THE INHIBITING EFFECT OF STREPTOCOCCUS LACTIS ON LACTOBACILLUS BULGARICUS

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The fact that certain species of bacteria have an inhibiting effect on the development of other species or varieties is too well known to need discussion. This effect is frequently observed on agar plates on which the development of the colonies of one species may be restricted or completely suppressed by a preponderance of colonies of another variety. Marmorek (1902) has shown that a broth in which a hemolytic streptococcus has grown is unfavorable to the growth of this same organism or to that of any other hemolytic streptococcus. McLeod and Govenlock (1921) have demonstrated that pneumococci inhibit such bacteria as coli, dysentery, and staphylococci, as well as other pneumococci, and that this inhibiting property may be destroyed by heating the cultures to 85°C. Gundel (1927) found that coli not only inhibited the growth of anthrax but under certain conditions completely destroyed it. He also found that this effect could be prevented by shaking the culture with such adsorbing materials as kieselguhr, charcoal, etc.

It is usually assumed that the lactic streptococci and bulgaricus, which differ in their action on milk in degree rather than in kind, grow together satisfactorily and even have a mutually favorable action. However, if a bulgaricus culture is used to increase the acidity of ordinary buttermilk the action is slow and may even fail entirely. If milk is inoculated with equal quantities of lactic[1] and bulgaricus cultures or only slightly predominating

[1] For the sake of brevity *Streptococcus lactis* cultures are referred to as lactic.

quantities of lactic a typical bulgaricus fermentation will ensue. If, however, the lactic inoculation greatly predominates, the bulgaricus culture will be suppressed and the fermentation will be of the streptococcus type. These facts are shown in table 1.

This inhibiting property is apparently common to lactic streptococci although some of the cultures used have been more effective than others in retarding the growth of bulgaricus.

TABLE 1

The influence of varying inoculation on the relative growth of Streptococcus lactis and Lactobacillus bulgaricus in milk

INOCULATION LACTIC:BULGARICUS	CELLS ON MICROSCOPIC FIELD LACTIC:BULGARICUS	LACTIC ACID AT 24 HOURS
		per cent
1:1	1:3.32	1.46
2:1	1:0.512	1.23
4:1	1:0.074	0.90
6:1	1:0.051	0.87
8:1	1:0.002	0.91
10:1	1:0.025	0.89

TABLE 2

The retarded development of bulgaricus in a lactic culture

AGE OF CULTURE	ST. LACTIS	L. BULGARICUS
days	*cells per cc.**	*cells per cc.†*
2	677,000,000	Under 3,000,000
3	468,000,000	Under 3,000,000
5	4,850,000	Under 3,000,000
6	Under 10,000	43,906,000
8		128,690,000

* Plate count.

† Microscopic count.

These results seem to be at variance with the usual method of isolating bulgaricus from milk. High acid cultures are obtained by allowing milk to stand in a warm place after curdling occurs. Under these conditions cultures of the bulgaricus type usually develop after the lactic fermentation has taken place.

Table 2 shows the results of an experiment in which sterile milk was inoculated with a lactic culture and after twenty-four

hours' incubation at 30°C. was inoculated with bulgaricus and held at 37°C.

The restraining effect of the streptococci diminished as the cells died or the bulgaricus gradually overcame the unfavorable conditions.

The inhibiting effect of the streptococci is not due to the acidity of the medium. Bulgaricus will develop normally in milk acidified to a reaction as low as pH 5.0 or 4.8.

TABLE 3

Inhibiting action in milk cultures held at pH 6.0

AGE OF CULTURE	LACTIC AND BULGARICUS—BULGARICUS CELLS PER FIELD	BULGARICUS ALONE—BULGARICUS CELLS PER FIELD
hours		
3	0.05	3.90
4	0.15	10.25
5	0.15	19.00
6	0.35	60.25
8	2.55	90.70
9	2.15	169.40
10	2.40	Too many to count

TABLE 4

The removal of the inhibiting action from a lactic culture neutralized to pH 6.8

TREATMENT	CLOUDING	CELL
Heated, inoculated with bulgaricus		Lactic
Filtered, not inoculated		
Filtered, inoculated with bulgaricus	++	Bulgaricus
Filtered and heated, inoculated with bulgaricus	++	Bulgaricus

In table 3 is shown the development of bulgaricus in milk cultures in which the reaction was held at pH 6.0 by frequent neutralization of the acid developed.

The lactic culture was allowed to develop and was then inoculated with bulgaricus. A check flask was inoculated with bulgaricus alone and both were held at 37°C. with frequent neutralization to maintain a reaction of approximately pH 6.0.

The development of the bulgaricus in combination with the lactic was almost nil whereas the multiplication in the check flask

was rapid. The inhibition in this case was entirely independent of the acid.

Heating to the boiling point does not destroy the inhibiting property, but it is at least partially removed by filtering through an earthenware or plaster of Paris filter. The results of an experiment which illustrates this point are shown in table 4.

The medium used was digested casein broth in which bulgaricus grew rapidly. After the lactic culture had grown for two days the reaction was corrected to pH 6.8; one fraction was heated fifteen minutes in an Arnold sterilizer and another was filtered through a Chamberland filter. Part of the latter fraction was also heated. Bulgaricus failed to grow in the unfiltered heated portion but grew luxuriantly in both the heated and unheated filtered fractions.

Some of these experiments suggest the possibility that the inhibiting property of the streptococci is inherent in the cells or is carried with them. A lactic culture in glucose broth was centrifuged until a mass of cells was obtained. This was washed with sterile water and recentrifuged. The water was decanted off and enough of the cells transferred to yeast extract broth to give a heavy suspension.

After heating a few minutes in the Arnold sterilizer, this broth suspension of killed lactic cells was inoculated with bulgaricus and incubated at 37°C. Bulgaricus grew readily, which indicates that the dead cells at least do not have an inhibiting effect. In the centrifuging and washing the inhibiting principle must have been removed with the supernatant broth and wash water and therefore it must have been a soluble and possibly a diffusible substance. The correctness of this assumption was demonstrated by the preparation of a collodion sac which was partly filled with yeast extract broth and suspended in a flask of the same medium. The sac was inoculated with the lactic culture and incubated for forty-eight hours. The medium surrounding the sac was then inoculated with bulgaricus. A check flask was inoculated at the same time and after twenty-four hours the number of bulgaricus cells in each flask was determined by the plate method.

The check flask contained 640,000,000 per cubic centimeter

while the flask exposed to the action of any substance which might diffuse from the lactic culture contained only 82,000,000 per cubic centimeter.

It is very evident from this experiment, which has been repeated a number of times with similar results, that a specific substance was produced in the lactic culture which diffused through the collodion membrane in sufficient quantity to inhibit materially the growth of bulgaricus.

It is shown in another paper that this substance also has an inhibiting effect on the lactic culture itself and is probably an important factor in limiting the population of the culture.

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