VARIATION IN MORPHOLOGY OF COLONIES OF LACTOBACILLI[1]

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Attempts have been made by Barber and Frazier (1945) to correlate variation in the morphology of colonies of lactobacilli with changes in the biochemical and physiological properties of cultures arising from rough or smooth cells. These workers reported that cultures from rough colonies of *Lactobacillis bulgaricus* were less desirable Swiss cheese starter cultures than those from smooth colonies. More recently Rogosa and Mitchell (1950) have shown that the type of colony produced by certain strains of lactobacilli could be altered by changing the composition of the agar medium of plating. The results reported herein show how certain substances and certain environmental conditions affect the appearance of colonies from cultures of some species of lactobacilli.

METHODS

Cultures. The complete history of all cultures used is not known, but all have been classified on the basis of carbohydrate fermentations, acidity produced in milk, and maximum and minimum temperature of growth in milk. The following cultures were used: *Lactobacillus bulgaricus*, strain Ga; *Lactobacillus lactis*, strains X-37 and 136; *Lactobacillus helveticus*, strains H-77 and H-80; and *Lactobacillus casei*, strains 9 and ATCC 7469.

Media. The carrot liver medium was prepared as described by Barber and Frazier (1945). Peptonized milk medium contained 10 g Difco peptonized milk in 1,000 ml of distilled water at pH 6.6 to 6.8 before sterilization. The semisynthetic medium was that of Hoff-Jørgensen, Williams, and Snell (1947) and was used undiluted. Basal medium "B" contained 10 g Difco peptonized milk, 10 g glucose, 10 g lactose, 1.0 g sorbitan monooleate ("tween 80"), and 100 ml pancreatin-hydrolyzed casein (Roberts and Snell, 1946), adjusted to pH 6.6 to 6.8, and made up to 900 ml with distilled water. Ten ml of the solution to be tested or 10 ml of water were added to 90 ml of this medium just prior to its inoculation.

To determine the effect of treatments on types of colonies produced an 18- to 20-hour broth culture was diluted, aliquots were distributed in replicate plates, and agar containing the test materials was added. The plates were incubated at 37 C and after 48 hours the subsurface colonies were differentiated and counted.

Stock cultures were transferred monthly in carrot liver agar stabs and were held at 4 C between transfers. Working cultures were maintained by daily transfer of a 1 per cent inoculum into carrot liver broth, followed by incubation at 37 C.

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The colonies produced by cultures of lactobacilli, as illustrated in figure 1 of the paper by Barber and Frazier (1945), were fuzzy, disk, lumpy-disk, fuzzy-disk, and fuzzy-lumpy. In the present work only subsurface colonies were counted and for convenience lumpy, fuzzy-lumpy, and fuzzy-disk colonies were grouped as intermediate colonies. Fuzzy and intermediate colonies were considered to have "rough" tendencies, whereas lumpy-disk and disk colonies were considered to have "smooth" tendencies.

RESULTS

The type of colony from stock cultures of lactobacilli plated in carrot liver agar depended upon the species. Cultures of *L. casei* always produced smooth

TABLE 1

Effect of the pH of carrot liver agar and of atmosphere during incubation on percentages of dissociants from broth cultures of Lactobacillus lactis X-37 (at 37 C)

ATMOSPHERE DURING INCUBATION	INITIAL pH OF AGAR PLATING MEDIUM	DISSOCIANTS*			
		Fuzzy	Intermediate	Lumpy-disk	Disk
		%	%	%	%
Air	6.5	100.0	0.0	0.0	0.0
	5.7	99.5	0.5	0.0	0.0
	5.3	0.0	83.9	15.7	0.4
	4.9	0.0	73.1	26.9	0.0
Air	6.5	95.3	4.7	0.0	0.0
75% CO_2	6.5	1.2	67.1	31.7	0.0
75% N_2	6.5	96.1	3.9	0.0	0.0
Air	5.4	1.5	81.7	16.8	0.0
75% CO_2	5.4	0.0	61.8	29.8	8.4
75% N_2	5.4	0.0	68.4	31.0	0.6

