DISSOCIANTS OF LACTOBACILLI[1]

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Earlier work on the growth of *Lactobacillus bulgaricus* at low temperatures (Voss and Frazier, 1945) had indicated that dissociation might have been affecting the results. Therefore a study was begun of the dissociation of some of the lactobacilli and its effect on their characteristics in the hope that the information obtained would prove helpful in the production and use of starter cultures for cheese.

LITERATURE

Most workers agree that dissociation is the change of colony form from smooth to rough or rough to smooth, with or without the appearance of intermediate types of colonies, and whether or not other characteristic reactions of the organism are altered. Dissociation does not necessarily imply a change in other than the type of colony, but various characteristics are frequently reported as being correlated with different phases or types (Hadley, 1937). Because of the usual association of virulence and nonvirulence with smooth and rough cultures most of the reports in the literature deal with dissociation in pathogenic bacteria.

Although different types of colonies of lactobacilli had been observed and reported, it was not until 1930 that definite reference to dissociation, as such, was made. At that time Hadley, Bunting, and Delves (1930) reported rough, smooth, and intermediate types of colonies, and observed dissociation from rough to smooth in *Bacillus* (*Lactobacillus*) *acidophilus* of dental origin. Rogers (1934) produced almost a complete change from rough colonies to smooth colonies from a rough culture of *Lactobacillus acidophilus*. Kopeloff (1934) and Raney and Kopeloff (1934) observed that dissociation from rough to smooth was common with *L. acidophilus*. Tracy (1938) was able to obtain rough and smooth types of colonies from a culture of *Lactobacillus plantarum* in which the dissociation was smooth to rough. The rough culture was very stable but the smooth culture remained stable only if transfers in glucose broth were made once or twice a week. Longer aging induced dissociation to the rough state.

Numerous reports in the literature refer to the appearance of various types of colonies but do not specify that the changes in colony form were due to dissociation. Rodella (1901) observed smooth-edged and filamentous forms in lactic acid rods. Mereshkowsky (1905) reported disc-shaped and filamentous colonies in cultures of "acid-loving bacteria in the intestinal canal." Heinemann

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and Hefferan (1909) and White and Avery (1910) observed solid and woolly colonies in cultures of *Bacillus* (*Lactobacillus*) *bulgaricus*.

The majority of reports of colony differences have been concerned either with comparative studies of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* or with cultural characteristics of *L. acidophilus* alone. The smooth or disc type and the rough or fuzzy-edged type have been reported for both species by Kulp and Rettger (1924), Kulp (1929), Kopeloff and Kopeloff (1937), and others, but the appearance of different types of colonies was not referred to as dissociation.

It is apparent that the observation of changes in the colony form of lactobacilli is not infrequent, but not often has dissociation been recognized. Two definite types of colonies for several species of lactobacilli have been studied in this laboratory and are believed to represent dissociation from rough to smooth or the reverse.

METHODS

The following cultures were used: *Lactobacillus bulgaricus*, a culture usually carried in association with a film yeast by the Department of Agricultural Bacteriology of the University of Wisconsin and first obtained from a Swiss cheese maker in Brodhead, Wisconsin, by C. Gere of the United States Department of Agriculture about 1920; *Lactobacillus helveticus*, strain R-14-1, carried by the Department of Agricultural Bacteriology of the University of Wisconsin; *Lactobacillus lactis*, strain 39a, originally isolated by B. J. Davis of the United States Department of Agriculture about 1910; and *Lactobacillus casei*, strain 28, isolated from Swiss cheese by R. G. Benedict in 1941. The first three cultures were further purified by single cell techniques, but because this method was unsuccessful with *L. casei* this culture was subjected to single colony isolations.

Stock cultures were carried in test tubes of reconstituted skim milk, incubated at 37 C for 12 hours, stored in the refrigerator, and transferred regularly at weekly intervals. Cultures for experimental studies were transferred every twelve hours to keep each type of colony stable.

The regular plating medium consisted of the carrot liver agar described by Garey, Foster, and Frazier (1941), with some modifications. United States flake agar (1.2 per cent) was used in place of washed agar, and the liver extract was replaced by an infusion made by steaming one pound of ground beef liver in two liters of water until coagulation of the proteins was complete. This infusion was then filtered and sterilized. The pH of the medium was adjusted to 6.6 before sterilization. Carrot liver broth was prepared in the same manner with the omission of the agar.

