

Maple Sirup. XIX.

Flavor and Color Through Controlled Fermentation of Maple Sap

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C. O. Willits, H. A. Frank, and
R. A. Bell

Eastern Regional Research Laboratory,^a
Philadelphia 18, Pennsylvania

SUMMARY

Maple flavor and color in maple sirup were markedly enriched by fermenting sap with 4 strains of *Pseudomonas geniculata* (of 174 bacterial strains studied). These 4 strains imparted maple flavor characteristics 3-4 times that obtained in sirup made from unfermented sap. Fourteen other strains of the genus *Pseudomonas* also exhibited flavor-enhancing properties but to a lesser degree, as did one identified as a *Flavobacterium*. The 4 strains of *Pseudomonas* having strong flavor-inducing properties are all aerobic, grow at psychrophilic temperatures.

THE UNDESIRABLE EFFECTS of excessive microbial growth in maple sap have been known for many years (Edson *et al.*, 1912; Hayward and Pederson, 1946; Naghski and Willits, 1955). The most common defects of sirup attributed to microbial fermentation of maple sap are excessive darkening and increased caramel flavor. Unpleasant flavors in sirup often result from highly contaminated sap.

The role of microbial growth in maple sap with respect to desirable changes in sirup has been studied in this laboratory. Naghski *et al.* (1957) reported that the flavor and color of maple sirup were affected by controlled fermentation of sap with bacterial and yeast strains. Several bacterial strains intensified maple flavor, color, and caramel flavor. The increase in caramel flavor was related to the degree of sucrose inversion, especially when yeasts were present in the sap.

Controlled fermentations of maple sap were explored as a means of influencing several characteristics of maple sirup. Particular attention was directed to microbial strains (usually bacteria) that can intensify maple flavor. It was preferred that the flavor-intensifying strain should not result in excessive darkening of the sirup. This latter characteristic is essential because darker sirups are assigned lower-grade values, commanding lower prices. Further, strains are preferred that do not produce undesirable, acrid caramel flavor, which may mask the maple flavor. Since it was observed (Naghski *et al.*, 1957) that yeast contributed to a high inversion of sucrose, and in turn a high caramel flavor, we confined our investigations to bacterial strains.

Maple sap collected under normal field conditions is converted to a sirup of characteristic maple flavor. During the maple season, a variety of microorganisms

contaminate the sap. These follow a rather typical pattern, being predominantly psychrophilic bacteria in the early season, and then an increasing proportion of yeasts and some molds (Sheneman and Costilow, 1959). This study employed a large number of bacterial isolates from maple sap so that selection could be made of strains that enhance maple flavor. Toward this end, 174 bacterial strains were screened. The 19 strains considered to enhance maple flavor the most were then classified by investigating their significant morphological and biochemical properties.

EXPERIMENTAL METHODS

Maple sap. Sterile maple sap was collected, packaged in 1-gal. metal cans, frozen, and stored as described previously (Naghski and Willits, 1955). The following method, involving aseptic precautions with sterile equipment, was used in preparing lots of sterile sap suitable for inoculation with the test strains. Three gallons of frozen sap were thawed in the cans by allowing them to stand 8 hr at room temperature and then overnight at 18°C. Thawing was completed the following morning by applying heat with a 375-watt infrared industrial reflector lamp to the cans on a shaking machine. The melted sap was transferred aseptically to a sterile 5-gal. glass carboy. Aliquots were plated to check the sterility of each lot used.

Cultures. One hundred and seventy-four bacterial strains, isolated from maple sap, were used in this study. All strains were capable of growth under psychrophilic conditions, i.e., below 5°C. Nineteen strains that enhanced flavor the most (Table 1) were identified with methods suggested in the "Manual of Microbiological Methods" (Society of American Bacteriologists, 1957) and keys in "Bergey's Manual of Determinative Bacteriology" (Breed *et al.*, 1957). Stock cul-

Table 1. Flavor and color characteristics of maple sirup produced from sap fermented by selected bacterial strains.

