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Yeasts and Molds Associated with Spoiled Dried Prunes

Yeasts and molds isolated from spoiled dried prunes were identified. Sixty-two strains of yeasts included 21 of *Saccharomyces rouxii,* 11 of *S. mellis,* eight of *Torulopsis magnoliae,* five of *T. stellata,* four of *Candida krusei,* three of *Trichosporon behrendii,* two of *Pichia fermentans,* two of *P. membranaefaciens,* two of *C. chalmersi,* and one each of *S. rosei, S. cerevisiae, Sporobolomyces roseus,* and *C. parapsilosis.*

One hundred and twenty-four strains of molds which were identified included 56 strains of *Aspergillus glaucus,* 18 of *A. niger,* 41 of *Penicillium* spp., four other *Aspergillus* spp., two *Alternaria,* and one each of *Monilia* sp., *Chaetomella* sp., and *Mucor* sp.

Studies of the Osmophilic Nature of Spoilage Organisms

Eleven strains of yeast, representing species most frequently isolated from spoiled dried prunes, and 124 strains of molds isolated from the same source were studied for their osmophilic character.

Strains of *Saccharomyces rouxii, S. mellis,* and *Torulopsis stellata* were able to ferment in a medium containing 70 per cent soluble solids, but failed to grow in two weeks in a medium containing 75 per cent soluble solids. Strains of *T. magnolieae* and *S. rosei* were able to ferment in 65 per cent soluble solids but not in 70 per cent.

The growth rate of *Saccharomyces rouxii* was suppressed considerably when soluble solids were increased from 40

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Hirosato Tanaka and M. W. Miller

I. Yeasts and Molds Associated with Spoiled Dried Prunes[1,2]

INTRODUCTION

California produces about 99 per cent (165,000 tons) of the total United States output of dried prunes annually, about 70 per cent of which is used in this country, the rest being exported, mainly to Europe.

Originally, prunes were dried in the sun, a slow process that frequently resulted in microbial spoilage, particularly when rain fell during the drying season. Such spoilage was primarily the result of yeast fermentation and formation of molds. Today, dehydrators are used almost exclusively for prune drying, and many of the microbial spoilage problems have thereby been eliminated. Phaff *et al.* (1946) found that no viable yeasts and molds remained on prunes at the end of commercial dehydration. However, yeast and mold spoilage still makes unsalable a considerable amount of bulk-stored and processed, packaged prunes.

Information is scant on the nature of the spoilage organisms and the conditions favoring spoilage of processed prunes. Baker and Mrak (1938) and Mrak and Baker (1939) studied the yeast flora associated with "sugared" dried prunes and figs. Nearly half of the 230 isolates of yeast were haploid-type species of *Saccharomyces (Zygosaccharomyces)*. One fourth of the strains of *Zygosaccharomyces* that these workers isolated were similar to *Z. mandshuricus* (*S. acidifaciens*). The next largest group was *Z. cavarae* var. *beauveria (S. rouxii* var. *polymorphus)*.

No other publications deal with the yeasts and molds on dried prunes. However, many have dealt with the flora from products rich in sugar because they are interesting from the ecological as well as the practical point of view. Studies prior to 1948 were reviewed by Mrak and Phaff (1948).

The present report describes taxonomic studies of the organisms isolated from spoiled dried prunes.

MATERIALS AND METHODS

Sources of Spoiled Prune Samples

Spoiled samples were shipped to this laboratory from the Dried Fruit Association of California, the California Prune Advisory Board, and various processors. The samples were collected from bulk-stored and packaged, processed prunes that showed obvious microbial spoilage. They consisted of 15 lots of 30-pound, bulk-carton export packs, 13 pliofilm packages each containing 1 or 2 pounds of processed prunes, 18 lots of bin-stored prunes, and two samples of spoiled fresh prunes.

[1] Submitted for publication May 4, 1961.

[2] A report of work done under contract with the U.S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Western Utilization Research and Development Division of the Agricultural Research Service.

Media

Media used for isolating yeasts and molds from spoiled prunes were: potato-dextrose-agar, prepared as described by Lodder and Kreger-van Rij (1952); acidic malt extract agar, prepared by adjusting a medium of 5 per cent dried malt extract (w/v) and 2 per cent (w/v) agar to a pH between 3.3 and 3.5 with HCl just before the plates were poured; and malt extract agar with a high sugar content, composed of 10 per cent malt extract, 35 per cent (w/v) cerelose (industrial glucose), and 2 per cent (w/v) agar.

The molds were classified on Czapek's agar, with and without an additional 17 per cent sucrose, on potato-dextrose agar, and on 5 per cent malt extract agar.

