gray; no liquefaction. Cider: Surface ring; no clouding but with flakes adhering to walls of tube. Abundant flaky, grayish-white sediment. No action on glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Torula species.

Lab. Designation: B 5 x 10. Morphology: Spheres occurring singly, in pairs and in irregular clusters. Average size 0.8 micron. Gram-positive. Agar colonies: Rapidly developing, circular, smooth, convex, entire, amorphous, Subsurface colonies lens-shaped. Gelatine colonies: Rapidly developing, circular, flat, entire, amorphous, with saucer liquefaction. Potato: Moderate, filiform, smooth, glistening, opaque, creamy-white, later becoming light brown in color. Litmus milk: Slightly reduced. No coagulation or peptonization in 10 days. Broth: No surface growth, moderate clouding, viscid sediment. Nitrates not reduced. Dunham's solution: Ammonia strongly positive, indol negative. No diastatic action. Acid but no gas from glucose, lactose, sucrose, glycerol and mannite. Aerobic. Species?

Lab. Designation: B 5 Y 1. Morphology: Long rods occurring singly, in pairs and in chains, with rounded ends. Size of the majority 0.6 to 2 microns. No endospores observed. Motile by means of 1 to 6 peritrichous flagella. Gram-negative. Agar colonies: Rapidly developing, circular, smooth, convex, entire. Deep colonies, lens-shaped. Gelatine colonies: Rapidly developing, irregular, undulate, coarsely granular; spreading liquefaction. Agar slant: Abundant, filiform, convex, glistening, smooth, grayish-white, with odor resembling feces. Gelatine stab: Uniform, filiform, with stratiform liquefaction. Potato: Abundant, spreading wrinkled growth with complete disintegration of medium. Litmus milk: Alkaline, coagulated, peptonized, reduced. Broth: Wrinkled surface pellicle, no clouding, flaky sediment. Nitrates reduced in 10 days. Dunham's solution: Ammonia strongly positive, indol negative. Diastatic action strongly positive. Acid but no gas from glucose, sucrose. No action on lactose, glycerol and mannite. Aerobic. Near to Achromobacter lipolyticum (Huss) Bergey et al.

Lab. Designation: B 5 Y 5. Morphology: Long rods occurring singly and in pairs, with rounded ends. Average size 0.8 by 3 4 microns. Endospores present, polar, elliptical,

Species?

Lab. Designation: B 5 Z 10. Morphology: Spheres occurring singly, in pairs and in irregular clusters. Average size .6 micron. Gram-negative. Agar colonies: Rapidly developing, circular, smooth, convex, entire, amorphous. Deep colonies lens-shaped. Gelatine colonies: Rapidly developing colonies, circular, convex, entire, amorphous, white, with saucer liquefaction. Agar slant: Abundant, filiform, flat, smooth, creamy-white. Gelatine stab: Growth best at top, filiform, creamy-white, with stratiform liquefaction. Potato: Moderate, filiform, convex, glistening, creamy-white. Litmus milk: slightly acid and slightly reduced in 10 days; no coagulation or peptonization. Broth: No surface growth, moderate clouding, abundant viscid sediment. Nitrate reduced to nitrite in 7 days. Dunham's solution: Ammonia strongly positive, indo negative. Diastatic action positive. Acid with no gas from glucose, lactose, sucrose, maltose, glycerol or mannite. Aerobic. Species?

Lab. Designation: B 5 W 10. Morphology: Spheres occurring singly, in pairs and in irregular

to nitrite in 7 days. Dunham's solution: Ammonia strongly positive, indol negative. Diastatic action positive. Acid with no gas from glucose, lactose, sucrose, maltose, glycerol or mannite. Acrobic. Species?

Lab. Designation: B 5 W 10. Morphology: Spheres occurring singly, in pairs and in irregular clusters. Average size 0.8 micron. Gram-positive. Agar colonies: Rapidly developing, circular, convex, entire, amorphous. Deep colonies lens-shaped. Gelatine colonies: Rapidly developing, circular, convex, entire, amorphous; with saucer liquefaction. Agar slant: Abundant, filiform, flat, glistening, smooth, opaque, grayish white. Gelatine stab: Growth best at top, filiform with stratiform liquefaction. Potato: Moderate, filiform, convex, glistening, smooth, opaque, white. Litmus milk: Slightly acid to slight reduction; no coagulation or peptonization in 10 days. Broth: No surface growth, moderate clouding, abundant viscid sediment. Nitrates strongly reduced to nitrite in 7 days. Dunham's solution: Ammonia strongly positive, indol negative. Diastatic action: Potato somewhat decomposed. Action on starch agar plates somewhat doubtful. Acid but no gas from glucose, lactose, sucrose, glycerol and mannite. Aerobic. Species?

