Lactobacillus Plantarum, The Cause of “Yeast Spots” on Olives

REESE H. VAUGHN, WILLIAM D. WON, FRANCIS B. SPENCER, DEMOSTHENES PAPPAGANIS, I. OMAR FODA, AND PAUL H. KRUMPERMAN

Department of Food Technology, University of California, Davis California

Received for publication October 13, 1952

One of the most common abnormalities associated with olives pickled in California is known throughout the industry as “yeast spots.” This blemish, shown in figure 1, is characterized by the formation of raised white spots or pimples between the inner surface of the skin and the flesh of the olive. Although in general, the Sevillano variety most frequently shows this blemish, the spots may be found on all varieties and types of olives which have undergone fermentation in salt brines. Because such olives are considered unsightly, some loss of value of the pickled fruit may be experienced, although the olives may be perfectly normal and healthful in other respects.

These small white spots actually are colonies of microorganisms which have developed during the fermentation. Vaughn, Douglas and Gilliland (1943) reported that most of the pimples examined in the laboratory contained lactobacilli, some cultures of which were identified as Lactobacillus plantarum. Then a deliberate search for yeasts was initiated because general use of the term “yeast spots” indicated that yeasts actually had been observed in the pimpled olives. No yeasts were found. After it was apparent that most, if not all, of the spots contained bacteria, it was decided to determine the predominating types. These investigations are described in the following pages.

Experimental Methods

Samples

California olives collected for examination included Spanish type green olives, Sicilian type green olives, brined Greek type olives, storage fruit held in brines for subsequent preparation of ripe olives, and canned ripe olives. Authentic samples of Spanish green olives also were obtained.

An individual sample consisted of at least 10 spotted olives. Between 1942 and 1952, 115 different samples were examined. These included 40 Spanish type, 25 Sicilian type, 5 brined Greek type, 30 brined storage, 5 canned ripe and 10 authentic Spanish olive samples.

Microscopic Examination

To determine whether bacteria or other microbes were present, the contents of at least 10 spots on each sample of olives were subjected to microscopic examination. The desired olives were rinsed in several changes of sterile water. The individual pimples were opened with a sterile dissecting needle and the microbial contents smeared onto glass slides, fixed and stained with the Gram stain. The stained preparations then were examined under the oil immersion lens.

Isolation and Identification of the Bacteria

To attempt isolation of viable bacteria from the spots, selected olives were rinsed in several changes of sterile water and then immersed for 10 minutes in a 1.0 per cent solution of mercuric chloride. The excess mercuric chloride was removed by rinsing the olives in several changes of sterile water. Then the individual pustules were opened as described above and a portion of the contents touched to the surface of sterile liver infusion agar as when making a giant colony inoculation. The remaining portion was prepared for microscopic examination as described above. If, after incubation for 4 days at 30°C, in an atmosphere of nitrogen with 5 per cent carbon dioxide, the colonies had grown, their cells were compared with those contained in the original pimple. Those colonies whose cells were similar to those of the original inoculum were purified and identified by conventional means.

Results

The presence of bacteria. Bacteria but no yeasts were seen in the microscopic preparations made from the spotted olives collected for this study. The bacteria were always rod-shaped and varied in length from ellipsoidal to long forms. The majority of cells were gram positive. However, some samples contained both gram positive
and negative cells and a number of spots from old olives contained only gram negative rods.

At first it was very difficult to obtain viable cells from the spots but it was soon found that the number before microscopic observations were made. Consequently, most of the cultures were isolated from samples of California olives that were between 1 and 3 months old. Unfortunately it was not possible to recover viable cultures from the authentic Spanish samples or from other California olives that were over 9 months old.

Identification of the bacteria. Twenty-five separate isolates obtained from 25 different samples of olives were finally chosen for complete identification. All of

---

**Table 1. Characteristics of the bacterial cultures isolated from "yeast spots" of olives**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Initial pH* range</th>
<th>Compound*</th>
<th>ml N/10 NaOH to neutralize 10 ml of culture (range)</th>
<th>Compounds not fermented*</th>
<th>Type of lactic acid from glucose†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean—30 C</td>
<td>4.7 to 5.5</td>
<td>Arabinose</td>
<td>1.0-4.0</td>
<td>Xylose‡</td>
<td>Inactive (DL)</td>
</tr>
<tr>
<td>Range—25 C to 34 C</td>
<td></td>
<td>Glucose</td>
<td>3.4-7.0</td>
<td>Sorbitol</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fructose</td>
<td>3.4-6.5</td>
<td>Glycerol</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannose</td>
<td>1.4-3.1</td>
<td>Starch</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Galactose</td>
<td>4.0-7.0</td>
<td>Malic Acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sucrose</td>
<td>2.0-5.6</td>
<td>Citric Acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maltose</td>
<td>2.0-6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactose</td>
<td>2.8-5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raffinose</td>
<td>0.5-2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannitol</td>
<td>3.0-6.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Incubated at 30 C for 7 days.
† Incubated at 30 C for 2 weeks.
‡ Culture 185 required 4.2 ml N/10 NaOH per 10 ml for neutralization. 