* Fuzzy and intermediate colonies are considered "rough"; lumpy-disk and disk colonies are considered "smooth."

colonies; cultures of *L. bulgaricus* Ga and the two strains of *L. helveticus* always produced fuzzy or intermediate colonies; but stock cultures of *L. lactis* X-37 produced fuzzy, intermediate, and lumpy-disk colonies. Repeated selection of fuzzy colonies from *L. lactis* X-37 resulted in cultures that produced 100 per cent fuzzy (rough) colonies in carrot liver agar, and repeated selection of lumpy-disk colonies resulted in the production of about 50 to 80 per cent lumpy-disk and disk (smooth) colonies on plating. These dissociant cultures were relatively stable and could be maintained by the incubation at 37 C of daily transfers in carrot liver broth. However, over long periods of time, both "rough" and "smooth" cultures reverted so that the intermediate type of colony was produced in carrot liver agar.

Preliminary studies had shown that the morphology of colonies from cultures of *L. lactis* X-37 was affected by the plating medium. Since carrot liver agar

had been used in previous studies on dissociation of lactobacilli (Barber, 1944; Osterhoudt, 1947) and since the dissociative stage of stock cultures in this study was determined by the use of this medium, it was chosen as a reference medium. Although changes in the composition of the plating medium were accompanied by changes in the proportions of colony types, the total viable counts were unchanged; therefore the changes in the percentages of dissociants did not seem to be due to a selection of a few variant cells but probably represented a change in the entire population.

Because preliminary experiments had shown that incubation of plates at 37 C resulted in a slightly greater percentage of smooth colonies than incubation at

TABLE 2

Effect of the addition of acetate and succinate to carrot liver agar on percentages of dissociants from originally "rough" and "smooth" broth cultures of Lactobacillus lactis X-37 (at 37 C)

CARROT LIVER AGAR PLATING MEDIUM	DISSOCIANT CULTURES	DISSOCIANTS			
		Fuzzy	Intermediate	Lumpy-disk	Disk
		%	%	%	%
Nothing added	Originally smooth	98.0	1.0	0.0	0.0
Nothing added (CO_2)*		0.0	41.7	45.4	12.9
Plus acetate†		0.0	42.6	44.4	13.0
Plus acetate (CO_2)*		0.0	20.8	26.7	52.5
Plus succinate‡		0.0	32.6	49.5	17.9
Plus succinate (CO_2)*		0.0	33.6	34.2	32.2
Nothing added	Originally rough	100.0	0.0	0.0	0.0
Nothing added (CO_2)*		0.0	50.7	48.0	1.3
Plus acetate†		No growth			
Plus acetate (CO_2)*		0.0	26.8	37.0	36.2
Plus succinate‡		No growth			
Plus succinate (CO_2)*		0.0	35.6	32.9	31.5

* Plates were incubated under an atmosphere of 75 per cent carbon dioxide.

† Acetate was added to carrot liver agar to make the concentration 0.25 M.

‡ Succinate was added to carrot liver agar to make the concentration 0.1 M.

30 or 45 C, 37 C was employed routinely throughout the following experiments.

Effect of pH of plating medium. Since earlier work had indicated that the pH of the plating medium affected the appearance of colonies, carrot liver agar was adjusted to various acidities with sterile hydrochloric acid just prior to inoculation. The results in table 1 show that as the initial acidity of the agar increased, the percentage of smooth colonies from broth cultures of *L. lactis* X-37 increased considerably.

To determine whether the morphology of colonies was influenced by the pH maintained during their development as well as by the initial pH of the medium, highly buffered carrot liver agar was used as the plating medium. The results in table 2 show that acetate (0.25 M) or succinate (0.1 M) favored a marked increase

in the percentage of smooth colonies from an originally "smooth" culture of *L. lactis* X-37. However, an originally "rough" culture of this organism and cultures of *L. helveticus* H-77 and H-80 failed to grow in acetate or succinate agar under aerobic conditions. Similar results were obtained when propionate or a citrate-phosphate buffer replaced acetate. The pH of the original broth culture did not seem to affect the morphology of colonies in the agar plates.