Lab. Designation: B 5 W 11. Morphology: Spheres occurring singly and in pairs and in irregular clusters. Average size 0.8 micron. Gram-positive. Agar colonies: Rapidly developing, irregular, smooth, flat, undulate, amorphous. Deep colonies lens-shaped. Gelatine colonies: Rapidly developing, circular, convex, entire, amorphous, with saucer liquefaction. Agar slant: Abundant, filiform, flat, glistening, smooth, opaque, gray. Gelatine stab: Growth best at top, filiform, with crateriform to saccate liquefaction. Potato: Moderate, filiform, raised, glistening, opaque, smooth to contoured (later) white. Medium decomposed. Litmus milk: Slightly acid and reduced in 10 days. No coagulation or peptonization. Broth: No surface growth, moderate clouding, abundant, visicid sediment. Nitrates reduced to nitrites in Species?

Lab. Designation: VIc 10. Morphology: Spheres occurring singly, in pairs and in irregular clusters. Average size 0.7 micron. Gram-positive. Agar colonies: Rapidly developing, circular, smooth, convex, entire, amorphous. Deep colonies lens-shaped. Gelatine colonies: Slowly develop-

ing, circular, flat, entire, amorphous, with saucer liquefaction. Agar slant: Moderate, filiform, convex, glistening, smooth, opaque, creamy white. Gelatine stab: Growth best at top, filiform, with crateriform liquefaction. Potato: Moderate, filiform, glistening, opaque, creamy-white. Later growth contoured and light brown. Medium slightly decomposed. Litmus milk: Slightly acid and reduced in 7 days. No coagulation or peptonization in 10 days. Broth: No surface growth, moderate clouding, abundant viscid sediment. Nitrate reduced to nitrite in 7 days. Dunham's solution: Ammonia positive, indol negative. No diastatic action. Acid but no gas from glucose, lactose, sucrose, glycerol or mannite. Aerobic. Micrococcus species.

Lab. Designation: VIc 11. Morphology: Spheres occurring singly, in pairs and irregular clusters, Average size 0.8 micron. Gram-positive. Agar colonies: Rapidly developing, circular, smooth, convex, entire, amorphous. Deep colonies lens-shaped. Gelatine colonies: Rapidly developing, circular, flat, entire, amorphous, creamy-white, with saucer liquefaction. Agar slant: Abundant, filiform, glat, glistening, smooth, opaque, creamy-white. Gelatine stab: Growth best

veloping, circular, flat, entire, amorphous, creamy-white, with saucer inquetaction. Agar slant: Abundant, filiform, flat, glistening, smooth, opaque, creamy-white. Gelatine stab: Growth best at top, filiform, with saccate liquefaction. Potato: Moderate, filiform, convex, glistening, opaque, cream-colored to light brown later. Medium decomposed. Litmus milk: Slightly acid and reduced in 10 days. No coagulation or peptonization. Broth: No surface growth, moderate clouding, abundant viscid sediment. Nitrates strongly reduced to nitrite in 7 days. Dunham's solution: Ammonia strongly positive, indo negative. No diastatic action. Acid but no gas from glucose, lactose, sucrose, glycerol and mannite. Aerobic. Micrococcus species.

Ammonia strongly positive, indol negative. No diastatic action. Acid but no gas from glucose, lactose, sucrose, glycerol and mannite. Aerobic. Micrococcus species.

Lab. Designation: VIc 13. Morphology: Spheres occurring singly, in pairs, fours and irregular clusters. Average size 0.8 micron. Gram-positive. Agar colonies: Rapidly developing, circular, smooth, convex, entire, amorphous. Deep colonies lens-shaped. Gelatine colonies: Moderate, circular, convex, entire, amorphous, saucer liquefaction. Agar slant: Abundant, filiform, flat, smooth, opaque, white, viscid, with fecal odor. Gelatine stab: Uniform, filiform with saccate liquefaction. Potato: Moderate, filiform, convex, contoured, opaque, creamy-white. Medium decomposed. Litmus milk: No change. Broth: No surface growth, moderate clouding, viscid sediment. Nitrates strongly reduced to nitrite in 7 days. Dunham's solution: Ammonia strongly positive, indol negative. Diastatic action: Strongly positive in 4 days. Acid but no gas from glucose, lactose, sucrose, glycerol and mannite. Aerobic. Micrococcus species.

