Microbial Flora of the Mouth: Determination of Susceptibility to Dental Caries

PURPOSES

1. To become familiar with the organisms responsible for dental caries.
2. To perform experiments that demonstrate a host's susceptibility to caries formation.

PRINCIPLE

A variety of microorganisms are known to be involved in the formation of dental caries, including Lactobacillus acidophilus, Streptococcus mutans, and Actinomyces odontolyticus. These organisms in the oral flora produce organic acids, particularly lactic acid, by fermenting carbohydrates that adhere to the surface of the teeth. In the continued presence of lactic acid, dental enamel undergoes decalcification and softening, which result in the formation of tiny perforations called dental caries.

The actual mechanism of action of these organisms is still unclear. However, it has been noted that S. mutans excretes an enzyme called dextran transacrase (glycosyl transferase), which is capable of polymerizing sucroses into a large polymer, glucan, plus the monosaccharide fructose. This polysaccharide clings tenaciously to the teeth and forms dental plaque, in which streptococci reside and ferment fructose with the formation of lactic acid.

Similarly, L. acidophilus produces lactic acid as an end product of carbohydrate fermentation. Oral lactobacilli are capable of metabolizing glucose found in the mouth, producing organic acids that reduce the oral acid concentration to a pH of less than 5. At this pH, decalcification occurs and dental decay begins.

One of the best microbiological methods for determining susceptibility to dental caries is the Snyder test. This test measures the amount of acid produced by the action of the lactobacilli on glucose. The test employs a differential medium, Snyder agar (pH 4.7), which contains glucose and the pH indicator bromcresol green that gives the medium a green color.

Following incubation, Snyder agar cultures containing lactobacilli from the saliva will show glucose fermentation with the production of acid, which tends to lower the pH to 4.4, the level of acidity at which dental caries form. At this pH the green medium turns yellow. A culture demonstrating a yellow color within 24 to 48 hours is suggestive of the host's susceptibility to the formation of dental caries. A culture that does not change color is indicative of lower susceptibility.

MATERIALS

Cultures
Organisms of the normal oral flora present in saliva.

Media
Per designated student group: two Snyder test agar deep tubes.

Equipment
Bunsen burner, 1-in. square blocks of paraffin, sterile 1-ml pipettes, mechanical pipetting device, sterile test tubes, and glassware marking pencil.
PROCEDURE

1. Melt two appropriately labeled Snyder agar deep tubes and cool to 45°C.
2. Chew one square of paraffin for a period of 3 minutes without swallowing the saliva. As saliva develops, collect it in a sterile test tube.
3. Vigorously shake the collected saliva sample and transfer 0.2 ml of saliva with a sterile pipette into one of the Snyder test medium tubes that have been cooled to 45°C. Caution: Don’t let the pipette touch the sides of the tubes or the agar.
4. Mix the contents of the tube thoroughly by rolling the tube between the palms of your hands or by tapping it with your finger.
5. Rapidly cool the inoculated tube of Snyder agar in an ice-water bath.
6. Repeat Steps 3 through 5 to inoculate the second tube.
7. Incubate both tubes for 72 hours at 37°C. Observe cultures at 24, 48, and 72 hours. (See the picture to the right for examples of color changes.)

Tube 1: Uninoculated Snyder tube.
Tube 2: No color change indicates little or no susceptibility to forming dental caries.
Tube 3: Sight color change indicates mild susceptibility to forming dental caries.
Tube 4: Significant color change indicates moderate susceptibility to forming dental caries.
Tube 5: Complete color change indicates high susceptibility to forming dental caries.
OBSERVATIONS AND RESULTS

1. Examine the Snyder test cultures daily during the 72-hour incubation period for a change in the color of the culture medium. Use an uninoculated tube of the medium as a control. Record the color of the cultures in the chart.

2. Using Table 60.1 to interpret your observations, record your findings about susceptibility to caries in the chart.

**TABLE 60.1 Assessment of Susceptibility to Dental Caries**

<table>
<thead>
<tr>
<th>Caries Activity</th>
<th>24</th>
<th>48</th>
<th>72</th>
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<tbody>
<tr>
<td>Marked</td>
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<tr>
<td>Moderate</td>
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<td>Slight</td>
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<td>Negative</td>
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Source: Courtesy of Dilco Laboratories, Inc., Detroit, Michigan.

Positive: Complete color change; green is no longer dominant.

Negative: No color change or a slight color change; medium retains green color throughout.

**REVIEW QUESTIONS**

1. How would you explain the differential nature of the Snyder agar medium as used for the detection of dental caries?
2. How would you explain the mechanism responsible for the formation of dental caries by resident microorganisms?

3. What is the function of the paraffin in this procedure?

4. Based on your results, what is your tendency to form dental caries?

   Is this result consistent with your dental history?

5. Are all members of the resident flora of the mouth capable of initiating dental caries? Explain.

6. What is the ideal time of day to perform this procedure? Why?

REFERENCES

378 Experiment 60