Bacterial numbers were determined by direct microscopic counts and plate counts made with carrot liver agar. Acid production was determined by titration of a 10-ml sample with N/10 sodium hydroxide and acidity expressed as lactic acid. The pH was determined by the use of a quinhydrone electrode.

The water baths used for the incubation of cultures were equipped with mechanical stirrers and were thermostatically controlled in such a manner that the temperature remained constant within ± 0.1 C.

EXPERIMENTAL

Dissociation was recognized by the appearance of different types of colonies on carrot liver agar plates. All cultures produced the following distinctive colonies shown in the photographs in figure 1: (1) a fuzzy or rough colony which resembled wisps of cotton; (2) a disc or smooth colony which was elliptical in shape with entire edges, and the following intermediates—(3) an entire-edged, lumpy colony; (4) a lumpy colony with elliptical protrusions at one or both ends; (5) a disc-shaped colony with wispy filaments around the colony; and (6) a lumpy colony with wispy filaments around the colony. The rough and smooth

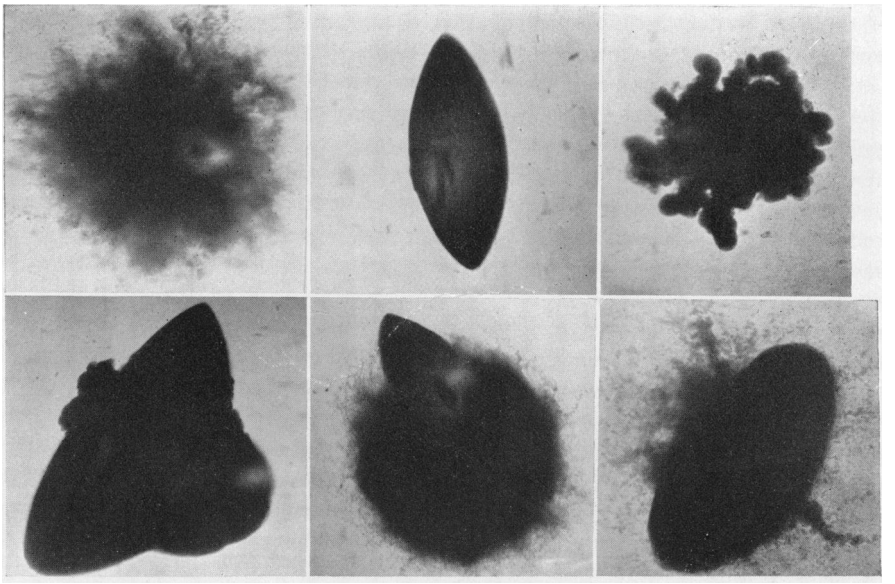

FIG. 1. DISSOCIANTS OF *Lactobacillus bulgaricus*
1. (Upper left) Fuzzy (rough) colony.
2. (Upper center) Disc (smooth) colony.
3. (Upper right) Lumpy (intermediate) colony.
4. (Lower left) Lumpy colony with elliptical protrusions.
5. (Lower center) Disc colony with wispy filaments.
6. (Lower right) Lumpy colony eith wispy filaments.

types of colonies were stable when transferred every 12 hours but the intermediate types always produced rough, smooth, and intermediate colonies. All cultures were comparatively unstable and dissociated if the method of transfer was altered appreciably. With each different lactobacillus, either the rough or the smooth form was the more stable and the culture tended to change toward that form.

The purified cultures gave the following types of colonies immediately after isolation: *L. bulgaricus* and *L. lactis*, lumpy (intermediate) colonies; *L. helveticus*, fuzzy (rough) colonies; *L. casei*, disc (smooth) colonies. After several transfers the cultures from the lumpy colonies gave rough, smooth, and intermediate colonies, whereas the fuzzy cultures gave smooth and intermediate

types only after special treatment, and the disc culture of *L. casei* remained smooth at all times.

Production of Dissociants

Since the time and the temperature of incubation affect the activity of a culture, they might also influence stability and dissociation.