Lab. Designation: VIdi. Morphology: Short rods, occurring singly and in pairs; size of majority .6 to 1.3 microns with central elongated thick-walled spores. Motile. Gram-negative. Agar colonies: Rapidly developing, irregular, smooth, flat, lobate, amorphous. Gelatine colonies: Rapidly developing, irregular, smooth, flat, lobate, amorphous, with a decided odor resembling lye. Gelatine stab: Growth best at top, fliform, medium fluorescent, stratiform liquefaction. Potato: Moderate, filiform to echinulate, brown. Litmus milk: Slightly coagulated without change of reaction, peptonized and reduced in 6 days. Broth: Membranous surface growth, strong clouding, decided odor resembling lye, viscid sediment. Nitrates reduced with production of gas in 6 days. No diastatic action. Acid but no gas from sucrose and glycerol. No action on glucose and lactose. Aerobic. Bacillus species.

Lab. Designation: VId 10. Morphology: Long rods occurring singly and in chains

ant, filiform, glistening, smooth, later wrinkled, grayish white, translucent; medium decomposed Litmus milk: Neutral, peptonized and reduced, but no coagulation in 10 days. Broth: Strong membranous surface pellicle. Slight clouding, no sediment. Nitrate reduced to nitrite in 7 days. Dunham's solution: Ammonia positive, indol negative. Diastatic action strongly positive in 7 days. Slight acidity but no gas in glucose, sucrose and glycerol. No action on lactose and mannite. Aerobic. Species?

Lab, Designation: VId 11. Morphology: Spheres occurring singly and in pairs. Average size 6 micron. Gram-positive. Agar colonies: Rapidly developing, circular, smooth, convex, entire, amorphous. Deep colonies lens-shaped. Gelatine colonies: Slowly developing, circular, flat, entire, filamentous: saucer liquefaction. Agar slant: Abundant, filiform, flat, glistening, smooth, opaque, yellow, viscid with odor resembling feces. Gelatine stab: Growth best at top, filiform; crateriform liquefaction. Potato: Moderate, filiform, smooth, convex, glistening, opaque, yellow; medium decomposed. Litmus milk: Slightly alkaline, peptonized, reduced, no coagulation. Broth: Floculent surface growth, moderate clouding, viscid sediment. Nitrate reduced to nitrite in 7 days. Dunham's solution: Ammonia positive, indol negative. Diastatic action strongly positive in 4 days. No action on glucose, lactose, sucrose, glycerol and mannite. Aerobic. Micrococcus species. Lab. Designation: VIIaa. Morphology: Short rods, occurring singly and in pairs with ends rounded. Size of majority 0.2 by 0.8 microns. Endospores absent. Motile by means of 1 polar flagellum. Gram-negative. Agar colonies: Slowly developing, punctiform to circular, smooth, flat, transparent, entire, amorphous. Deep colonies punctiform. Gelatine colonies: Rapidly developing, nunctiform to circular, smooth, translucent, creamy-yellow at first, approaching canary later, butyrous. Gelatine stab: Growth best at top, filiform; stratiorm liquefaction. Potato: Moderate, filiform, to spreading, f

in 10 days. Abundant production of ammonia in 10 days. No diastatic action. Acid but no gas in glucose, sucrose, glycerol and mannite. No action on lactose. Aerobic. Achromobacter species.

Lab. Designation: VIIa 4. Morphology: Rods occurring singly, with rounded ends. Limits of size 0 2 by 1 3 microns. Size of majority 0 2 by 1.6 microns. Sporangia absent; endospores absent. Motile by means of 1 to 4 peritrichous flagella. Gram-negative. Agar colonies: Rapidly developing, punctiform to round, smooth, convex, entire, amorphous. Deep colonies punctiform to lens-shaped. Gelatine colonies: Rapidly developing, punctiform to round, flat, entire; saucer lique-faction. Agar slant: Abundant, filiform, flat, glistening, smooth, translucent, yellowish-white, butyrous. Gelatine stab: Growth best at top, filiform; liquefaction napiform. Potato: Abundant, filiform, flat, glistening, smooth, translucent, yellowish-white, peptonized, reduced. No coagulation. Broth: Surface ring; strong persistent clouding, putrid

odor, abundant viscid sediment. Nitrates not reduced. Dunham's solution: Ammonia positive, indol negative. No diastatic action. No action on glucose, lactose, sucrose, glycerol and mannite.

