

BACTERIAL ADHESION TO PLASTIC TUBING WALLS

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Introduction

Several studies have indicated microorganisms as one cause of pre-nature stoppage of sap flow in tapped sugar maples (*Acer saccharum* Marsh.) (1,2,3). It is also well documented that heavily contaminated maple sap produces syrup of dark amber color grade (4,5). Microorganisms alter the sap biochemistry at the tap hole and in the sap collecting and storing systems. The process is accelerated as temperature warms, favoring microbial growth in sap(5).

The advent of plastic tubing systems to collect sap has eliminated several problems associated with the traditional bucket system. Insects, leaves and debris are prevented from entering the sap. Tubing systems increase sap flow either due to natural vacuum formation on a slope, or with vacuum pump, and reduce the labor needed to gather the sap. Both of these considerations benefit the maple producer. However, plastic tubing systems also present some problems of their own. Sap quality problems arise if the lines sag and the sap lingers within the tubings or the large conduits. In addition, the warming effect of the sun increases the temperature within the tubing (6) to optimum levels for microbial growth and sap flow may decrease because of "organic buildup" on the internal tubing walls (7). This buildup is a result of the adhesion of microorganisms to the tubing walls (8). They can clog the tubing connection at the spout and junctions as well as other critical areas.

Several species of microorganisms have been shown to be able to adhere specifically to a surface when optimal nutritional and environmental conditions are met. One example is the adhesion of *Streptococcus sanguis* and *Streptococcus mutans* to dental surfaces to form a film known as plaque (9). Fouling of submerged wastewater treatment equipment by *Pseudomonas* species and other marine bacteria has also been documented (9). Adherent bacterial biofilms, composed of *Pseudomonas* species, have also been found in human bone disease (10).

Early research documented microorganisms associated with maple tree tap-

holes (2). *Pseudomonas*, *Achromobacter*, and *Flaobacterium* genera were identified. *Pseudomonas geniculata* (presently known as *Pseudomonas fluorescens* biotype G) was the identified bacteria they most often encountered. Unpublished research performed at the University of Vermont Maple Research Laboratory on the microbial ecology of the sugar maple has confirmed that these groups are the ones we most often have identified on the tree bark, wood, and in contaminated sap.

In 1983, with partial funding from the Vermont Chittenden County Maple Sugar Makers Association, we initiated a project to study the microorganisms associated with maple sap collecting tubing systems. The results of that study (8) indicated that bleach cleaned-and-stored tubing systems were devoid of viable microorganisms when examined after 9 months of open-to-air storage. Any contamination of tubing systems during sap flow would therefore originate from tapping techniques and handling of tubing during installation. Species of the genus *Pseudomonas* were found to be involved in adherence to the inner walls of the plastic tubing when examined after sap flow (8).

In 1984, again with partial funding from the Vermont Chittenden County Maple Sugar Makers Association, we conducted this follow-up study to identify the species within the *Pseudomonas* family that were actually involved in the adhesion. Except for our 1983 study, no previous research has documented the numbers and types of microorganisms specifically adhering to plastic tubing walls.

Methodology:

Three tapholes were opened in a large maple tree (27 inch in diameter at breast height) with a 7/16 inch drill bit at breast height to a depth of 5 cm. The sterile tap was bored with sterilized equipment using an aseptic tapping technique (5).

Three different tap and tubing designs were used to study the numbers and species of microorganisms adhering to the internal walls of sap collecting tubing. The three systems consisted of 1) nonsterile tap with nonsterile (nonautoclaved) tubing, 2) nonsterile tap with sterile (autoclaved) tubing and 3) sterile (aseptically bored) tap with sterile

(autoclaved) tubing. The dropline for each tap was branched at a point 60cm from the spout into three separate 30 cm tubing lines. The three lines converged into a single tubing that was connected to a sterilized collection flask. The flask had an overflow to prevent contamination. All three systems were assembled using Lamb's Naturalflow² tubing. This tubing had been washed with a 10% bleach solution immediately following the previous sap season, and was stored during the summer and fall in a large, dark, screened and aerated shed.

For each system, each of the three separate lines was used for only one of three collection times: early season (3/21), mid season (3/31), or late season (4/6). One of the 30 cm sections of tubing was removed, using sterile scissors, from each system at each collection time. The sections were cut on both sides to a length of 25 cm and clamped shut on both ends. At each collection time a sample of the sap collected from the tubing systems into a sterile flask was aseptically transferred to a sterile bottle, and transported on ice along with the tubing samples to the Maple Laboratory.