Strain	Classification	Flavor rating ^a	Color rating (U. S. grades)
4	<i>Pseudomonas geniculata</i>	4	B
11	<i>Pseudomonas geniculata</i>	3	B
25	<i>Pseudomonas geniculata</i>	3	A
222	<i>Pseudomonas geniculata</i>	3	Fancy
387	<i>Flavobacterium rhenanum</i>	2	Unclassified
249	<i>Pseudomonas geniculata</i>	2	B
397	<i>Pseudomonas geniculata</i>	2	B
605	<i>Pseudomonas fluorescens</i>	2	Fancy
1	<i>Pseudomonas geniculata</i>	1	A
9	<i>Pseudomonas convexa</i>	1	A
111	<i>Pseudomonas geniculata</i>	1	A
236	<i>Pseudomonas geniculata</i>	1	A
392	<i>Pseudomonas geniculata</i>	1	A
525	<i>Pseudomonas geniculata</i>	1	A
559	<i>Pseudomonas fluorescens</i>	1	A
612	<i>Pseudomonas geniculata</i>	1	A
169	<i>Pseudomonas geniculata</i>	1	AA
507	<i>Pseudomonas geniculata</i>	1	Fancy
221	<i>Pseudomonas geniculata</i>	1	Fancy

^a Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

^a Flavor is assigned values of 1-4, indicating the maple flavor intensity developed in each sirup sample.

tures of all strains were maintained on tryptone-glucose-yeast extract (TGE) agar slants.

Fermentation procedure. Inocula were growth washed from the surfaces of TGE agar plates incubated 24 hr at 27°C. By dilution of inoculum the initial concentration in the sap was kept at about 10^4 organisms per ml. The inoculated sap was incubated 1 week in a cold room (ca. 1–3°C). Concentration of organisms, estimated by plating with TGE agar, was then generally $20\text{--}60 \times 10^6$ per ml.

Maple sirup evaluation. After one week of fermentation, each lot of inoculated sap was evaporated to sirup as described previously (Naghski *et al.*, 1957). After the sirup was adjusted to standard density (65.5° Brix), percent invert sugar (Naghski and Willits, 1956) was estimated, and the amount of color was estimated with color standards for maple sirups established by the United States Department of Agriculture.

RESULTS AND DISCUSSION

The flavor evaluation was made by a trained five-member panel. After preliminary screening, all samples with an off-flavor (caramel or molasses) were discarded. The remaining samples were then compared to a "standard" sirup made from unfermented sap. Sirups judged to have the same or slightly higher maple flavor levels were assigned a value of one and were not subject to further tests. The remaining eight samples, which possessed an enrichment of flavor greater than that of the control, were then evaluated by the paired method of comparison. This permitted grouping the samples into three groups of different flavor levels, values of 2, 3, and 4.

None of the 19 bacterial strains yielded sirup of excessive caramel flavor. Invert sugar content of all sirups was below 2%. This agrees with previous results that showed a high correlation between high invert sugar and excessive caramel flavor (Naghski *et al.*, 1957).

Eleven of the nineteen organisms enriched flavor slightly over that of the control sirup (made from unfermented sap). The sirups from the 8 remaining strains, with a flavor enrichment rating greater than 1, were rated 2, 3, and 4, in accordance with the maple flavor produced as a result of each fermentation (Table 1).

Pseudomonas geniculata, strain 4, rated 4, the highest of the 174 strains tested. Three other strains of *P. geniculata* (11, 25, and 222) rated 3. That all four of the superior strains are *P. geniculata* may be of some significance. However, since representatives

of other species of *Pseudomonas*, and indeed one species of *Flavobacterium*, also contribute to maple flavor, specificity of contribution to maple sirup flavor is not supported. All members of this group of flavor intensifiers are capable of strictly aerobic growth at psychrophilic temperatures.

Table 1 shows that fermented sap generally yields darker sirups. This is especially true of sirups with higher flavor values. Strains 222 and 605 were exceptions, producing only light-colored sirups.

The possibility of using specific strains for achieving sirups of given specifications is not unreasonable. Flavor production in cured ham by a psychrophilic bacterium has been reported by McLean and Sulzbacher (1959). Occasionally, maple producers find that their sirups lack sufficient flavor. Viable cultures or, more likely, dried but active preparations could well be used to develop flavor in sirups from maple saps that are deficient in the necessary flavor precursors. Selection of the proper strain would avoid excessive darkening.

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