Acrobic. Achromobacter species.

Lab. Designation: VIIa 5. Morphology: Short rods, occurring singly with rounded ends. Limits of size 0.4 by 0.4 to 15 microns. Size of majority 0.4 by 0.8 micron. Endospores absent. Motile by means of one polar flagellum. Gram-negative. Agar colonies: Rapidly developing, one of the polar flagellum, and the polar flagellum. Deep colonies proposed convex entire, to undulate, finely granular. Deep colonies processors

Lab. Designation: VIIa 5. Morphology: Short rods, occurring singly with rounded ends. Limits of size 0.4 by 0.4 to 15 microns. Size of majority 0.4 by 0.8 micron. Rapidly developing, round to irregular, smooth, convex, entire, to undulate, finely granular. Deep clonies punctiform to round to lens-shaped. Gelatine colonies: Rapidly developing, to round, to irregular, flat, entire, saucer liquefaction. Agar slant: Moderate, filiform, flat, glistening, smooth, to provide the personal properties of the provided of the provided flat, glistening, smooth, gliste

nitrites in 10 days. Strong production of ammomia in 10 days. Diastatic action feeble in 26 days. Acid but no gas from glucose, sucrose, and mannite. No action on lactose or glycerol. Aerobic. Bacillus species.

Lab. Designation: VIIc 10. Morphology: Long rods occurring singly and in chains, with rounded ends. Limits of size 0.5 to 0.6 by 2 to 3 microns. Size of majority 0.5 by 2.4 microns. Endospores absent. Motile by means of numerous peritrichous flagella. Gram-positive. Agar colonies: Moderate, circular, smooth, convex, entire, finely granular. Deep colonies rhizoid, with filamentous edges. Gelatine colonies: Slowly developing, circular, convex, entire, filamentous internal structure, with cup liquefaction. Agar slant: Abundant, spreading, flat, glistening, contoured (later wrinkled) opaque, white, viscid. Gelatine stab: Growth best at top, arborescent, with napiform liquefaction. Potato: Moderate, filiform, glistening, smooth, (later wrinkled) convex, opaque, white (later turning pink). Medium decomposed. Litmus milk: Neutral, not coagulated, strongly peptonized and reduced in 10 days. Broth: Flocculent surface growth, slight clouding, viscid sediment. Nitrates strongly reduced to nitrite. Dunham's solution: Ammonia strongly positive, indol negative. Diastatic action: Strongly positive. Acid but no gas from glucose, sucrose, glycerol and mannite. No action on lactose. Aerobic. Achromobacter species.

Lab. Designation: VIIc 11. Morphology: Short and long rods, occurring singly and in pairs, with ends rounded. Limits of size 0.6 by 1 to 2.7 microns. Size of majority 0.6 by 2.3 microns. Endospores absent. Motile by means of 1 to 6 peritrichous flagella. Gram-positive. Agar colonies: Rapidly developing, circular, flat, entire, filamentous structure; cup liquefaction. Agar slant: Abundant, spreading, flat, glistening, rugose, opaque, gray, viscid. Gelatine stab: Uniform, arborescent, with no liquefaction. Potato: Moderate, filiform, smooth, convex, glistening, translucent, pink (later brown color) wrinkled afte

Scanty, filiform, convex, smooth, glistening, opaque, white. Litmus milk: No change. Broth: No surface growth, moderate clouding, flocculent sediment. Nitrate broth: Not reduced. Dunham's solution: Ammonia strongly positive, indol negative. Diastatic action: Positive. Acid but no gas from glucose, sucrose, glycerol and mannite. No action on lactose. Aerobic. Micrococcus