Effect of carbon dioxide and nitrogen. When cultures of *L. lactis* X-37 were plated in carrot liver agar and incubated under an atmosphere of 75 per cent carbon dioxide (table 1), the percentages of smooth colonies were increased. Similar results were obtained with cultures of *L. helveticus* H-77 and *L. helveticus* H-80, but carbon dioxide failed to alter the percentages of dissociants from cultures of *L. bulgaricus* Ga. Since the percentages of dissociants from *L. lactis* X-37 were similar whether carrot liver agar at pH 6.5 was incubated under an atmosphere of 75 per cent nitrogen or aerobically, anaerobic conditions appeared to have little effect on the morphology of the colonies.

The addition of 2.5 per cent calcium carbonate to carrot liver agar failed to produce results similar to those obtained with carbon dioxide. However, preliminary experiments showed that an increase in the carbon dioxide concentration was accompanied by an increase in the percentage of smooth colonies from cultures of *L. lactis* X-37. The pH of uninoculated agar decreased with a corresponding increase in the carbon dioxide content of the atmosphere; e.g., immediately after agar was removed from an atmosphere of 50 per cent carbon dioxide the pH was 5.5; on removal from an atmosphere of 75 per cent carbon dioxide the pH was 5.3; under aerobic conditions the pH of the agar was 6.5. Therefore, it appeared that the effect of carbon dioxide might have been due to the increased acidity of the agar. However, when the initial pH of the agar was reduced, incubation of cultures of *L. lactis* X-37 under carbon dioxide or nitrogen resulted in a greater percentage of smooth colonies than incubation aerobically (table 1). Therefore it appears that anaerobic conditions favored smooth colonies only when the initial pH of the medium was reduced, and the effect of carbon dioxide might have been due to its dual role in the maintenance of anaerobic conditions and in the reduction of the pH of the medium.

The percentages of dissociants from cultures of *L. lactis* X-37 were not affected by the presence of 10 μg of biotin, 100 μg of pyridoxal hydrochloride, or 0.2 g of aspartate per liter of carrot liver agar; and carbon dioxide was equally effective in the presence or absence of the foregoing substances each of which has been related to the carbon dioxide requirements for some lactobacilli. However, since carrot liver agar is a complete medium, the conclusion that carbon dioxide fixation was not involved in smooth colony formation would not be valid. On the other hand, no evidence was obtained that carbon dioxide fixation was involved in the formation of smooth colonies.

Effect of carbon dioxide, nitrogen, and buffers. When originally smooth cultures of *L. lactis* X-37 were plated in carrot liver acetate agar and incubated under carbon dioxide, the percentage of smooth colonies was increased considerably (table 2). With succinate in place of acetate, carbon dioxide had little effect on

the total percentage of "smooth" colonies but favored an increase in the percentage of disk colonies. The originally rough cultures, which failed to grow in carrot liver acetate (or succinate) agar, grew when carbon dioxide was present (table 2), and the percentage of smooth colonies was markedly greater in the presence of acetate and carbon dioxide than in the presence of carbon dioxide alone. Cultures of *L. helveticus* H-77 and *L. helveticus* H-80 gave results with acetate and carbon dioxide similar to those with rough cultures of *L. lactis* X-37. The pH of the uninoculated medium was found to be the same whether incubated aerobically or under carbon dioxide. Thus the effect of carbon dioxide did not appear to be entirely one of alteration of the pH of buffered media.