Transfers from a stock culture of *L. bulgaricus* which produced entirely lumpy colonies into tubes of carrot liver broth were incubated at 24.5, 37, and 48 C. Transfers and plates were made after every three days at 24.5 C, every 12 hours at 37 C, and every 24 hours at 48 C until seven such transfer periods had elapsed. The results of three trials, summarized in table 1, indicated that changing the temperature of incubation to either above or below the optimum for growth favored a change from intermediate to rough or smooth or both on repeated

TABLE 1

Effect of incubation temperatures of 24.5, 37, and 48 C on the percentage of dissociants of Lactobacillus bulgaricus in carrot liver broth

NUMBERS OF TRANSFERS	DISSOCIANTS*					
	24.5 C		37 C		48 C	
	Smooth	Rough	Smooth	Rough	Smooth	Rough
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	2.4	0	0	0	35.6	5.6
2	21.2	18.2	0	0	26.6	7.7
3	28.7	35.7	0	0	7.0	39.6
4	51.5	18.2	0	0	6.0	31.3
5	55.6	44.4	0	0	15.3	40.5
6	41.2	0	0	0	18.7	73.7
7	84.5	2.2	0	0	13.5	78.8

* The usual type of colony was intermediate and the dissociants were smooth and rough colonies.

transfer, but at 37 C little change in the type of colony was evident. The highest temperature favored in general an increase in the percentage of rough colonies, whereas the lowest temperature favored an increase in the percentage of smooth colonies.

To try the effect of aging cultures at 37 C for different periods, transfers and plates were made at 12-, 24-, 36-, and 48-hour intervals for at least 7 transfers. The results of three trials, summarized in table 2, showed that as the time interval between transfers was increased beyond 12 hours, the percentages of rough and smooth colonies increased at the expense of the intermediate colonies. The percentage of the latter type remained constant throughout the 14 transfers at 12-hour intervals, but when the time between transfers was increased to 24, 36, and 48 hours, smooth and rough types appeared after the fourth, second, and first transfers, respectively. The percentage of smooth and rough colonies varied to some extent throughout the remainder of the experiment but never increased

sufficently to bring about a complete change to one type of colony. Prolonged aging at 37 C for 72 hours, however, resulted in entirely smooth colonies. Since no change in the type of colony was evident when the time between transfers was 12 hours, it woud seem that, as far as stability of the type of colony was concerned, the best transfer period at 37 C was every 12 hours.

The aging of carrot liver broth cultures at 37 C proved a satisfactory method for obtaining dissociants of *L. bulgaricus* and was used to obtain the smooth, rough, and intermediate dissociants of *L. lactis* and *L. helveticus*. In these studies at no time was it possible to bring about a change from the smooth type of colony of *L. casei* to any other type. However, 8 other strains of *L. casei* were plated out and all of the dissociants observed with *L. bulgaricus* were found to be present.

TABLE 2

Effect of the length of time between transfers on the percentage of dissociants of Lactobacillus bulgaricus in carrot liver broth

(Incubation was at 37 C)

NUMBER OF TRANSFERS	DISSOCIANTS*							
	Incubation period							
	12 hours		24 hours		36 hours		48 hours	
	Smooth	Rough	Smooth	Rough	Smooth	Rough	Smooth	Rough
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	9.8	7.7
3	0	0	0	0	6.9	3.5	9.1	16.5
4	0	0	0	0	7.1	12.1	21.9	12.3
5	0	0	5.4	13.2	18.1	13.3	52.7	2.9
6	0	0	5.8	14.7	19.1	18.4	23.4	3.7
7	0	0	2.4	10.6	28.5	11.6	36.4	3.0

* See footnote to table 1.

Several other procedures to obtain dissociants of cultures of lactobacilli were tried. It was found that such methods as irradiation by X-rays or ultraviolet light, subjecting cultures to various heat treatments, and associative growth with a film yeast could be used to obtain the smooth dissociants from either the intermediate or rough dissociants. In all cases it appeared that any treatment favored the development of the more resistant type of culture and that in the foregoing procedures the smooth type was the most resistant.

Differences Between Dissociants

The morphology of the dissociants in preparations stained with methylene blue showed marked differences between the smooth and rough types. The rough type usually produced long chains of 5 or more cells with increased chaining when grown at temperatures other than the optimum, whereas the smooth type

always occurred in short chains of 2 to 4 cells, even when grown at temperatures unfavorable for the best growth of the culture. Metachromatic granules were evident in all smooth cultures but were seldom observed in the rough cultures. Granulation was more evident in the smooth cultures when the conditions for best growth were unfavorable, such as low or high temperature of incubation or prolonged aging at 37 C, but granules always were evident even in young cells of cultures grown at 37 C.