Upon arrival at the Lab the tubing samples were immediately opened at one end, filled with 10 ml of phosphate buffered saline (PBS) with a sterile pipette, and resealed. The sealed tubings were then agitated for 30 seconds to loosen any lightly attached microorganisms that might have been present. -Flushing Technique:

The PBS solution was then removed from the tubings and serial dilution platings were performed on each sample for growth determination and count of bacteria and yeast (11).

Swabbing Technique:

The flushed tubing sections were then cut to a length of 10 cm. The inner surface of the tubings was sampled by scraping it with a sterile cotton swab. The cotton end of the swab was then placed in 10 ml of PBS solution and shaken vigorously. This technique was

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²Lamb Tubing System. Robert M. Lamb, Bernhards Bay, NY. Use of name of manufacturer does not imply endorsement by the Vermont Agricultural Experiment Station over others not named.

designed to remove any microorganisms strongly adhering to the tubing walls. Dilution platings were performed on this solution as above.

Sap Control:

Dilution platings were performed on the collected sap samples in the same manner as was done with the flushing and swabbing of tubing samples.

Tubing Control:

A 10 cm section of new, unused tubing served as the tubing control. The section was flushed and swabbed to determine if any microbial contamination was present.

Identification of bacterial isolates to the specie level was attempted using appropriate morphological and biochemical identification techniques (12).

The ability of an organism to adhere was assessed by comparing the microorganism count (CFU/ml), as well as the number of isolates found in the sap control, to the microorganism count and number of isolates found in the flushing and swabbing of tubing samples.

Results and Discussion:

The section of new tubing that served as a control yielded neither bacteria nor yeast from both the flushing and swabbing techniques. This indicates that new tubing, as we previously found for used, bleach-cleaned, and stored tubing (8), should not contaminate sap too early in the season with viable microorganisms.

Of the 115 microbial isolates from all tubing systems and sap controls, 59.2% were fluorescent pseudomonads, 29.6% were yeasts, 4.3% were non-fluorescent pseudomonads, and 6.9% were other bacteria.

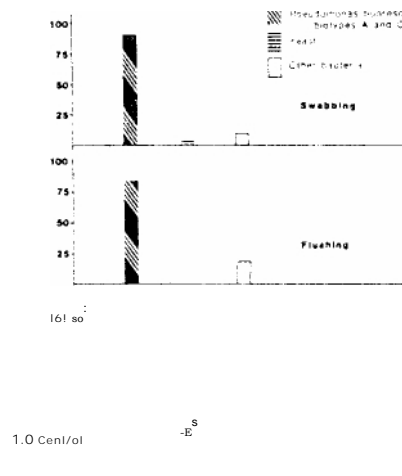
Of the total isolates, 62.6% were recovered from sap control. Identification of these isolates indicated that 40.4% were *Pseudomonas fluorescens* (biotypes A and G). 45.8% were yeast, and 14% were other bacteria (Fig. 1).

Of the total isolates, 9.6% were recovered from flushing the tubings. Of these isolates 81.8% were *Pseudomonas fluorescens* (biotypes A and G) and 18.2% were other bacteria (Fig. 1).

Of the total isolates 27.8% were recovered from swabbing the tubing walls. Of these isolates 90.6% were *Pseudomonas fluorescens* (biotypes A and G), 3.2% were yeast and 6.2% were other bacteria (Fig. 1). The number of recovered bacteria (*Pseudomonas fluorescens*) should have decreased proportionally from sap control to flushing to swabbing if adherence to the tubing walls had not occurred.

Conclusions:

It is difficult to derive statistically significant conclusions with a small sample size. However, results from this study showed significant adhesion of *Pseudomonas fluorescens* biotypes A and G to the walls of all three tubing systems during sap flow only after mid-season. No adherence of living yeast was found. This indicates that the internal tubing walls provide an environment for attachment and growth that is best suited for *Pseudomonas fluorescens*. After being deposited on the tubing walls these bacteria reproduce rapidly. Such a buildup of microorganisms eventually leads to a slowing or clogging of sap flow, and further contamination of sap. This study substantiates our previous work (8) that adhesion is not detectable on tubing walls until microbial load in the sap approaches a high level.



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