To determine whether anaerobic conditions alone were responsible for the growth of the rough cultures in acetate agar and for the increase in the percentage of disk colonies from smoooth cultures, rough and smooth cultures of *L. lactis* X-37 were plated in carrot liver acetate agar and incubated under carbon

TABLE 3

Effect of carbon dioxide and nitrogen on the percentages of dissociants in carrot liver acetate agar from broth cultures of Lactobacillus lactis X-37 (at 37 C)*

DISSOCIANT CULTURE	ATMOSPHERE DURING INCUBATION	DISSOCIANTS			
		Fuzzy	Intermediate	Lumpy-disk	Disk
		%	%	%	%
Smooth	Air	0.9	34.8	36.9	27.4
	75% CO_2	0.0	23.6	32.6	43.8
	75% N_2	1.4	33.7	47.3	17.6
Rough	Air	No growth			
	75% CO_2	0.0	17.2	33.6	49.2
	75% N_2	0.0	28.6	31.2	41.2

* Acetate was added to carrot liver agar to make the final concentration 0.25 M.

dioxide or nitrogen. The results in table 3 show that nitrogen allowed the growth of the originally rough cultures in acetate agar and that the percentages of dissociants were about the same in the presence of nitrogen or carbon dioxide. However, the percentages of dissociants from the originally smooth cultures were about the same in air and under nitrogen, but under carbon dioxide the percentage of smooth colonies increased considerably. Thus it appears that anaerobic conditions were responsible for the growth of the originally rough cultures and for the increase in the percentage of smooth colonies from these cultures in acetate agar, but the presence of carbon dioxide was necessary for the increase in the percentage of smooth colonies from the originally smooth cultures.

Effect of semisynthetic media containing peptonized milk. Earlier work (McDonald, 1948) had shown that cultures of *L. lactis* X-37 formed rough colonies in a semisynthetic agar of Hoff-Jørgensen, Williams, and Snell (1947). It also was noted that the addition of Difco peptonized milk to the semisynthetic agar

favored an increase in the percentage of smooth colonies. Treatment of peptonized milk solutions with activated carbon did not completely nullify the favorable effect of this ingredient. Previous data had shown also that peptonized milk agar supported the growth of *L. lactis* X-37 and that the percentages of dissociants were approximately the same as the percentages in carrot liver agar. These data seemed to indicate that some components of the semisynthetic medium were responsible for the changes in the morphology of the colonies when peptonized milk and semisynthetic media were combined.

Effect of ingredients of semisynthetic medium. In an attempt to determine which of the ingredients of the semisynthetic medium was responsible for the changes

TABLE 4

Effect of the incubation of cysteine agar plates under carbon dioxide on percentages of dissociants from broth cultures of Lactobacillus lactis X-37 (at 37 C)

DISSOCIANT CULTURE	ATMOSPHERE	AGAR PLATING MEDIUM	DISSOCIANTS			
			Fuzzy	Intermediate	Lumpy-disk	Disk
			%	%	%	%
Smooth	Air	Basal "B"*	5.5	87.4	7.1	0.0
		Basal "B" + cysteine (0.007 M)	0.0	50.0	35.4	14.6
	75% CO_2	Basal "B"	0.0	59.4	13.3	27.3
		Basal "B" + cysteine (0.007 M)	0.0	54.2	14.7	31.3
Rough	Air	Basal "B"	75.0	25.0	0.0	0.0
		Basal "B" + cysteine (0.007 M)	2.4	83.3	11.9	2.4
	75% CO_2	Basal "B"	3.6	84.1	10.1	2.2
		Basal "B" + cysteine (0.007 M)	0.0	43.3	29.9	26.8

* Basal "B" agar contained peptonized milk, glucose, and lactose 10 g ea.; pancreatin-hydrolyzed casein, 100 ml; and sorbitan monooleate, 1.0 g per 900 ml of medium. The addition of 100 ml of test solution prior to use gave a final volume of 1 liter.

in the morphology of colonies, each of the components of the semisynthetic medium was added to a base of peptonized milk agar. The addition of glucose, lactose, pancreatin-hydrolyzed casein, or sorbitan monooleate, or the addition of these components together, did not affect the appearance of colonies. However, since growth was more rapid in these media than in plain peptonized milk agar, further experiments were conducted with basal medium "B," which contained peptonized milk, glucose, lactose, pancreatin-hydrolyzed casein, and sorbitan monooleate. The remaining ingredients were tested individually except that the B vitamins, the inorganic salts, and the purines and pyrimidines were tested as mixtures.