Minimum and maximum temperatures for growth. The minimum and maximum temperatures for growth were determined for the different dissociants by inoculation of enriched litmus milk (Sherman and Hodge, 1940), and incubation at various temperatures. Duplicate tubes were used and three different trials were conducted. All cultures produced a curd at 30 and 37 C after 12 hours.

TABLE 3

Temperature range for growth of dissociants of Lactobacillus bulgaricus

TEMPERATURE	ROUGH		SMOOTH	
	Change in milk	Time of change	Change in milk	Time of change
°C		*days*		*days*
15	–		C	5
18	–		C	4
21	A	2	C	2
25.5	C	1	C	1
30	C	1	C	1
37	C	1	C	1
45	C	1	C	1
51	C	1	C	1
53	A	7	–	
54.5	–		–	

A = acid.
C = curd.
– = no growth at 15 and 18 C in 60 days or no growth at 53 C or 54.5 C in 7 days.

In all cases the smooth cultures grew at lower temperatures than the rough or original cultures and the rough cultures grew at higher temperatures than the smooth or original cultures.

The results, summarized in table 3, showed that the smooth cultures of *L. bulgaricus* grew at temperatures of 15, 18, and 21 C, producing curd in 5, 4, and 2 days, respectively, whereas the original and rough cultures did not grow below 21 C, and at that temperature only slight acid was evident after 2 days and no curd developed in 60 days. At higher temperatures the smooth and original cultures produced a curd in 1 day at 51 C but did not grow above that temperature, and the rough culture produced acid but no curd at 53 C in 7 days.

Survival after heat treatment. Flasks containing 250 ml of reconstituted skim milk were inoculated with the desired culture using 0.5 per cent of a 12-hour culture. The flasks were then subjected to the desired heat treatment for a

period of 30 minutes. Direct microscopic and plate counts were made immediately before and after the heat treatments, and from these counts the percentage of survival was calculated. In this study the following temperatures were used for heat treatments: 55, 57.5, 60, and 63 C.

The results of numerous trials (table 4) showed that the smooth culture was more resistant to heat treatments than either the rough or original cultures. An increase in the temperature of heat treatment brought about a decrease in the percentage of survival of all cultures, but in every instance the smooth culture was the most resistant.

Longevity at refrigeration temperatures. Peppler and Frazier (1941a, 1941b) have shown that the heat resistance and the later growth and acid production are not changed appreciably after short periods of storage at refrigeration temperature. Kopeloff, Etchells, and Kopeloff (1934) found that the rough strains of *L. acidophilus* lost viability more rapidly at 4 C or 9 C than did the smooth strains. Graham (1943) showed that the associative growth of *L.*

TABLE 4

Effect of heating for 30 minutes at different temperatures on the percentage of survival of dissociants of Lactobacillus bulgaricus

TEMPERATURE OF HEATING	ORIGINAL CULTURES	ROUGH CULTURES	SMOOTH CULTURES
	Survival	Survival	Survival
°C	*per cent*	*per cent*	*per cent*
55	65.3	56.7	94.4
57.5	38.4	51.5	83.2
60	37.2	36.5	72.6
63	32.4	33.1	68.6

bulgaricus with certain film yeasts prolonged the viability of the lactobacillus for many months. With this information in mind a study of the percentage of viability at refrigeration temperatures with and without the addition of a film yeast was undertaken for both smooth and rough cultures.

Smooth and rough cultures were inoculated into duplicate bottles containing 100-ml amounts of skim milk. Half the bottles also were inoculated with a culture of the film yeast *Candida krusei.* All bottles were incubated at 37 C for 24 hours and then stored at 4 C for 5 weeks. Stained preparations and plates were prepared after the incubation period and at weekly intervals throughout the holding period. The percentage of viable cells was calculated for each sample on the basis of the count before storage at 4 C.

The results, summarized in table 5, showed that the cultures without the addition of the film yeast lost viability rapidly and after 5 weeks contained less than 0.0001 per cent viable cells. Associative growth of the lactobacilli and the film yeast resulted in prolonged viability of the lactobacilli. The smooth cultures contained a higher percentage of viable cells after 5 weeks at 4 C than did the rough cultures. The rough cultures produced intermediate colonies

when grown in association with the film yeast and contained about as many viable cells as the smooth cultures through the first 3 weeks of storage. Then there was a rapid decrease in the number of viable cells until at 5 weeks the percentage of viable cells was less than that of the smooth culture.

