A CONTRIBUTION TO THE BACTERIOLOGY OF SILAGE[1]

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The fermentation which ensilage undergoes during its curing process was looked upon a few decades ago as being entirely of microbic origin, and we find, in the older literature on the subject, frequent reference to the alcoholic, acetic acid and lactic acid ferments which were supposed to exist in the ensilage. The evidence upon which such statements were based was, as far as can be ascertained, merely the occurrence in silage of the products characteristic of the action of such organisms. At the present time, due chiefly to the work of Babcock and Russell (1900, 1901), opinion has swung in the opposite direction to such an extent that microörganisms are now generally considered of little if any significance in the normal fermentation of silage.

That most microbiologists in this country do not consider bacteria of significance in the formation of silage is indicated by a review of the recent textbooks on the subjects of general and agricultural bacteriology. Marshall (1911) and Jordan (1914) make no mention of silage, although other related fermented products are discussed. Russell and Hastings (1915) state that the fermentation is believed to be due to the respiration of the living plant cells. Lipman (1911) outlines the respiration theory of Babcock and Russell and states that silage may be made under conditions which exclude bacterial action.

[1] This paper, originally entitled "The Occurrence of Aciduric Bacilli in Corn Silage," was prepared for publication when a paper appeared by Hunter and Bushnell bearing a somewhat similar title. This article, slightly changed so as to contain reference to their work, is published only to confirm the observations of Hunter and Bushnell.

He further states that the question as to whether bacteria have any beneficial function can not be answered at the present time. Conn (1901) gives the old ideas concerning the supposed rôles of microörganisms, and then reviews the work of Babcock and Russell (1900, 1901). His views on the subject may be well exemplified by the following quotation:

> From all these facts it becomes clear that while this method of preparing food is due to a fermentation, it can not be attributed to the growth of microörganisms. It certainly involves other factors, and it is uncertain whether bacteria, or other microörganisms, have anything to do with the process as normally carried out.

Since the work of Babcock and Russell fifteen years ago, little has been published on the processes involved in the curing of ensilage. E. J. Russell's work (1908) was in substantial agreement with that of Babcock and Russell, though he thought it possible that bacteria play a minor part. Esten and Mason (1912) considered the process entirely bacteriological. Three chief fermentations were thought to take place: the lactic acid, alcoholic and acetic acid fermentation. The lactic acid fermentation was thought to be due to organisms similar to those concerned in the souring of milk. It was also believed by these workers that yeasts cause an alcoholic fermentation and that acetic acid bacteria then oxidize the alcohol so formed to acetic acid. Samarani (1913) concludes that the acetic acid fermentation in silage is due to the respiration of the plant cells, while the lactic acid fermentation is due to bacterial action. The organisms responsible for the latter process were identified by Samarani as a bacillus and a coccus which occurred in about equal proportions. The former he designated as the *B. acidilactici* of Hueppe, and the latter was considered identical with the common streptococcus of milk.

The rôle of acid producing bacteria of the Bulgarian type in such fermented products as sauerkraut, milk beverages, cheese, etc., is well known. That they should function in silage would appear probable, but until very recently no data were at hand which gave any evidence for such a belief. Although

suggestions that these organisms may be of importance in the ensiling of foods are found in the literature (Heinze, 1913), such suppositions have not been based upon established facts. The lactic acid bacteria mentioned by Esten (1910) as important in silage were inhibited by the presence of only 0.45 per cent of lactic acid when grown in corn juice. Gorini (1906) made a detailed study of the bacteria of silage and mentioned *Streptococcus lactis-acidi* and *B. lactis-acidi* as the most important of the acid-forming organisms. None of the organisms which he described had the property of forming a high degree of acidity. Löhnis (1907) in his classification of the lactic acid bacteria described a number of strains of the aciduric bacteria but made no mention of a group derived from silage. Stevenson (1911) studied the distribution of the high acid bacteria but did not report silage as one of the sources from which they were obtained. Heinemann and Hefferan (1909) noted silage as one of the substances from which they had isolated cultures of *B. bulgaricus*. The recent paper by Hunter and Bushnell (1916) however is the first report, so far as the writer is aware, of the constant occurrence and probable importance of organisms of the *B. bulgaricus* group in silage.

OBSERVATIONS

The notes recorded in this paper are those which have been made on ensilage, more or less incidentally, during the past year. It was first noted on April 26, 1915, that sterilized milk inoculated with silage developed a high acidity. After incubation for ten days at 37°C. the milk was found to contain an acidity of 2.3 per cent calculated as lactic acid. This observation indicated the presence of organisms belonging to the group of aciduric bacilli, and these bacteria were isolated from the milk culture by plating on ordinary lactose agar. Their occurrence has been repeatedly verified in samples of corn ensilage from four different silos taken at various stages during the feeding season.

That the high acid-producing organisms not only exist in

silage but that they occur in large numbers is shown by the fact that sterile milk usually develops a high acidity when inoculated with dilutions of silage representing only 1/1,000,000 of a gram of the moist material, or when inoculated with 1/1,000,000 of a cubic centimeter of the juice expressed therefrom. The occurrence of the high-acid organisms in such large numbers has been observed in silage over nine months old.