The addition of cysteine to the basal medium resulted in a marked increase in the percentage of smooth colonies, as shown in table 4, but the addition of the other ingredients had little or no effect. Cysteine was active whether it was added to the medium and sterilized together with the other components or was added to the sterile medium as a solution sterilized by filtration. The addition of cystine also favored smooth colonies, but the low solubility of cystine prevented its addition as a filtered solution, and it is probable that an equilibrium was established between cysteine and cystine as a result of sterilization in the presence of the other components of the medium. The amount of cysteine added to the medium did not affect the total percentage of smooth colonies (lumpy-disk plus disk), although the percentage of disk colonies was increased considerably as the concentration of cysteine was increased from 0.02 to 2.0 per cent. However, at no time were the percentages of dissociants altered so that all of the colonies could be classified as smooth.

The effect of cysteine on the appearance of colonies might have been due to its reducing action, but when sodium thioglycolate or ascorbic acid was substituted for cysteine, the morphology of colonies of *L. lactis* X-37 was not affected. These results seemed to indicate that the effect of cysteine was not due to its reducing action alone.

When methionine was substituted for cysteine, little effect on the percentages of dissociants was noted until the concentration added was twice that of the cysteine used. Even at this high concentration methionine did not favor so great a change as cysteine, and only the originally smooth cultures of *L. lactis* X-37 were affected; the rough cultures did not respond to methionine.

When cysteine was replaced by alanine or serine, a slight increase in the percentages of smooth colonies was obtained. Serine was more effective than alanine, but neither was so effective as cysteine. The effect of serine may have been due to its conversion to cystine by lactobacilli (cf. Hift and Wallace, 1949). This was partially confirmed in that the addition of filtered serine to a medium that contained homocystine (sterilized with the other components) resulted in an increase in the percentage of smooth colonies comparable to that obtained with cysteine. The addition of either serine or homocystine alone was not effective. The lack of effect of homocystine might have been due to the low concentration used, although the molar concentration was in the same order of magnitude as that of cystine in other experiments.

Effect of cysteine plus carbon dioxide. Since cysteine and carbon dioxide were both shown to affect the percentages of dissociants from cultures of *L. lactis* X-37, the combined effect of these substances was studied. When cultures were plated in the presence of cysteine and incubated under carbon dioxide the results in table 4 were obtained. The effect of cysteine was equal to that of carbon dioxide with both rough and smooth cultures but only with the rough cultures were the effects additive. (Note that the cultures were termed rough and smooth on the basis of the predominant types of colonies in carrot liver agar at the beginning of these studies and that the percentages of dissociants in basal medium "B" are not necessarily the same as those in carrot liver agar.)

Effect of combined addition of cysteine, acetate, and sorbitan monooleate. Preliminary experiments had shown that the addition of cysteine to carrot liver agar did not affect the percentages of dissociants from cultures of *L. lactis* X-37. It has been shown above that the addition of cysteine to a basal medium of peptonized milk, pancreatin-hydrolyzed casein, and sorbitan monooleate favored the development of smooth colonies and that the addition of acetate to carrot liver agar also favored smooth colonies. However, since the procedure used in the preparation of pancreatin-hydrolyzed casein involved the addition of acetate, the possibility was investigated that both acetate and cysteine were responsible for the effect shown by cysteine in basal medium "B." Since sorbitan monooleate has been shown to influence the development of smooth colonies of some lactobacilli (Rogosa and Mitchell, 1950) its effect was studied also.

Agar media with peptonized milk as a base were prepared with acetate, cysteine, and sorbitan monooleate added singly or together, as shown in table 5.