Proteolytic activity. Frequently, clear zones were observed around the smooth colonies on carrot liver plates. These zones remained clear after the addition of 1 per cent hydrochloric acid, which would indicate that the clearing was not due to acid production by the organisms but to proteolysis.

Eagles and Sadler (1932) stated that the trichloracetic acid soluble nitrogen in cheese was composed entirely of protein decomposition products resulting from the action of enzymes upon the protein during the ripening process. Proteolytic activity was further shown by the determination of nitrogen in flasks of milk plus calcium carbonate inoculated with smooth and rough cultures of *L. bulgaricus* after incubation at 37 C for 6 weeks. Total nitrogen and trichloracetic

TABLE 5

Percentage of viable cells of rough and smooth cultures of Lactobacillus bulgaricus with and without the addition of a film yeast after incubation for 24 hours at 37 C and then storage at 4 C for five weeks

CULTURE	VIABLE CELLS			
	Time of storage			
	1 week	2 weeks	3 weeks	5 weeks
	per cent	*per cent*	*per cent*	*per cent*
Rough	84.3	1.87	<0.008	<0.0001
Smooth	21.0	1.85	<0.008	<0.0001
Plus film yeast				
Rough	65.1	68.1	65.5	13.2
Smooth	58.5	57.2	57.6	38.2

acid soluble nitrogen were determined by the semimicro Kjeldahl method of Gunning, as described in the fifth edition of Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (1940), modified by the use of hot aeration distillation for the collection of NH_3.

The results of the foregoing nitrogen determinations showed that the trichloracetic acid soluble nitrogen content of milk inoculated with smooth cultures was 15.3 mg per g as compared to 9.46 and 3.11 mg per g for the rough culture and an uninoculated control, respectively. These results gave added proof that the smooth culture was more proteolytic than the rough culture.

Rates of growth and acid production. The value of a starter culture in the manufacture of Swiss cheese lies in its ability to withstand the rather harsh heat treatment received during the early steps of manufacture and its ability to initiate growth and acid production as soon as possible after the "dip." The methods developed by Elliker and Frazier (1938) and Voss (1943) have proved satisfactory for the determination of this activity. Therefore measurement of the rates of

growth and acid production of the dissociants and the original cultures was believed to represent a satisfactory method of comparison in a search for important differences between these cultures.

For each series of comparisons 500-ml flasks containing 250 ml of sterile reconstituted skim milk were inoculated with a 1:1 mixture of sodium citrate and the inoculating culture which had been incubated at 37 C for 12 hours. The flasks were then subjected to the desired heat treatment and incubated at the desired temperature. The rates of growth and acid production were followed by sampling at definite intervals and determination of the microscopic count and the pH of the sample.

