Incidence of Heat-Resistant Molds in Eastern Orchards and Vineyards

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Received for publication 26 October 1970

Over 70% of the samples of fruit, vegetation, and soil obtained in surveys of New York orchards and vineyards were contaminated with heat-resistant molds. The counts generally were low, under one per gram. Byssochlamys fulva was the most common isolate. Other isolates were identified as B. nivea, Paecilomyces variotii, Aspergillus fischeri, A. fischeri var. spinosus, A. fumigatus, Penicillium verruculatum, and P. ochro-chloron.

The first reported spoilage outbreak of thermally processed fruit caused by Byssochlamys occurred in Great Britain during the early nineteen thirties, and for a number of years the mold appeared to be restricted to that country (1). More recently the organism has been isolated from other areas including continental Europe (3, 5), Canada (8), and the West Coast of the United States (2). Although spoilage outbreaks have occurred in fruit processed in the Northeast, it has not been known whether Byssochlamys was endemic to eastern orchards or whether it was introduced via food ingredients imported from other regions.

The objective of this research was to determine the extent to which Byssochlamys is present in the orchards and vineyards of this region and to learn something about its distribution. This information is important to the food processor because it would appear that the best method for preventing spoilage of certain fruit products is to minimize the opportunity for contamination.

MATERIALS AND METHODS

The samples were obtained from surveys made in Ontario and Chautauqua counties during the 1968 and 1969 growing seasons. Details of the culturing methods have been published (7). Essentially, the procedure consisted of blending the sample, usually 50 to 100 g, in 16°Brix Concord grape juice until the mixture was homogeneous. The material then was heated 1 hr at 70 C to select for the heat-resistant molds and to activate dormant spores. The Concord juice enhanced this activation. After heating, the homogenate was distributed into petri dishes, approximately 10 ml per plate. Equal volumes of double-strength potato-dextrose-agar (pH 3.5) were then added and the material was mixed. Colonies were counted after incubation for 48 hr at 32 C. Negative plates were incubated an additional 48 hr before being discarded.

Several precautions were taken to assure that the heat-resistant molds did, in fact, originate from the field samples and not our laboratory. The samples were processed in an isolated laboratory that had not been used for other mold studies, and in many surveys the tared, sterile, screw-cap blender jars were carried to the orchards and vineyards.

Confirmation that the counts represented populations of heat-resistant molds was achieved by transferring representative colonial types from each sample into culture tubes containing 5 ml of 15°Brix Concord grape juice. After incubation for 28 days at 32 C, to assure ample time for ascospore formation (6), the pellicle and broth were blended in a Sorvall Omnimixer homogenizer. The homogenate was then heated for 1 hr at 70 C before the plating of appropriate dilutions on acidified potato-dextrose-agar.

Identification of the isolates was based on macroscopic and microscopic observations of cultures propagated at room temperature and 32 C in various media including potato-dextrose, Czapek, and malt-extract agars.

The homogenates of 28 day grape juice cultures were used in the studies on heat activation of dormant spores. The material was washed three times in distilled water and then suspended in 95% ethanol for 20 min (one part aqueous spore suspension per nine parts ethanol). This treatment served to destroy the heat-labile structures such as conidia and hyphae without affecting viability and dormancy of the ascospores (7).

RESULTS AND DISCUSSION

A total of 99 samples of various fruit, vegetation, and soil were obtained from 15 vineyards, 9 orchards, and the receiving platform of 1 grape processor during the two growing seasons. Over 70% were found to be contaminated with heat-resistant molds (Table 1). In general, the incidence

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1 Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper no. 1841.
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Orchard D

Byssochlamys .

Paecilomyces varioti.

Blackberries, plums, decayed.....

Vegetation from

vineyard floor

Grape pomace.....

Apples from ground.

Plums, decayed.

Blackberries, cherries, peaches, raspberries, apricots, pears.

Orchard and vineyard soil.....

| Sample                                      | Fraction contaminated | Molds/100 g (geometric mean) | Predominant species* |
|--------------------------------------------|-----------------------|------------------------------|----------------------|
| Grapes, sound.                             | 19/25                 | 3.2                          | A, D                 |
| Grapes, decayed.                           | 18/20                 | 72                           | C, A                 |
| Vines, leaves, etc., on trellis.           | 5/10                  | 13                           | A                    |
| Vegetation from vineyard floor             | 5/8                   | 150                          | A, I                 |
| Grape pomace                               | 4/4                   | >100                         | I                    |
| Apples from ground.                        | 5/8                   | 4.1                          | A                    |
| Plums, decayed.                            | 4/6                   | 63                           | C, D, I              |
| Blackberries, cherries, peaches, apricots, pears. | 4/10                  | 3.8                          | A                    |
| Orchard and vineyard soil.                 | 8/8                   | 260                          | A, C                 |

* Listed in order of isolation frequency. A = Byssochlamys fulva, C = Aspergillus fischeri, D = Penicillium vermiculatum, I = Paecilomyces variotii.

of the organisms was low, usually under one per gram. The highest populations were in soil and in materials collected from the ground. Apples which gave an average figure of only 4.1 per 100 g were an exception, perhaps because of their relatively large weight-to-surface ratio.

Only a limited number of sound fruits other than grapes were cultured, because it was assumed that decayed materials would be more likely to yield the organisms. Of seven sound fruit samples, five were negative, whereas single samples of raspberries and peaches gave counts of 11 and 5.6 per 100 g, respectively.