TABLE 5

Effect of the addition of cysteine, acetate, and sorbitan monooleate to agar on percentages of dissociants from a broth culture of Lactobacillus lactis X-37 (at 37 C)

PEPTONIZED MILK AGAR PLATING MEDIUM	DISSOCIANTS			
	Fuzzy	Intermediate	Lumpy-disk	Disk
	%	%	%	%
No additions	1.6	55.7	42.7	0.0
Plus cysteine (0.01 M)	2.1	53.5	43.3	2.1
Plus sorbitan monooleate (0.1%)	0.0	47.0	50.3	2.7
Plus cysteine and sorbitan monooleate	0.0	59.9	40.1	0.0
Plus acetate (0.25 M)	0.0	53.2	35.6	11.2
Plus cysteine and acetate	0.0	40.4	28.3	31.3
Plus acetate and sorbitan monooleate	0.0	50.8	37.7	11.5
Plus cysteine, acetate, and sorbitan monooleate	0.0	50.0	32.7	17.3

When dissociant cultures of *L. lactis* X-37 were grown in these media, cysteine and sorbitan monooleate whether used singly or together exerted little effect in the absence of acetate. Acetate, however, favored smooth colonies when it was used alone; cysteine increased this effect slightly; but sorbitan monooleate did not affect the results. When cultures of *L. lactis* 136 were plated under the foregoing conditions, acetate favored smooth colonies, and cysteine increased this effect slightly; but sorbitan monooleate reduced the effect slightly. When other cultures of lactobacilli were used, different results were obtained. *L. helveticus* H-77 and H-80 failed to grow in the presence of acetate in the concentrations used. Smooth colonies of *L. helveticus* H-77, but not of *L. helveticus* H-80, were favored by cysteine or sorbitan monooleate.

Regardless of the method used (except repeated selection from carrot liver agar) the smooth colonies proved to be unstable and always resulted in broth cultures that gave rough and intermediate colonies in carrot liver agar. At no time was it possible to obtain stable smooth cultures from smooth colonies

arising as a result of alterations of the agar media or of the conditions under which they developed.

DISCUSSION

The appearance of several types of colonies from cultures of lactobacilli has been observed by numerous investigators, and recent evidence has indicated that some of the variations are due to the medium employed rather than to the inherent characteristics of the organisms. The present investigation showed that the composition of the agar media affected the appearance of the colonies and that environmental conditions (anaerobiasis and reaction) seemed to have a greater effect than specific components of the medium.

The morphology of colonies of *L. lactis* X-37 was affected by the hydrogen ion concentration of the agar and was dependent upon the acidity of the medium at the time growth was initiated and during logarithmic growth. When cells initiated growth at an unfavorable pH and when no attempt was made to maintain a favorable pH in the vicinity of the colonies during their development, the rough tendencies of the cells seemed to be favored. Braun (1946) has shown that the pH of broth media influenced the percentage of dissociants from cultures of *Brucella abortus*. However, the pH of broth had no effect on the dissociants of lactobacilli in the present studies. Since a suitable pH for the development of smooth colonies was maintained by any of several buffers, this effect was not similar to that reported by McIlroy, Axelrod, and Mellon (1948), in which case acetate was essential for the maintenance of mucoid, virulent strains of hemolytic streptococci.

Carbon dioxide, since it lowered the pH of agar media, also favored smooth colonies, and part of the effect could be attributed to the buffering action of bicarbonate. However, since the percentage of smooth colonies was greater in a medium of low pH incubated under nitrogen than on incubation in air, it appears that anaerobic conditions also were important for the development of smooth colonies.

Two species of *L. helveticus* that produced rough colonies in carrot liver agar and rough cultures of *L. lactis* X-37 did not grow in highly buffered agar unless anaerobic conditions prevailed. Just why these organisms required a lowered oxygen tension is not known, but a similar effect of oxygen has been reported by Brodie and Shepherd (1950), who demonstrated that rough cultures of intestinal pathogens were unable to initiate growth in certain bile salt agar media unless the oxygen content of the atmosphere was reduced.

The reason for the favorable effect of cysteine is not clear, but since thioglycolate or ascorbate failed to replace cysteine, its activity did not appear to be due to its reducing ability. Cystine replaced cysteine, but under the conditions of the experiments part of the cystine probably was converted to cysteine; homocystine was relatively ineffective, but serine and homocystine together favored smooth colonies. The evidence seemed to indicate that cysteine or cystine was specific, but the mechanism of action is not known. However, it is apparent that cysteine was more effective in buffered medium than in a poorly buffered one.