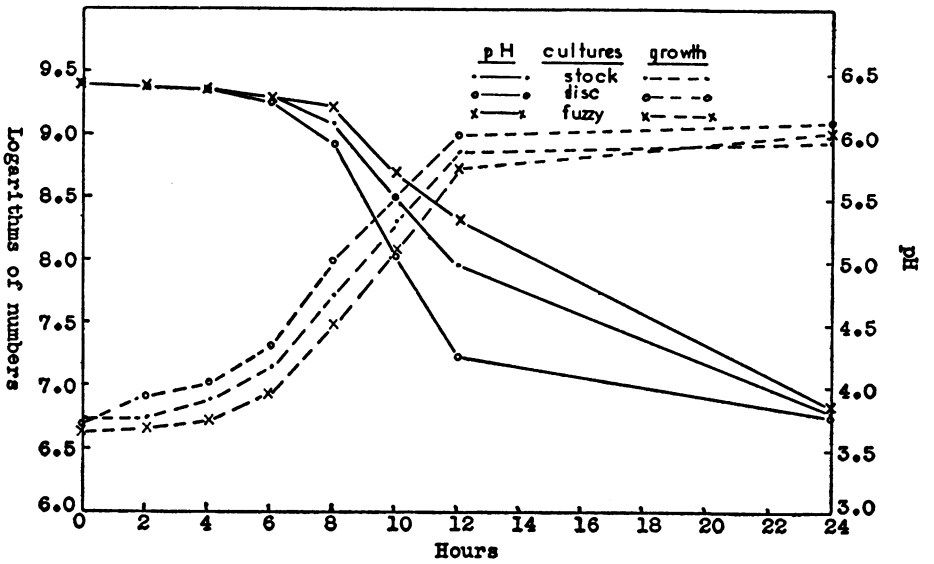

FIG. 2. GROWTH AND ACID PRODUCTION OF DISSOCIANTS OF *Lactobacillus bulgaricus* AT 37 C AFTER HEATING FOR 30 MINUTES AT 63 C

Since, in the process of making Swiss cheese, cultures are subjected to high temperatures while the curd is being heated to 53 C, while it is being held at that temperature, and while the first part of the slow cooling occurs, the differences between the dissociants after heat treatment are of importance. A heat treatment at least as rigorous as that used in the manufacture of Swiss cheese was desired, and therefore a treatment at 63 C for 30 minutes was chosen.

An example of the results obtained in these studies is shown in figure 2 and table 6. The activity of the smooth culture was markedly greater than that of the rough culture when incubated at 37 C after the preliminary heat treatment. The pH began to drop after 6 hours and was appreciably lower than that of the rough culture at 12 hours. At the end of 24 hours, however, the final pH of all cultures was about the same. Growth of the smooth culture as indicated by the increase in numbers was more rapid in the early hours of incubation. Similar results were obtained when incubation temperatures of 47.5 C and a

gradual drop in temperature from 53 C to 37 C over a period of 14 hours were used. These results would seem to indicate that the smooth dissociant might be the desirable culture for use as a starter culture in the manufacture of Swiss cheese.

TABLE 6

Growth and acid production of dissociants of Lactobacillus bulgaricus at 37 C after heating at 63 C for 30 minutes

CULTURE TIME	STOCK		ROUGH		SMOOTH	
	Bacterial numbers	Acidity	Bacterial numbers	Acidity	Bacterial numbers	Acidity
hours	*millions/ml*	*pH*	*millions/ml*	*pH*	*millions/ml*	*pH*
0	5.35	6.40	4.64	6.40	5.02	6.40
2	5.65	6.38	4.73	6.38	7.27	6.38
4	7.70	6.35	5.13	6.35	10.2	6.35
6	14.2	6.29	8.27	6.31	20.6	6.26
8	51.7	6.08	30.6	6.22	98.7	5.91
10	201.0	5.50	129.0	5.69	319.0	5.05
12	731.6	4.96	532.2	5.34	982.8	4.23
24	872.9	3.77	983.0	3.84	1250.0	3.77

	STOCK	ROUGH	SMOOTH
	per cent	*per cent*	*per cent*
Titratable acidity of inoculating cultures	0.96	0.87	0.97
Final titratable acidity of inoculated cultures	1.19	1.06	1.17

DISCUSSION

Dissociation in these studies has been defined as a change in colony form from rough to smooth or smooth to rough, with or without the appearance of intermediate types of colonies and whether or not changes other than colony form were evident. There is little doubt that dissociation takes place in cultures of lactobacilli since a culture which developed from the progeny of a single cell and produced lumpy (intermediate) colonies immediately after isolation later was observed to produce rough, smooth, and intermediate colonies.

The methods utilized in obtaining dissociants of the various lactobacilli were apparently enrichment or selective procedures which favored the development of the more resistant dissociants and the suppression of the less resistant ones. In most cases it would appear that the smooth dissociant was more resistant than the rough or intermediate types and therefore was present in greater numbers. However, growth at a higher temperature than optimum favored the rough type. The smooth dissociant, on the other hand, was more resistant to aging, incubation at low temperatures, various heat treatments, and irradiation by ultraviolet light or X-rays, and these procedures could be used to obtain cultures of the smooth type.

Cheese makers and field men in the Swiss cheese industry have expressed the

opinion that a starter culture showing the organisms in short chains and containing many granules is the most desirable for the manufacture of cheese. This description might be considered to be of the smooth dissociants observed in this study, inasmuch as the smooth culture consistently showed only short chains even at high temperatures, and always showed the presence of granules even in young cells. The physiological differences between the dissociants of these lactobacilli emphasize the similarity between the smooth dissociant and a good starter culture.