Isolation and Purification of Yeasts and Molds

Small fragments of tissue were removed from the surface of spoiled prunes with forceps or needles dipped and flamed in 95 per cent ethanol. The fragments were then plated on the isolation media listed.

Yeasts were purified by making suspensions from separated colonies and restreaking on malt-extract agar or on malt-extract agar plates of high sugar concentration.

Molds were transferred to fresh media from the outer margin of colonies. When necessary, conidia from the outer margin of the colonies were suspended in sterile water and streaked on isolation media. Purification was accomplished by repeated transfers.

Identification of Yeasts

Yeasts were classified by the methods described by Lodder and Kreger-van Rij (1952), modified as follows: Albimi yeast autolysate (0.5 per cent w/v) was used in the fermentation media. Durham rather than Einhorn tubes were used in the fermentation tests. Yeasts that gave ambiguous results with the auxanographic technique were reexamined in liquid media (Wickerham and Burton, 1948) to ascertain whether the carbohydrate compound in question had been assimilated.

RESULTS AND DISCUSSION

Yeasts

Sixty-two strains of yeasts (38 sporogenous, 24 asporogenous) isolated from the spoiled dried prune samples are listed below.

TAXONOMIC DESIGNATION	NO. OF CULTURES ISOLATED
Sporogenous yeasts:	
Pichia fermentans Lodder 1932	2
P. membranaefaciens Hansen 1888.	2
Saccharomyces cerevisiae Hansen 1883	1
S. mellis (Fabian et Quinet) Lodder et Kreger-van Rij 1928	11
S. rosei (Guilliermond) Lodder et Kreger-van Rij 1913	1
S. rouxii Boutroux 1883	21
Sporobolomyces roseus Kluyver et van Niel 1924–'25	1
Asporogenous yeasts:	
Candida chalmersi Langeron et Guerra 1938	2
C. krusei (Cast.) Berkhout 1910	4
C. parapsilosis (Ashf.) Langeron et Talice 1928	1
Torulopsis magnoliae Lodder et Kreger-van Rij 1952	8
T. stellata (Kroemer et Krumbholz) Lodder 1931	5
Trichosporon behrendii Lodder et Kreger-van Rij 1952	3
Total isolates	62

Most isolates proved to be haploid-type species of *Saccharomyces* (previously classified as subgenus *Zygosaccharomyces*) and two species of *Torulopsis*. Also found were several diploid species of *Saccharomyces, Pichia, Candida,* and *Trichosporon,* and one of *Sporobolomyces.*

Sporogenous Yeasts

Genus Saccharomyces. Of the 34 cultures isolated, 21 were *S. rouxii,* 11 *S. mellis,* 1 *S. rosei,* and 1 *S. cerevisiae.* Three strains of *S. rouxii* and three strains of *S. mellis* did not sporulate with the methods employed. Character-

istics other than sporulation, however, agreed very well with the standard description of these species (Lodder and Kreger-van Rij, 1952). It is worth noting that 11 strains of *S. rouxii* and five strains of *S. mellis* were obtained from isolation media containing about 40 per cent (w/v) sugar.

Eighteen of 21 strains of *S. rouxii* showing a delayed fermentation of sucrose (three to four weeks after inoculation) confirmed the observations of Pappagianis and Phaff (1956).

Lodder and Kreger-van Rij (1952) abolished the genus (subgenus) *Zygosaccharomyces* and included it in the genus *Saccharomyces*. According to their classification, currently accepted by many microbiologists, many different species of *Zygosaccharomyces* associated with products rich in sugar are now included in the species *Saccharomyces rouxii* or in *S. mellis*. In the present investigation, 32 of 62 isolates from spoiled prunes were identified with these two haploid-type species of *Saccharomyces*.

Genus Pichia. Two strains of *Pichia fermentans* and two of *P. membranaefaciens* were isolated. These species have often been isolated as contaminants in natural fermentations and spoilage of various foodstuffs.

Asporogenous Yeasts

The asporogenous yeast flora included two interesting osmophilic yeasts: *Torulopsis magnoliae* and *T. stellata*. The former, originally isolated from the flower of a magnolia in Holland, has also been isolated from 65° Brix orange juice concentrates by Kitchel and Miller (1960). *T. stellata* was associated with the juice of partially vine-dried grapes (Kroemer and Krumbholz, 1931) and with souring figs (Mrak *et al.*, 1942).

Genus Torulopsis. Two species of *Torulopsis* were included. Eight strains of *T. magnoliae* and five strains of *T. stellata* were isolated. All strains were osmophilic and grew slowly on media with low concentrations of soluble solids. Good growth was obtained on a solid medium containing 10 per cent (w/v) malt extract and at least 10 per cent (w/v) of cerelose. These organisms are considered to be potential spoilage organisms on the basis of frequency of isolation and the results of osmophilic studies (Tanaka and Miller, 1961).