Of the sound grape samples, 13 were collected at the receiving platform of a processor and thus represented commercially harvested fruit. All were contaminated with heat-resistant molds. Five of the samples were of mechanically harvested fruit. There was some indication that this material was the more heavily contaminated in that it gave a geometric mean count of 12 per 100 g compared to 4.8 per 100 g with the handpicked fruit. Although it is not known whether the results were typical, our ability to isolate B. fulva from the soil surfaces of a harvester that had been idle for several days lent support to the data. It is very possible that harvesters can be a significant source of contamination when their sanitation has been overlooked.

B. fulva was the species isolated most frequently (Table 1). Although it was not the predominant contaminant of certain materials such as the decayed plums, it was recovered from all sample types. Other molds that survived the selective treatment at 70 C included B. nivea, Paecilomyces variotii, and species of Aspergillus and Penicillium (Tables 1 and 2). It is of interest that B. nivea, which is the most common contaminant of California grapes (2), did not predominate on any of our samples.

Many of the isolates were studied to determine whether spores were responsible for their heat resistance, whether they exhibited a dormancy that could be broken with heat, and whether grape juice enhanced spore activation. This work was completed before the cultures had been identified and, therefore, it was reassuring to find later that those grouped as a given species had generally responded in a similar manner to the different tests. Thus, the 10 cultures of B. fulva that were tested were similar to the isolate in Table 2 in that

| Table 1. Incidence of heat-resistant molds in New York orchards and vineyards |

| Sample                                      | Fraction contaminated | Molds/100 g (geometric mean) | Predominant species* |
|--------------------------------------------|-----------------------|------------------------------|----------------------|
| Grapes, sound.                             | 19/25                 | 3.2                          | A, D                 |
| Grapes, decayed.                           | 18/20                 | 72                           | C, A                 |
| Vines, leaves, etc., on trellis.           | 5/10                  | 13                           | A                    |
| Vegetation from vineyard floor             | 5/8                   | 150                          | A, I                 |
| Grape pomace                               | 4/4                   | >100                         | I                    |
| Apples from ground.                        | 5/8                   | 4.1                          | A                    |
| Plums, decayed.                            | 4/6                   | 63                           | C, D, I              |
| Blackberries, cherries, peaches, apricots, pears. | 4/10                  | 3.8                          | A                    |
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| Table 2. Some properties of the different thermotropic species that were isolated |

| Species                                      | No. of isolates | Observations on representative culture homogenates |
|----------------------------------------------|-----------------|-----------------------------------------------------|
| Byssochlamys fulva                          | 35              | Hemocytometer count/ml                                |
| B. nivea                                     | 5               | Viable counts/ml                                     |
| Paecilomyces variotii                       | 9               |                                                      |
| Aspergillus fischeri                        | 8               |                                                      |
| A. fischeri var. spinosus                   | 5               |                                                      |
| A. fumigatus                                 | 3               |                                                      |
| Penicillium vermiculatum                    | 16              |                                                      |
| P. ochro-chloron                            | 4               |                                                      |

* Heated suspensions were held in a water bath for 60 min.
heating in grape juice produced a significant increase in the viable count and that heating for 1 hr at 80 °C afforded counts considerably higher than those obtained at 60 °C. The counts at 70 °C (not shown) were still higher by a factor of two-to threefold, indicating some spore destruction at 80 °C. The fact that the maximal viable count agreed quite well with the microscopic spore count indicated that the individual spores present in the homogenate were mainly ascospores rather than heat-labile conidia.

_B. nivea_ and _P. variotii_ responded in a manner similar to _B. fulva_ in that activation was stimulated by heating in grape juice. The _B. nivea_ spores, however, were considerably less heat-resistant as evidenced by the significant reduction in viable count at 80 °C. The isolates identified as _P. variotii_ appeared identical to _B. fulva_ except that asci were not observed. We suspect that they actually are poor ascospore-producing strains of _B. fulva_ and that the low viable counts of heated suspensions reflected the ascus-ascospore populations, levels that would not have permitted them to be readily detected under the microscope.

The aspergilli and penicillia differed from the above species in that grape juice had little or no effect and viable counts usually were not greatly increased by heating at 60 °C. _A. fischeri, A. fischeri_ var. _spinosus_, and _P. vermiculatum_ exhibited considerable heat resistance in that their homogenates gave viable counts of over 10^4 per ml after being subjected to heating for 1 hr at 80 °C. The three _A. fumigatus_ cultures were also relatively resistant when first isolated and, therefore, are believed to be strains of _A. fischeri_ that later lost the ability to form asci. _A. fischeri_ has been isolated in other searches for heat-resistant molds (4).

The structures responsible for the limited resistance of _P. ochro-chloron_ remain to be defined since there appeared to be little correlation between the microscopic spore count and the viable population of heated suspensions. Non-alcohol treated homogenates gave similar low counts when heated at 80 °C, indicating that a sensitivity to ethanol was not responsible for the low viable recoveries.

The ubiquitousness of _Byssochlamys_ in New York orchards and vineyards raises the question as to why spoilage has not been a more serious problem. Preliminary studies indicate that many of the isolates would survive the thermal processes commonly given fruits and fruit products. One possible explanation is that the initial low incidence of spores on sound fruit, combined with the effect of certain processing steps, results in an extremely low level of contamination by the time the product is ready for the heat exchanger or retort. King et al. (2) have shown that a high percentage of spores are removed by the type of filtration given commercial grape juice. Washing, fluming, blanching, peeling, and pressing are some of the other operations that would be expected to remove spores from contaminated fruit.

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