The smooth dissociants were able to grow at lower temperatures than the rough dissociants. The work of Voss and Frazier (1945) has shown that growth of the organisms at near their minimum temperature improves the heat resistance of the culture, which consequently may be more desirable for use in the manufacture of Swiss cheese. The results obtained here might explain why this is a superior starter culture in that the smooth type not only grew at a lower temperature but also was more resistant to heat treatments than the other dissociants. Continued serial transfers at low temperatures have been shown to result in increased percentages of the smooth dissociant. It therefore may be that the growth at low temperatures of a culture of an unknown type would result finally in predominance of the smooth dissociant and consequently in a better or more active culture for use in cheese manufacture.

Occasionally it is necessary to store cultures at refrigeration temperatures for short periods of time. Voss (1943) and Peppler and Frazier (1941a, 1941b) have shown that the activity of a culture is not changed by short periods of storage at refrigeration temperatures. This work showed that the smooth type had greater longevity than the rough type when stored at 4 C for 5 weeks. This might be considered important in that the smooth type would not be lost during storage at low temperatures.

The smooth type was more proteolytic than the rough type and might be considered more desirable for cheese manufacture in that this proteolysis, although not great, possibly could aid in the ripening of the cheese.

It already has been noted that the smooth type is more resistant to heat treatments than the other types as shown by a greater percentage of survival. The greater percentage of survival was accompanied by an increase in the rate of growth and acid production over other types. This was observed with incubation temperatures of 37 C or 47.5 C and a gradual drop in temperature from 53 to 37 C similar to that observed in the manufacture of cheese.

Since the smooth type possesses these desirable characteristics for use as a starter culture, it is of interest to note that this type of culture may be obtained by such enrichment treatments as growth at temperature near the minimum, prolonged aging at 37 C, subjection to heat treatments of 63 C for 30 minutes, and to some extent by irradiation by ultraviolet light or X-rays. The rough type, on the other hand, is favored by incubation at temperatures near the maximum, a procedure which should be avoided if the smooth type is desired. Once the smooth type has been obtained, a uniform and stable culture can be maintained by incubation at 37 C and transfer to fresh medium every 12 hours.

Experience has shown that continuous transfer every 12 hours may tend to cause deterioration of the culture, however, and at least once a week a return should be made to the culture grown continuously at 19 C.

SUMMARY

Cultures of *Lactobacillus bulgaricus*, *L. helveticus*, and *L. lactis* were purified by single cell procedures, and a culture of *L. casei* was purified by a colony isolation. These cultures were subjected to various treatments in attempts to induce dissociation. Physiological differences between dissociants were determined.

All cultures produced rough (fuzzy), smooth (disc), and intermediate types of colonies.

The rough and smooth dissociants were stable only when transferred every 12 hours at 37 C; and the intermediate types were stable at no time and gave intermediate, rough, and smooth colonies.

Dissociation was chiefly from rough to smooth, but the reverse could take place.

An incubation temperature below the optimum for growth favored the development of the smooth type, whereas a temperature above the optimum favored the rough type.

Prolonged incubation at 37 C favored the development of rough and smooth types from the intermediate type.

Rough cultures produced long chains with few metachromatic granules, whereas the smooth cultures produced short chains with many metachromatic granules.

The minimum temperature for growth of smooth cultures was 3 to 6 degrees lower than that of the rough cultures, and the maximum temperature for growth of the rough cultures was 2 to 4.5 degrees higher than that of the smooth cultures.

The percentage of survival after heat treatments was greater for the smooth dissociants than for the rough dissociants.

The smooth cultures showed greater longevity than the rough cultures when stored at 4 C, both with and without the addition of a film yeast.

The smooth dissociants were more proteolytic than the rough dissociants.

The smooth dissociants after heat treatment and incubation at 37 C, 47.5 C, and a gradual drop in temperature from 53 to 37 C grew and lowered the pH more rapidly than did the rough dissociants.

These studies would indicate that the smooth dissociant of cultures of lactobacilli might make a more desirable starter culture for use in the manufacture of Swiss cheese than other dissociants or mixed dissociants.

REFERENCES

Assoc. Official Agr. Chem. 1940 Official and tentative methods of analysis of the Association of Official Agricultural Chemists. Washington, D. C.

