BACTERIA CONCERNED IN THE RIPENING OF CORN SILAGE

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The production of silage on the farm may be regarded as a means of conserving valuable food material. During the process of fermentation which takes place in the silo the fresh fodder is so altered as to prevent spoilage and to conserve its food value. Experience as well as experiment has shown that a highly nutritious and palatable food is thus made available during the winter months, when fresh fodder cannot be obtained.

The authors make no claim to have solved all the problems connected with the fermentation of silage. Many details must remain for future investigation and all that can be claimed is progress. Hunter and Bushnell and Sherman lay stress on the activity of lactobacilli in the ripening of silage and the present work confirms the findings of these authors, namely that lactobacilli are important factors, but that they probably reach the maximum of their activity during late stages of fermentation.

Opportunity presented itself to investigate silage from three silos. Two of these are constructed of wood with concrete foundation, while the third is concrete throughout. The investigation, as might be anticipated, shows that although the fermentative process in general is similar in different silos, it is by no means identical. That is to say, after a definite lapse of time the silage of one silo may be fermented in larger measure than that in another silo. Furthermore, the same stage of fermentation does not necessarily obtain throughout the same silo. No relation to the difference in construction could however be detected.

The silage in the three silos was removed from the top as it was required as food for cattle. Therefore the samples obtained for investigation had not only fermented during progressively longer periods of time, but since the surface layers were periodically removed samples had to be procured from increasing depths. It is conceivable, therefore that anaerobic fermentation becomes more pronounced as silage ripens.

METHODS

Samples were taken from the silos in November, January, February, March, April, May and June. A few days elapsed between sampling the different silos in order not to crowd the work but this short interval probably made no material difference in the relative stage of fermentation. Ten grams of each sample were infused with 50 cc. of cold physiologic salt solution. From this infusion stains were prepared and inoculations made in milk with addition of brom-cresol-purple as indicator, into flasks and fermentation tubes containing 2 per cent glucose broth, and on agar. The last medium was used for surface and stab cultures. The infusion of silage was titrated with $N/20$ NaOH with phenolphthalein as indicator and then together with the inoculated media was incubated at 37°C.

RESULTS

Stains from the silage infusion showed enormous numbers of microorganisms. No attempt was made to enumerate them or separate the types, but the predominating types were noted and cultivated. Yeast cells were invariably present and frequently formed a solid film on the surface of the incubated infusion. The acidity of this infusion was never more than 3 per cent normal, but it is probable that the salt solution did not dissolve all the acid contained in the silage. If the juice had been pressed from the silage and then diluted five times its volume with salt solution the acidity would probably have proved higher than the values obtained. After 24 hours incubation of the silage infusion the acidity rose to about three

times the original amount, but after forty-eight hours a marked decrease of acidity was observed coincident with the development of a heavy film consisting of yeast cells.

A bacillus of the colon-aerogenes group was present in large numbers, but only in smears prepared from silage during early stages of fermentation. During late stages the bacillus may still have been present, but if so was numerically insignificant. Streptococci and lactobacilli were always present in early as well as in late samples. The streptococci were readily isolated by plating, but the lactobacilli did not grow readily on ordinary media and were isolated by the method employed by Heineman and Hefferan, namely by repeated transfers through milk and final plating on whey agar.

The streptococci isolated varied somewhat morphologically. In some cases the diplococcus form was predominant, while in others chain formation was observed. However, the diplococcus form was found chiefly in early samples of silage, while chain formation was abundant in later samples. Chains were prominent also in stock cultures prepared from the isolated streptococci, but the short form reappeared when fresh subcultures were prepared. The fact has been repeatedly recorded, that during the most active period of growth, streptococci, especially saprophytic ones, appear chiefly in diplococcus form, while after the maximum growth has passed chain formation becomes more evident. The size of individual cells of streptococci confirms the assumption that they are more active in early silage than in the ripened product. From early samples the cells were uniformly small and in later samples they were large and frequently appeared swollen. These abnormal forms disappeared when young generations grew on inoculated media.

The amount of acid formed in glucose broth by the streptococci was determined by titration with $N/20$ NaOH. The quantity thus determined never exceeded 5 per cent normal acid in five days. Milk was readily coagulated by most strains of streptococci isolated, in some cases within twenty-four hours.

The lactobacilli observed occurred singly, showed granular staining with methylene-blue in many instances and were rather difficult to isolate. They grew slowly on media containing 2

per cent glucose and the colonies formed on whey agar were always very small. However, from the latest samples a streptobacillus was isolated which grew with relative abundance on glucose media and produced up to 11.5 per cent acid in glucose broth in six to seven days. This bacillus appeared in long filaments composed of rather short bacilli. The acid produced consisted chiefly of lactic acid. It should be emphasized that this strepto-bacillus was never observed in early samples, although it probably was present in small numbers.

Milk inoculated with two loopfuls of silage infusion coagulated promptly, usually within twenty-four hours and when inoculated with the infusion of early samples considerable amounts of gas were produced. In some instances the gas formation was so vigorous as to break up the coagulum. As ripening of the silage progressed the amount of gas formed in milk decreased and finally in the last stages of fermentation no gas appeared. Stains from the coagulated milk, prepared after twenty-four hours incubation, showed that in early stages organisms of the colon-aerogenes group were present in abundance, accompanied by large numbers of streptococci, but as the ripening process proceeded streptococci became more prominent and finally were present practically in pure culture. The milk cultures were not incubated for a sufficient length of time to permit lactobacilli to supersede streptococci, excepting when lactobacilli were searched for.