---

**Fig. 2. Cross-section of an olive showing a subcutaneous colony of bacteria. Gram's stain.**
these cultures were gram positive, non-motile, catalase negative rods, 0.8–1.0 x 3.0–5.0 microns in size; did not produce significant quantities of acetic acid or carbon dioxide from glucose; and did not reduce fructose to mannitol. The specific characteristics which identified them as belonging to the homofermentative species *Lactobacillus plantarum* are shown in table 1. It will be noted that there is a very close relationship between the olive cultures and other isolates of *L. plantarum* described by Orla-Jensen (1919) and Pederson (1936).

"yeast spots" although the fruit is fresh, the spots do not contain colonies of microorganisms and on lye treatment they disappear. Therefore, it is assumed that the bacteria which finally develop into subcutaneous colonies gain entry through these enlarged pores.

Finally, it should be emphasized that olives are not the only food product subject to this bacterial spotting. Similar bacterial blemishes have been observed in commercial packs of Italian peppers (pepperoncini) and pickled green tomatoes.

**FIG. 3.** Freshly harvested Sevillano olives with enlarged stomatal openings. The prominent white areas disappear when the air has been displaced and reappear only when bacteria have developed during fermentation. (See figure 1).

**DISCUSSION**

Attempts to discover methods for control of this spotting of olives were not successful. Circumstantial evidence hints that prevention would be very difficult. The subcutaneous colonies of bacteria (figure 2) develop within the first few weeks after the olives have been placed in brine. Evidently, any factor which would influence the entrance of the bacteria through the pores or other breaks in the skin of the fruit might contribute to the development of these colonies.

Olives of the Sevillano variety are particularly prone to have enlarged stomatal openings in their skin. As shown in figure 3, these enlarged pores appear to be like

**ACKNOWLEDGEMENTS**

The use of funds from grants-in-aid to the University of California by the California Olive Association and the State of California Olive Advisory Board are gratefully acknowledged.

**SUMMARY**

Microscopic and cultural study of the blemish of olives commonly known as "yeast spots" revealed the abnormality is caused by bacteria rather than by yeasts. The bacteria which could be isolated were identified as representatives of the species *Lactobacillus plantarum.*
ACETONE-BUTANOL FERMENTATION OF STARCHES

REFERENCES


A Microbiological Process Report

Acetone-Butanol Fermentation of Starches

Samuel C. Beesch

Publicker Industries, Incorporated, Philadelphia, Pennsylvania

Received for publication October 14, 1952

The production of acetone and butanol by the Weizmann fermentation process has previously been described in some detail by Killeffer (1927), Gabriel (1928), Gabriel and Crawford (1930) and Prescott and Dunn (1949). The complete description of the modern industrial process using starch-containing material and its resulting problems has not been previously reported. The following data are presented in an effort to bring up-to-date the literature on this subject.

Microorganisms Used

Clostridium acetobutylicum is the organism commonly used in the industrial acetone-butanol fermentation of starch mashes. It is a spore-forming rod. Its inactive stage consists of a rod containing a spore, or a free spore by itself. There are several other types of organisms similar to C. acetobutylicum which deserve some mention. They are Clostridium roseum and Clostridium felsineum. These organisms, when grown in a corn or starch mash, produce a pink-colored mash and produce yields of acetone and butanol equivalent to about one-half to three-quarters of that produced by C. acetobutylicum. C. felsineum is used in the flax retting industry for its enzymatic powers of hydrolyzing the pectin, causing a separation of the fiber from the cortex and wood. Jean (1939) has patented the use of this culture to ferment an admixture of garbage and grains to n-butyl alcohol, acetone and ethyl alcohol.

Numerous strains of C. acetobutylicum have been isolated. Some are characterized by different fermentations of carbohydrates, different ratios of solvents, immunity to bacteriophage attacks and visual characteristics of the fermentation. The isolation of new cultures of C. acetobutylicum may be accomplished in many ways. One consists of the introduction of a small amount of soil, manure, roots of leguminous plants, cereals, decayed wood, corn stalks, sewage or river-bottom-mud, into a sterile 4 per cent corn mash tube. The corn mash tube is then heated for two minutes at 212°F in a boiling water bath or Arnold steamer to destroy any vegetative forms present; only the spores survive. These tubes are then cooled to 98°F and are allowed to incubate at 98°F in a constant temperature incubator for several days. Usually, in 24 hours’ time the tubes begin to produce gas and the corn starch undergoes fermentation. By noticing the head or cap (if one is formed) or by smelling the tube, one experienced in the art can determine with considerable certainty whether an acetone-butanol organism is present. If neither of the above-mentioned criteria is evident, the usual custom is to discard the tube. If, however, the culture has a sweet butylic odor or the corn has formed a head on top, we then proceed to plate out the culture. This involves making dilutions of the unknown culture and adding these dilutions to Petri plates containing sterile malt agar, prepared especially for the isolation of such cultures. These plates are then incubated under strict anaerobic conditions for 48 to 72 hours at 98°F. Upon observation of the plates, numerous colonies are picked from the plate and carried through a series of tests in corn mash, finishing up with a quantitative test on 8 per cent to 10 per cent corn

1 Present address: Chas. Pfizer & Co., Brooklyn 6, N. Y.