Sorbitan monooleate was found to favor smooth colonies of only some species of lactobacilli and to be unfavorable for smooth colonies of other species. Rogosa and Mitchell (1950) found that sorbitan monooleate favored smooth colonies of several species of lactobacilli, but it should be noted that colonies called smooth by these authors would be termed intermediate and lumpy-disk by us, and that colonies we have called rough may have been classified as smooth by them.

It appears, therefore, that there were two types of smooth variant colonies produced by some strains of *L. lactis* and *L. helveticus*; one type was relatively stable but the other type was unstable. The stable type appeared spontaneously in carrot liver agar and successive selection of these stable smooth colonies yielded cultures that produced a high percentage of smooth colonies and that could be maintained in the smooth state for fairly long periods of time. The unstable variants appeared only under certain environmental conditions or in the presence of certain compounds in the agar medium and reverted to the parent type when the specific conditions required for their establishment were removed. Thus it appears that the use of colony type as the sole criterion for the determination of the dissociative stage of a culture could prove very misleading if it were assumed that colony type was accompanied by changed physiological and biochemical properties.

SUMMARY

The morphology of colonies from strains of *Lactobacillus lactis* and *Lactobacillus helveticus*, but not of *Lactobacillus casei* and *Lactobacillus bulgaricus*, has been shown to depend upon the composition of agar media and upon environmental conditions during growth.

Smooth colonies from susceptible strains were favored in agar of low initial pH and in agar highly buffered near pH 5.0. Smooth colonies were favored also by anaerobic conditions, especially if the pH of the agar was simultaneously reduced. Cysteine or cystine favored smooth colonies but sorbitan monooleate was of doubtful value.

The total number of colonies from cultures was unaffected by any of the treatments, and it appears that the smooth colonies did not arise at the expense of rough colonies through a selective action. The smooth colonies that arose under special conditions were unstable and could not be kept smooth when they were returned to a reference medium of carrot liver agar.

REFERENCES

BARBER, F. W. 1944 Dissociants of lactobacilli. Ph.D. thesis, University of Wisconsin.

BARBER, F. W., AND FRAZIER, W. C. 1945 Dissociants of lactobacilli. J. Bact., **50**, 637–649.

BRAUN, W. 1946 Dissociation in *Brucella abortus:* a demonstration of the role of inherent and environmental factors in bacterial variation. J. Bact., **51**, 327–349.

BRODIE, J., AND SHEPHERD, W. 1950 The effect of the gas-phase on differential inhibition of intestinal bacilli. J. Gen. Microbiol., **4**, 102–105.

HIFT, H., AND WALLACE, G. I. 1949 A study on the synthesis of cystine by some lactic acid bacteria. J. Biol. Chem., **177**, 927–931.

HOFF-JØRGENSEN, E., WILLIAMS, W. L., AND SNELL, E. E. 1947 Preferential utilization of lactose by a strain of *Lactobacillus bulgaricus*. J. Biol. Chem., **168**, 773–774.

MCDONALD, I. J. 1948 Effect of environment on dissociation of *Lactobacillus bulgaricus* M.S. thesis, University of Wisconsin.

MCILROY, A. P., AXELROD, A. E., AND MELLON, R. R. 1948 The influence of sodium acetate upon the dissociation of a strain of hemolytic streptococcus. J. Bact., **56**, 547–553.

OSTERHOUDT, J. 1947 Effect of variation in substrate on the dissociants of *Lactobacillus bulgaricus*. M.S. thesis, University of Wisconsin.

ROBERTS, E. C., AND SNELL, E. E. 1946 An improved medium for microbiological assays with *Lactobacillus casei*. J. Biol. Chem., **163**, 499–501.

ROGOSA, M., AND MITCHELL, J. A. 1950 Induced colonial variations of a total population among certain lactobacilli. J. Bact., **59**, 303–308.