The aciduric bacilli may also be readily isolated by the direct plating of the silage on lactose agar on which this type can be grown. In fact these organisms constitute a majority of the bacteria found in ensilage during the latter part of the curing process. Unfortunately, it was not possible to make a study of the silage during the first two months when the most important bacterial processes undoubtedly take place. Plate counts made on lactose agar of the juice expressed from silage between three and six months of age have given counts ranging from a few thousand to over 1,000,000 bacteria per cubic centimeter. As is well known, most types of the aciduric bacilli do not grow well on agar plates, and it would seem very probable that the actual number of such organisms is considerably greater than is indicated by the plate count. That this supposition is correct is shown by the observation that these organisms may be present in numbers approximating 1,000,000 per cubic centermeter of juice,as revealed by the dilution method, when the plate count shows only 15,000.

Counts made on silage juice by the direct microscopic method of enumeration have shown the presence of from 1,500,000,000 to 4,800,000,000 bacterial cells per cubic centimeter, most of which were slender rods. Many of the organisms observed under the microscope were, in all probability, dead, since autolysis would undoubtedly be greatly retarded in such an acid medium. However, these observations indicate that immense bacterial activity had taken place.

The morphological and cultural characteristics of the acid producing bacilli which have been isolated agree with those reported by Hunter and Bushnell (1916). The rods were of variable size, but the growth characters of the cultures thus

far collected are very similar in so far as the cultural observations have been made. The colonies on agar appear exactly like those of the *B. lactis-acidi* group and, in the presence of a fermentable carbohydrate, they are surrounded by the characteristic haze. The development of colonies is not so rapid as with organisms of the *B. lactis-acidi* type, but on prolonged incubation they usually develop to a greater size. The readiness with which this group of bacteria grows on ordinary laboratory media differentiates it quite sharply from the typical *B. bulgaricus* of milk.

Not only do these organisms cause a high acid fermentation in milk but they have a similar action in corn juice in which they grow very rapidly. In the table given below are the data

TABLE I

Acidity produced by silage organisms in milk and in corn juice

CULTURE NUMBER	PER CENT ACID AS LACTIC ACID	
	Milk	Corn Juice
1	1.36	1.36
2	2.25	1.67
3	1.38	1.21
4	1.53	1.67
5	2.34	1.35
6	1.36	1.53
7	1.51	1.69
8	2.28	1.25
9	1.39	1.44
10	1.44	1.69
11	2.25	1.24
12	1.34	1.55
13	1.39	1.64
14	1.53	1.51

obtained with fourteen cultures grown in milk and in corn juice. The corn juice used was obtained from green plants at about the tasselling stage. The juice was expressed from the stalks by pressure, heated for a few minutes in the autoclave, filtered through filter paper, tubed and sterilized. The cultures were incubated twelve days at 37°C.

The observations reported in this paper would appear to indicate that acid producing bacteria might play a part in the fermentation of silage. How much of a factor they are in ordinary silage can not be answered from the meager data which have been collected. From the evidence presented by various workers, there can hardly be any question but that cell respiration is of vital importance in the fermentation of normal silage, but that this may be supplemented greatly by the action of bacteria certainly appears reasonable. It would seem that microörganisms might be responsible for the fermentation which takes place in silage made from shocked corn. The ensiling of shocked corn and corn stover, a practice which has been in vogue to a limited extent in some localities for years, in which we would expect the plant cells to be inactive, must be largely dependent, it would seem, upon the action of bacteria.

A laboratory test on this point was made by ensiling some corn stover with double the amount, by weight, of water in a glass jar. The stover used had been shredded and baled and was about fifteen months old. After one month at laboratory temperature the jar was opened and the ensilage examined. The material had a clean acid odor quite typical of ordinary silage, but on comparison of the two it was found to lack a certain richness in aroma so characteristic of silage put up in the usual way The juice expressed from the stover silage had an acidity of 1.35 per cent, calculated as lactic acid, and a bacterial count on lactose agar of 1,700,000,000 organisms per cc. of which 600,000,000 were of the high acid producing type. A direct microscopic examination of the juice revealed a count of 11,000,000,000 bacteria per cc.

The subject of the fermentation in stover silage is under further investigation at this station.

The constant occurrence of a group of organisms in silage with characteristics which differentiate its members from other related groups is of interest from a biological as well as from the practical viewpoint. The question naturally arises as to how they gain entrance to the ensilage, or what is their habitat in nature. Observations made in this connection indi-

cate an intimate relationship between the corn plant and this group of aciduric bacilli. Juice expressed from corn cut a few weeks prior to the time it was ready for the silo underwent an acid fermentation, when kept in the laboratory, with the production of a high degree of acidity and an aroma resembling that of good silage. From this were then isolated cultures of organisms similar to those obtained from silage. Sterile milk inoculated with bits of corn stover, either from the leaves or pith, always develops large amounts of acid and from it the typical organisms may be obtained. On a sample of shredded corn stover nearly a year and a half old the acid forming bacilli were found in numbers approximating 1,000 per gram as determined by dilutions in sterile milk.

SUMMARY

The data presented in this paper suggest the probable importance of a group of acid-tolerant, acid-producing bacilli in the curing of corn silage.

The organism concerned, while closely related to the *B. bulgaricus* group of milk and the *B. acidophilus* group of the intestines, appears to differ somewhat from the typical members of these groups, notably by its comparatively abundant growth on ordinary laboratory media.

The microscopic examination of silage juice demonstrates the presence of immense numbers of bacterial cells (always over one billion per cubic centimeter), most of which are bacilli which resemble morphologically the high acid producing bacilli described above.

The aciduric bacilli of silage are constantly found in quite large numbers on corn fodder, so that silage made from corn is always amply seeded with these organisms.

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