Genus Candida. Four strains of *C. krusei,* two of *C. chalmersi,* and one of *C. parapsilosis* were isolated. These organisms, rather widespread in nature, are probably secondary contaminants.

Genus Trichosporon. Three strains of *T. behrendii* were isolated.

Genus Sporobolomyces. One strain of *S. roseus* was isolated.

Molds

One hundred and twenty-four strains of molds were isolated from the dried prune samples: 56 of *Aspergillus glaucus* (45.2 per cent); 18 of *Aspergillus niger* (14.5 per cent); 41 of *Penicillium* spp. (33 per cent); four other *Aspergillus* spp.; two *Alternaria* spp.; one each of *Monilia* sp., *Chaetomella* sp., and *Mucor* sp. Investigations on the osmophilic characteristics of these molds later revealed that the complex of species comprising the *Aspergillus glaucus* group contains by far the most important spoilage molds of prunes (Tanaka and Miller, 1961).

Among the 124 strains of molds isolated from the spoiled dried prunes, those of *Aspergillus glaucus* were by far the most common (56 strains). These are among the most important fungi responsible for spoilage of food products of low water activity. They cause spoilage in jams, jellies, soft sugars, honey, fruitcake, etc. Thom and Raper (1941) stated that the molds present in moldy food products of minimum moisture content are likely to belong to the *A. glaucus* members.

The strains of *Aspergillus niger* and certain species of *Penicillium* also might be considered to be potential spoilage organisms since they were frequently isolated from the spoiled prunes

and were found to be moderately osmophilic.

SUMMARY

Sixty-two strains of yeasts were isolated from spoiled dried prunes: 21 of *Saccharomyces rouxii,* 11 of *S. mellis,* eight of *Torulopsis magnoliae,* five of *T. stellata,* four of *Candida krusei,* three of *Trichosporon behrendii,* two of *Pichia fermentans,* two of *P. membranaefaciens,* two of *C. chalmersi,* and one each of *S. rosei, S. cerevisiae, Sporobolomyces roseus,* and *C. parapsilosis.*

One hundred and twenty-four strains of molds were isolated from the spoiled dried prunes. These included 56 strains of *Aspergillus glaucus,* 18 of *A. niger,* 41 of *Penicillium* spp., four other *Aspergillus* spp., two *Alternaria,* and one each of *Monilia* sp., *Chaetomella* sp., and *Mucor* sp.

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to 60 per cent, was very slow at 65 per cent, and not detectable at 70 per cent soluble solids.

Colony diameters of the mold strains were measured daily for up to seven days, on solid media containing 40, 50, and 60 per cent soluble solids. Strains of *Aspergillus glaucus* showed pronounced osmophilic characteristics whereas those of *A. niger* and *Penicillium* spp. were somewhat less osmophilic. Strains in the genera *Alternaria, Monilia,* and *Chaetomella* showed no osmophilic characteristics. The single strain of *Mucor* grew luxuriantly on 40 per cent soluble solids, barely grew on 50 per cent, and not at all on 60 per cent.

The effect of increasing the soluble-solids content in a medium on the lag phase and the growth rate was least for strains of *Aspergillus glaucus,* confirming the definite osmophilic nature of this group of molds, and greatest for strains of *Penicillium. A. niger* strains were affected in an intermediate fashion on the same media.

Relation of Equilibrium Relative Humidity to Potential Spoilage

The soluble-solids content of fresh prunes was studied in fruits picked by hand at three stages of maturity. Although fruits were carefully sorted for uniformity of size and color, the soluble-solids content of individual fruits varied greatly at each stage of maturity.

Changes in the moisture content of dried prunes placed in atmospheres of various relative humidities at 20° and 30° C were followed for 25 weeks. Prunes attained equilibrium in 25 weeks in atmospheres of 76 per cent relative humidity or lower.

Dried prunes equilibrated in atmospheres of various relative humidities were inoculated with selected strains of *Aspergillus glaucus, A. niger, Penicillium* sp., and *Saccharomyces rouxii.* At 20° C the strains of *A. glaucus* and *S. rouxii* grew on prunes equilibrated at relative humidities as low as 76 per cent, but not at 69 per cent, in a four-month period. The strains of *A. niger* and of *Penicillium* sp. grew well on prunes equilibrated at 93 per cent relative humidity but failed to grow on those equilibrated at 87 per cent. At 30° C, only the strain of *S. rouxii* grew on prunes equilibrated at 85 per cent relative humidity. All strains tested, however, grew at 97 per cent.

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