Lab. Designation: VIId 10. Morphology: Long rods, occurring singly and in chains, with ends rounded. Limits of size 0.5 by 1.7 to 2.8 microns. Size of majority 0.5 by 2.5 microns. Endospores absent. Motile by means of 6 peritrichous flagella. Gram-positive. Agar colonies: Slowly developing, circular, smooth, flat, with filamentous edge and filamentous internal structure. Deep colonies like a tuft of cotton. Gelatine colonies: Slowly developing, punctiform, to round, flat, with filamentous edge and internal structure and cup-shaped liquefaction. Agar slant: Abundant, spreading, flat, glistening, contoured to rugose, gray, viscid. Gelatine stab: Uniform, flitform, pliquefaction crateriform: Potato: Abundant, filiform, glistening, smooth to wrinkled, translucent, convex, gray to light brown (later). Litmus milk: Alkaline at surface, no coagulation, peptonization and reduction in 10 days. Broth: Surface pellicle, slight clouding, floculent sediment. Nitrates strongly reduced to nitrite and ammonia in 7 days. Dunham's solution: Ammonia strongly positive, indol negative. Diastatic action strongly positive in 4 days. Acid but no gas from glucose, sucrose, glycerol and mannite. Slight acidity from lactose. Aerobic. Achromobacter species.

Lab. Designation: VIId 11. Morphology: Long rods occurring singly with rounded ends.

tive, indol negative. Diastatic action strongly positive in 4 days. Acid but no gas from glucose, sucrose, glycerol and mannite. Slight acidity from lactose. Aerobic. Achromobacter species.

Lab. Designation: VIId 11. Morphology: Long rods occurring singly with rounded ends. Limits of size 0.5 by 1.8 to 2.6 microns. Size of majority 0.5 by 2.3 microns. Endospores absent. Motile by means of 1 to 6 peritrichous flagella. Gram-positive. Agar colonies: Rapidly developing, circular, smooth, convex, with filamentous edge and filamentous internal structure. Gelatine colonies: Slowly developing, punctiform to circular, convex, entire amorphous, with saucer lique-faction. Agar slant: Abundant, spreading, flat, glistening, rugose, opaque, gray, cretaceous consistency and odor resembling feces. Gelatine stab: Uniform, filiform to arborescent. Lique-faction crateriform. Potato: Abundant, filiform, convex, glistening, translucent at first pink, later brown. Litmus milk: Slightly alkaline, no coagulation, strongly peptonized and reduced in 7 days. Broth: Membranous surface growth, moderate clouding, viscid sediment. Nitrates strongly reduced to nitrites and ammonia in 7 days. Dunham's solution: Ammonia strongly positive, indol negative. Diastatic action strongly positive in 4 days. Acid but no gas from glucose, sucrose, glycerol and mannite. No action on lactose. Aerobic. Achromobacter species.

Lab. Designation: VIIIb 1. Morphology: Short and long rods, occurring singly and in pairs with truncate ends. Limits of size 0.5 by 1.5 to 4.5 microns. Size of majority 0.5 by 2.4 microns. Sporangia present, elliptical. Endospores present, elliptical in shape, mostly free. Motile by means of 3 to 6 peritrichous flagella. Gram-positive. Agar colonies: Rapidly developing, round to irregular, rough, flat, lobate, coarsely granular. Deep colonies round. Gelatine colonies: Rapidly developing, punctiform to spreading, flat, dull, smooth, white, butyrous. Gelatine colonies: Rapidly developing, punctiform, liquefaction, napiform. Potato: Abu

Bacillus species. Lab. Designation: Lab. Designation: VIIIca. Morphology: Long rods occurring singly and in pairs, with rounded ends. Size of majority 1 by 2.8 microns. Thick walled, central spores, size of majority 0.6 by 0.8 micron. Non-motile. Gram-positive. Agar colonies: Rapidly developing, circular, smooth, flat, entire, amorphous. Gelatine colonies: No growth. Agar slant: Moderate, spreading, flat, dull, contoured, gray. Potato: Abundant, brown, glazed. Litmus milk: Neutral, coagulated, peptonized, reduced in 4 days. Broth: Membranous surface growth, moderate clouding, flocculent sediment, viscid on agitation. Nitrates reduced to nitrite in 7 days. Positive diastatic action. Acid but no gas from glucose, lactose, sucrose and glycerol. Aerobic. Bacillus species

species.

species.