Eagles, B. A., and Sadler, W. 1932 Cheese-ripening studies. Nitrogen distribution in Kingston cheese-ripening. J. Dairy Research, **3**, 227–240.

Elliker, P. R., and Frazier, W. C. 1938 Factors affecting the activity and heat resistance of Swiss cheese starter cultures. I. Influence of time and temperature of incubation. J. Dairy Sci., **21**, 801–813.

GAREY, J. C., FOSTER, E. M., AND FRAZIER, W. C. 1941 The bacteriology of brick cheese. I. Growth and activity of starter bacteria during manufacture. J. Dairy Sci., **24**, 1015–1025.

GRAHAM, V. E. 1943 The maintenance of viability in *Lactobacillus bulgaricus* cultures by growth in association with certain yeasts. J. Bact., **45**, 51.

HADLEY, F. P., BUNTING, R. W., AND DELVES, E. A. 1930 Recognition of *Bacillus acidophilus* associated with dental caries: a preliminary report. J. Am. Dental Assoc., **17**, 2041–2058.

HADLEY, P. 1937 Further advances in the study of microbic dissociation. J. Infectious Diseases, **60**, 129–192.

HEINEMANN, P. G., AND HEFFERAN, M. 1909 A study of *Bacillus bulgaricus*. J. Infectious Diseases, **6**, 304–318.

KOPELOFF, L. M., ETCHELLS, J. L., AND KOPELOFF, N. 1934 Bacteriological changes in acidophilus milk at room and ice box temperature. J. Bact., **28**, 489–500.

KOPELOFF, L. M., AND KOPELOFF, N. 1937 Optical activity of lactic acid produced by *L. acidophilus* and *L. bulgaricus*. J. Bact., **33**, 331–334.

KOPELOFF, N. 1934 Dissociation and filtration of *Lactobacillus acidophilus*. J. Infectious Diseases, **55**, 368–379.

KULP, W. L. 1929 Preliminary report on a method for the differentiation of *L. acidophilus* and *L. bulgaricus*. J. Bact., **17**, 358–361.

KULP, W. L., AND RETTGER, L. F. 1924 Comparative study of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. J. Bact., **9**, 357–394.

MERESHKOWSKY, S. S. 1905 Zur Frage über die Rolle der Mikroorganismen im Darmkanal. Acidophile Bakterien. Zentr. Bakt. Parasitenk., I, Orig., **39**, 380–389.

PEPPLER, H. J., AND FRAZIER, W. C. 1941a Factors affecting the activity and heat resistance of Swiss cheese starter cultures. III. Effect of variations in time and temperature of incubation and of storage on activity of cultures. J. Dairy Sci., **24**, 603–610.

PEPPLER, H. J., AND FRAZIER, W. C. 1941b Factors affecting the activity and heat resistance of Swiss cheese starter cultures. IV. Effect of variations in time and temperature of incubation and of storage on heat resistance of cultures. J. Dairy Sci., **24**, 611–623.

RANEY, M. E., AND KOPELOFF, N. 1934 Dissociation and filtration studies with *L. acidophilus*. J. Bact., **27**, 45–46.

RODELLA, A. 1901 Ueber die sogenannten säureliebenden Bacillen im Säuglingstuhle. Zentr. Bakt. Parasitenk., I, Orig., **29**, 717–724.

ROGERS, L. A. 1934 The constancy of essential characters of *Lactobacillus acidophilus*. J. Bact., **27**, 41–42.

SHERMAN, J. M., AND HODGE, H. M. 1940 The value of certain tests in the differentiation of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus*. J. Bact., **40**, 11–22.

TRACY, R. L. 1938 Comparative metabolism of the R and S variants of *Lactobacillus plantarum* (Orla-Jensen). J. Bact., **36**, 467–479.

VOSS, J. G. 1943 Influence of growth at low temperature on heat resistance and activity of thermoduric lactic acid bacteria. Ph. D. thesis, Univ. Wisconsin.

VOSS, J. G., AND FRAZIER, W. C. 1945 Influence of incubation at low temperatures on heat resistance of Swiss cheese starter cultures. J. Dairy Sci., **28**, 545–553.

WHITE, B., AND AVERY, O. T. 1910 Observations on certain lactic acid bacteria of the so-called bulgaricus type. Zentr. Bakt. Parasitenk., II, **25**, 161–178.