In a recent paper Gorini calls attention to the presence of butyric acid bacilli in silage and warns of the possibility of their influence on the product. Since anaerobic conditions obtain, at least in deep layers of silage, and since the temperature may be relatively high, conditions for the growth of butyric acid bacilli are not unfavorable. Gorini suggests that the temperature be not permitted to go beyond 50°C. nor below 30°C. in order to favor the growth of lactic ferments (lactobacilli and streptococci) and thus antagonize butyric acid bacilli. In our limited investigation no evidence of the presence of butyric acid bacilli was observed, although they may have been present in small numbers. Several anaerobic cultures prepared from the silage infusion invariably gave a growth of streptococci, practically in pure culture.

DISCUSSION

As stated before the investigation reported in this paper does not warrant very definite conclusions and the following suggestions are offered tentatively. It is a well known fact that the groups of colon-aerogenes bacilli, of streptococci and of lactobacilli are widely distributed in nature and especially on fodder. Lacto-bacilli were found by Heineman and Hefferan in cornmeal and by Sherman on fresh corn leaves. The presence of these groups in silage may therefore be considered as established. This condition recalls observations on the so-called normal souring of milk and the activity of streptococci and lactobacilli during cheese ripening. Assuming that the colon-aerogenes group is present in fresh silage a limited growth of these organisms would produce an initial fermentation with acid and gas as the chief products. This assumption is supported by the fact that considerable pressure is developed during the ripening of silage and by our observation that gas is formed in abundance in milk cultures prepared from samples of silage in early stages of ripening. The colon-aerogenes group is soon suppressed by the streptococci. Acid is the chief product of this group. Milk cultures showed a progressive decrease of gas formation a fact which indicates the gradual suppression of the colon-aerogenes group. Streptococci are then crowded out by lactobacilli, but probably never disappear entirely as they could be isolated readily from silage in its last stages of fermentation. It is suggested that at least two groups of lactobacilli are active. The first group is of the slow-growing type, the bacilli occurring singly and showing granular staining with methylene-blue. The second group of lactobacilli is of the more rapid-growing order and is readily isolated from later stages of fermenting silage.

Wyant inoculated corn silage experimentally with cultures of *Streptococcus lacticus* and *Lactobacillus bulgaricus* and obtained a product which according to the author's description resembled normal silage. However, after a period of four to five weeks lactobacilli were not isolated, while streptococci were recovered without difficulty. This shows that the silage had not passed

beyond the second phase of fermentation. According to the investigation reported in this paper the third phase of fermentation, when lactobacilli are active, does not occur during the first weeks of the ripening process.

The investigation came to a conclusion because the silage was practically exhausted in the silos whose product was available. The remaining silage contained considerable quantity of acid and the owners of the silos hesitated to use this remnant for fodder. It does not seem, however, that the acidity was great enough to cause injury to the cattle. Fair samples could not be obtained from these remnants.

It has been stated that stains from early samples of silage showed the presence of large numbers of microorganisms of various types. During the progress of the work the number of types was gradually reduced and the microscopic picture of stains from the last stages of ripening differed materially from that obtained from early stages, inasmuch as streptococci and lactobacilli were clearly predominant and other forms had disappeared in very large measure. No doubt the result is explained by the accumulation of acid in the final product. The disappearing bacteria may influence the ripening process by consuming oxygen and thus create favorable conditions for facultative anaerobes, such as streptococci and lactobacilli.

CONCLUSION

The authors as a result of this investigation offer as a tentative hypothesis of the ripening process of corn silage the following: The fermentation consists of three phases brought about by three groups of bacteria. The initial phase is of short duration and is caused by members of the colon-aerogenes group of bacteria. It is accompanied by acid and gas formation. The second phase is carried on by streptococci and is accompanied by moderate acid formation. The third phase is the result of the activity of lactobacilli. The third phase may possibly be subdivided into minor phases owing to the presence of different strains of lactobacilli. It should be added that the assumption

of some investigators that the first process of silage ripening is a respiratory process (Babcock and Russell) is not excluded by the hypothesis advanced and does not interfere with it. Furthermore, mention should be made of the fact that at no time was growth of yeasts in the silage observed, although yeast cells were invariably present. Growth of yeast was observed only when an extract of silage was incubated at 37°C. There is therefore in this investigation no indication of an alcoholic or acetic acid fermentation as suggested by Esten and Mason.

It should be emphasized that the assumption of different phases of silage fermentation does not mean that the phases are distinctly separated, or that a period of rest occurs when one phase passes into the next one, or finally that the same phase is operative throughout the whole quantity of silage. The authors are inclined to the opinion that conditions of ripening are not the same in different parts of the silage and that unless samples are taken from definite regions, if this were possible, the progress of ripening would appear somewhat obscured. It is suggested further that the process of ripening may differ in kind and in degree in different silos, owing to variations in construction, in moisture content, and to climatic conditions. However, the fundamental principles and the groups of organisms active in the fermentation of silage are similar under a variety of conditions.

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