Lab. Designation: VIIIc 4. Morphology: Short and long rods, occurring singly and in pairs, with ends rounded. Limits of size 0.8 by 2.4 to 8 microns. Size of majority, 0.8 by 3 microns. Sporangia present, elliptical. Endospores present, central elliptical. Some free spores. Motile by means of 8 to 12 peritrichous flagella. Gram-positive. Agar colonies: Rapidly developing, round to irregular, rough, flat, lobate, coarsely granular. Deep colonies round. Gelatine colonies: Rapidly developing, punctiform to round, flat, entire; saucer to spreading liquefaction. Agar slant: Moderate, filiform to spreading, flat, dull. smooth, translucent, becoming opaque, white, butyrous. Gelatine stab: Growth best at top, filiform, white; liquefaction saccate. Potato: Abundant, filiform to spreading, flat, dull rugose, orange (later salmon pink), membranous, medium reddened and softened. Litmus milk: Completely reduced, neutral reaction, with alkaline ring on top. Completely peptonized. Not coagulated. Broth: Surface pellicle slight clouding, putrid odor, abundant flaky sediment. Nitrates strongly reduced to nitrites and ammonia in 7 days. Dunham's solution: Ammonia strongly positive, indol negative. Diastatic action: Starch decomposition strongly positive in 1 day. Acid but no gas from glucose and sucrose. No action on lactose, solution: Ammonia strongly positive, indol negative. Diastatic action: Starch decomposition strongly positive in 1 day. Acid but no gas from glucose and sucrose. No action on lactose, glycerol or mannite. Aerobic. Bacillus species.

Besides the foregoing more or less complete descriptions of organisms isolated, the following gives a summary of partial descriptions of organisms also isolated and mentioned in Table XI. These descriptions were those made at the time of isolation. A complete study was not made because for some reason they died or were lost before pure culture studies could be made. They are included here only for their statistical value.

B. M. 1. B. M. 1b: Slender rod, non-liquefying acid colony on litmus gelatine. B. M. 1c: Small misseagues white conclusions are reasonable to the statistical value.

B M 1. B M 1b: Slender rod, non-liquefying acid colony on litmus gelatine. B M 1c: Small micrococcus, white, small, round, raised surface colonies on litmus gelatine. B M 1d: Short rods, yellow, round surface colonies on litmus gelatine plate. B M 1h: Aroma producing streptococcus of Str. lactis group. B M 1j: Similar to B M 1h.

B M 2. B M 2a: Short thick rods, subsurface acid colony on litmus milk agar plates; casein liquefier on plates but not in tube of litmus milk. Caused acid coagulation of milk in litmus milk

tubes. Died before study could be made. B M 2b: A typical Str. lactis. Died before study. B M 2c: Thick rods of variable length. Probably surface colony of same organism as B M 2a. B M 2d: A typical Str. lactis.

B M 3. B M 3e and B M 3f, both typical Str. lactis.

B M 4. B M 4b, B M 4f, B M 4h, B M4i, B M 4j and B M 4l typical Str. lactis.
B M 5. B M 5a: Morphology not noted. Round, porcelain white colonies. B M 5c: Morphology not noted. Small, pitted, liquefying colonies on gelatine.
B M 6. B M 6a: Morphology not noted. Liquefier on milk agar plates. Round, surface,

milk-white colony

B M 7. B M 7a: Short thick rod, in pairs. Did not curdle or reduce litmus milk. Acid producer. Surface colony, round, small, white on litmus milk agar plates. Died soon after isolation. B M 7b: Similar to B M 7a, except subsurface lens-shaped colony. Died soon after isolation. B M 7c: Very minute micrococcus. Small white colony in bottom of liquefied pit on gelatine plates. Died soon after isolation. B M 7d: Small rod, no spores observed. Small yellowish white pitted liquefier on gelatine plates. Died before cultural study was made. B M 7e: A micrococcus.

Small, round, raised, yellow surface colony on gelatine plates.

B M 8. B M 8b: Similar to B M 8a, except smaller colony. Died before cultural study was made. B M 8d: Yeast, similar to B M 8c, except larger colony. Died before cultural study was

made.

B M 9: Oidium. Died before cultural study was made. Also picked several typical

B M 9. B M 9: Oidium. Died before cultural study was made. Also picked several typical Str. lactis colonies which died soon after isolation.

B M 11. B M 11a: Rods of variable size. No spores seen. Medium-sized, round, white, surface colony. Died before cultural study was made.

