A SUGAR-TOLERANT MEMBER OF THE COLON-AEROGENES GROUP

LAWRENCE H. JAMES

Food Research Division, United States Department of Agriculture, Washington, D. C.

Received for publication July 26, 1929

Investigations of the decomposition of raw sugars have demonstrated the ability of microörganisms of various types to grow in concentrated sugar solutions. Owen (1911) and other workers showed that certain members of the aerobic spore-forming group, capable of growth in high sugar concentrations, were common in sugar refineries and were responsible for the decomposition of raw sugars and sugar products. While studying the deterioration of cane sugar in Louisiana, Kopeloff and Kopeloff (1919) isolated highly sugar-tolerant[1] molds which were responsible for certain types of sugar deterioration in storage.

In 1918 Browne isolated strains of torula, monilia, and a rod-shaped bacterium from raw sugars. He found these microorganisms were the most common forms present in Cuban raw sugars. Other organisms were found also, but were not studied. He concluded that no one type of microörganism alone was responsible for the deterioration of commercial raw sugars, but that bacteria, molds, and budding fungi should be suspected. He concluded further that these microörganisms were unable to grow in saturated solutions and suggested the remedy which sugar producers have since found effective in preventing deterioration losses.

[1] In describing the characteristics of microörganisms, careful distinction should be made between the words "resistant" and "tolerant." The former should be used in considering the ability of an organism to combat conditions which are tending to destroy it; while "tolerant" should be used to describe the ability of an organism not only to resist outside injurious influences, but also to be capable of growth or metabolism under the conditions in question.

Shutt (1925) in studying the bursting of chocolate-coated creams found that yeast present in a candy factory grew and produced gas in commercial fondant, the filling for chocolate-coated creams.

While studying in the Department of Agriculture the relation of sugar-tolerant organisms to certain industrial practices, Church, Paine and Hamilton (1927) isolated sugar-tolerant yeasts and demonstrated their relation to the fermentation of fondant. They concluded that these microörganisms were responsible for the bursting of chocolate-coated cream candies.

From these and many other studies, it is evident that strains of organisms capable of growth in sugar solutions of rather high density may be encountered among the bacteria, yeasts, or molds. The further studies of this nature are carried the more we are forced to believe that highly sugar-tolerant organisms, highly salt-tolerant organisms, and highly heat-tolerant organisms are widely distributed in nature. Knowledge of the biochemical phenomena involved in the metabolism of these organisms, however, is woefully lacking.

Following the isolation of spoilage thermophiles from sugar (Cameron, Williams and Thompson, 1927, and James, 1928a), a survey of the microbial flora of commercial sugars was made in this laboratory (James, 1928b). In this survey the results of many of the tests for putrefactive anaerobes were unsatisfactory because of a liberation of gas in the anaerobic media to which the original test sugar solution had been added, but in which putrefactive anaerobes could not subsequently be found. This indicated the presence of organisms capable of growing under anaerobic conditions only in the presence of fairly large concentrations of sucrose. This conclusion was supported by the occasional development of gas bubbles in deep agar tubes containing 10 per cent sucrose, which were run on a few samples to test for sugar-tolerant anaerobes. Some time after the survey had been completed, some of the original anaerobic tubes were removed from cold storage, and further studies of their microbial contents were made.

Time did not permit a study of more than a few of the tubes,

and most of the organisms have died since. In the course of the tests, however, an organism which possessed interesting characteristics was isolated from a deep tube of Kopeloff's agar containing 10 per cent sucrose. It produced abundant gas within twenty-four hours, incubation at 37°C.

Cultural studies of this organism have shown it to be a Gram-negative, non-spore-forming, short, plump rod. It grows best under aerobic conditions, but in the presence of sugar grows well anaerobically. It is actively motile, ferments lactose with acid and gas production, and, as judged by its cultural and morphological characteristics, belongs to the species designated by Weldin (1927) as *Aerobacter cloacae*. We do not consider it a new species of the colon-typhoid group, but only one which through long exposure to adverse conditions has acclimated itself to its surroundings, until now it can grow anaerobically in fairly concentrated sugar solutions. It produces abundant gas in both liquid and solid media containing sucrose in concentrations from 1 to 30° Brix. In a 35° solution its growth is not as luxurious as in the lower concentrations, but it still shows considerable development. Gelatin is not liquefied.

In considering the tolerance of microörganisms in concentrated solutions, careful attention should be given to the methods of preparation of the test solutions and to the treatments accorded the microörganisms. Tests of an organism's ability to do certain things cannot be fairly made unless it is given every opportunity to adapt itself to the conditions. Inoculations into media containing 20, 40, 60 or more degrees Brix of sucrose should not be made from ordinary broth or agar cultures. The highest concentration showing growth should be used as inoculum, and, then, not more of the culture than necessary should be added, in order to avoid diluting the test medium more than a fraction of a degree Brix. Media containing more than the usual 1 per cent of sugar should always be controlled by saccharometer or polariscope readings, and not made up by weight or volume alone.

SUMMARY

An actively motile, sugar-tolerant organism resembling *Aerobacter cloacae* has been isolated from granulated cane sugar. It

actively ferments sucrose in a concentration of 30° Brix, shows delayed fermentation of sucrose in a concentration of 35° Brix, and does not ferment or grow in a concentration of 40° Brix.

REFERENCES

BROWNE, C. A. 1918 The deterioration of raw cane sugar: A problem in food conservation. Jour. Ind. and Eng. Chem., **10**, 178–90.

CAMERON, E. J., WILLIAMS, C. C., AND THOMPSON, R. J. 1927 Sources of thermophilic contamination in the canning of peas and corn. Jour. Bact., **10**, 37–46.

CHURCH, M. B., PAINE, H. S., AND HAMILTON, J. 1927 Sugar-tolerant yeasts in chocolate-coated creams. Jour. Ind. and Eng. Chem., **19**, 353.

JAMES, L. H. 1928a Note on the distribution of thermophilic spoilage bacteria. Iowa State College, Jour. Sci., **2**, 155–8.

JAMES, L. H. 1928b The bacterial content of raw and commercial sugars. Food Industries, November, 1928.

KOPELOFF, N., AND KOPELOFF, LILLIAN 1919 The deterioration of cane sugar by fungi. Louisiana Exp. Sta. Bul. 166.

OWEN, W. L. 1911 A recently discovered bacterial decomposition of sucrose. Jour. Ind. and Eng. Chem., **3**, 481–6.

SHUTT, D. B. 1925 Yeast contamination as a source of explosion in chocolate-coated candies. Sci. Agric., **6**, 118–9.