B M 12. B M 12a: Oospora. Culture lost. B M 12b: Oospora. A peculiar oospora colony differing from B M 12a. More compact colony, raised, umbonate, white on top, yellow near surface of medium. Culture lost. B M 12c: Fusarium. Culture lost.

B M 13. B M 13a: Oidium. Culture lost. B M 13b: Large micrococcus. Large, white surface colony. Died soon after isolation.

B M 14. B M 14c: Micrococci, singly or in pairs. White surface colony. B M 14e: A very minute organism; difficult to determine whether it was a short rod or a coccus. Nearly white, soft, surface colony. B M 14f: A very minute coccus, in pairs. Probably the same as B M 14e, same colony appearance. colony appearance.

B M 15. B M 15e: Large micrococcus, in pairs and irregular clusters. Bright lemon-yellow colony. Probably identical with B M 15a. B M 15i: Short rods, about size of Esch. coli. White colony, grows poorly on agar. Produced acid in litmus milk but not enough to cause curdling. B Very minute micrococcus. Light lemon-colored colony. No change produced in litmus

milk.

B M 16. None.
B M 17. B M 17b: Cocci in pairs like Str. lactis. Lens-shaped medium-sized subsurface colony.

Casein liquefier on litmus milk agar plates.

B M 18. B M 18.3: Small gram-negative rod occurring singly and in pairs. Large, round, grayish, smooth, convex, glistening, surface colony. B M 18-2: Very short gram-positive rods, occurring singly and in pairs. Large, round, surface colony. Light reddish-brown in color, convex, smooth, glistening.

grayish, smooth, convex, glistening, suriace colony. B M 18-2: Very short gram-positive rods, smooth, glistening singly and in pairs. Large, round, surface colony. Light reddish-brown in color, convex, smooth, glistening.

O B 3. O B 3c: Small yeast or large coccus. No budding observed. Variable in size. Small yellowish lens-shaped colony. O B 3d: Micrococcus. Large, round, lemon-yellow, surface colony. O B 5d: Micrococcus, with a tendency to form in pairs and tetrads. White, lens-shaped, white, subsurface colonies.

B 5x 5y-5w-5z. B V x 2: Small white colony. B V x 3: Pink colony; B Same as O B 8b, i. e., poorly staining, budding, yeast-like cells. Lens-shaped, white, subsurface colonies.

B 5x 5y-5w-5z. B V x 2: Small white colony. B V x 3: Pink colony; B V g4: Very small gram-negative coccus. White, small, lens-shaped, subsurface colony B V g4: Very small gram-negative cod. Small, white lens-shaped subsurface colony. B V 24: Small gram-negative rod. Small, white, lens-shaped subsurface colony. B V 24: Small gram-positive rod. Small, white, lens-shaped surface colony. B V w4: Small gram-positive rod. Small, white, lens-shaped subsurface colony. B V I a9: Very small gram-negative rod. Large, translucent, glistening, whitish, circular surface colony. B V I a10: Large gram-positive rods with cylindrical centrally placed spores. Grayish, spreading, rhizoid colonies. B V I a10x: Gram-positive coccus, occurring singly, in pairs or in chains. Small elliptical, light brown subsurface colony. Edge entire. B V I a12: Very small gram-positive rods (very short, almost cocci) occurring singly or in chains. Grayish, transparent, concentrically ringed, round, filmy, surface colony. B V I b: Gram-negative short rod. White irregularly spreading surface colonies. B V I a10: Short thick rod, gram-negative. Flat ovoid colony. B V I b8: Gram-positive coccus, occurring singly, in pairs or in chains. Small, light brown, elliptical, subsurface colony. B V I c9: Small gram-negative rod. Small, white, lens-shaped subsurface colony. B

spindle-shaped sporangia, with central oval spores. Irregular, large, spreading, dirty-white surface colonies. Culture lost. B 7 d10: Gram-positive rods, occurring singly or in short chains. Large, cream-colored, opaque, irregular, wrinkled, surface colonies. B 7 d11: Gram-positive rods, with rounded ends, occurring singly or in pairs and chains. Concentrically ringed, grayish, translucent, subsurface colonies.

with rounded ends, occurring singly or in pairs and chains. Concentrically ringed, grayish, translucent, subsurface colonies.

B 8a-b-c-d. B8 al: Str. lactis. B8 a2: Long gram-positive rods (a few gram-negative). No spores seen. Nearly round, transparent surface colonies. B8 a3: Gram-positive rods, some long, slender, not containing spores, some shorter and plumper, containing oval to cylindrical spores, of same diameter as sporangia. Possibly a mixed culture. Large spreading colony. B8 a4: Large, gram-negative rods, with a tendency to produce long threads or filaments. Large, round, "fuzzy" subsurface colonies. B8 a5: Small, gram-negative rods; large, round yellow surface colony. B8 a10: Gram-variable coccus, occurring singly and in pairs. Small, elliptical, smooth-edged, light brown subsurface colonies. B8 a11: Gram-positive rods, occurring singly, in pairs and in short chains. Large, irregular, translucent, concentrically ringed, grayish, glistening, contoured colony of slimy consistency. B8 b2: Str. lactis. B8 b3: Very minute oval, gram-negative rods. Large, canary-yellow, irregular surface colony. Alkali producer, brom-thymol blue lactose agar slants and in litmus milk, with partial reduction of litmus and yellow precipitate in bottom of tubes. B8 b4: B8 b10: Gram-variable coccus, similar to B8 a10 in microscopic and colony appearance. B8 b11: Gram-positive rod. B8 c1: Gram-positive rods, fairly long, slightly curved, some gram-negative individuals. No spores observed. Small, lens-shaped, yellowish-white, subsurface colonies. B8 c10: Mostly gram-positive, large, spore forming rods, occurring singly or in chains. Spores round, terminal or sub-terminal. Spores larger in diameter than sporangia. Surface colony, large, with irregular margins, opaque, light brown area in center. Subsurface colony, small, elliptical or irregular, opaque, light brown with edges entire.

B8 d1: Str. lactis. B8 d2: Str. lactis. B8 d3: Gram-positive, budding yeast-like cells. Colonies pink, large, round, glistening. Produces alkali

The foregoing data are not susceptible of much detailed discussion, but a short summary of the types of organisms found may be helpful. Altogether 210 cultures were isolated from the 45 butters under study. Of these 123 were studied in considerable detail. The remaining 87 were lost for one reason or other before a complete study could be made of them. As already stated, most of these butters also contained Str. lactis, which usually was not picked, because Str. lactis was regarded as a normal constituent of good butter as well as of off-flavored butter.

The 210 cultures were distributed as follows: 91 rods, 50 torulae 49 cocci, exclusive of streptococcus of which 19 were picked. The only molds

picked were Oospora, of which there were 11 cultures.

Of the rods, 21 were aerobic spore formers, 16 of which were subjected to detailed study. Of the remaining 70 rods, only 30 survived the vicissitudes of cultivation in the laboratory. Of these, 13 were gram-positives and 17 were gram-negatives. At the time of isolation many of the cultures were stained by Gram's method. Of the 70 nonspore formers, 60 were stained by Gram's These 60 cultures were exactly evenly divided into gram-positive and gram-negative groups of 30 each.

Of the 30 nonspore forming rods studied in detail, 24 were able to liquefy

gelatine, while 6 lacked this characteristic.

Of the 49 cocci, exclusive of streptococci, there were 21 gram positives and 10 gram negatives, while 10 were not determined and there were 21 liquefying and 7 non-liquefying, while the remainder were undetermined. Of the 23 cultures which were fully studied, there were 14 gram positives, 11 liquefying and 3 non-liquefying and 9 gram negative, 8 liquefying and 1 non-liquefying.

There remains to be mentioned the 50 cultures or torulae, of which 17 were

gelatine liquefiers, 28 non-liquefiers and 5 not determined.

The 11 cultures of Oospora (or Oidium) while small in number, represent a very conspicuous flora in off-flavored butters, especially of market butters. They always appear in the lower dilutions of the platings but one should remember that they are enormous in size compared to the bacterial cells and consequently are potentially able to play an important role in producing off-flavors far in excess of what their mere numbers on the plates

might indicate.

Taking this cultural study as a whole, it must be admitted that at first glance there is little evidence that bacteria play much of a part in butter spoilage after the butter is manufactured, since the same organisms have not appeared uniformly on every plating as the butters aged. Closer examination of the data, however, seems to indicate that certain organisms may be the predominant flora at one plating while a totally different flora is predominant at a later examination. This fact in itself suggests that there is a decided rise and fall in the different types of organisms which occur in off-flavored butters and is rather strong evidence for the bacterial spoilage of butter.