ZYGOSACCHAROMYCES PINI, A NEW SPECIES OF YEAST ASSOCIATED WITH BARK BEETLES IN PINES

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INTRODUCTION

The present work was undertaken to identify the yeasts that occur in blue-stained wood of trees infested by certain bark beetles. The general association of yeasts and yeastlike organisms with the blue-staining fungi isolated from the wood of beetle-infested trees has been noted by Rumbold (8) and it was with material supplied by her that this study was begun.

Cultures of yeasts were isolated from two species of beetles, Dendroctonus brevicomis Lec. from the Western States and D. frontalis Zimm. from the Southern States, received at the Forest Products Laboratory during 1932 and 1933. It was found that a heretofore undescribed zygosaccharomycete yeast was generally associated with both species of beetles and the wood of trees infested by them. Some anascosporous, mycelium-forming yeasts not described in this paper, were also frequently present.

During the summer of 1934 a more extensive study of the flora of the beetles of the Southern States confirmed the previous findings for Dendroctonus frontalis. In addition it was found that other bark beetles indigenous to the region, Ips grandicollis (Eich.), I. calligraphus (Germ.), and I. avulsus (Eich.), carry the same zygosaccharomycete.

A brief mention of the occurrence of this zygosaccharomycete has been made in a paper by Bramble and Holst (1). Since experimental work is being continued on the function of the yeast in relation to the blue-staining fungi, beetles, and the host, it seemed advisable to present the results of a determinative study at this time. This paper gives a technical description of the organism and a report of its known distribution.

HISTORICAL REVIEW

The first mention of the association of yeasts with blue-staining fungi and bark beetles appears to be that of Grosmann (3), made in 1930. She reported having isolated three nonfermenting types of yeast: (1) A budding yeast having hat-shaped ascospores arising parthenogenetically, (2) a yeast similar to the first type but forming mycelium, and (3) a type forming mycelium but anascosporous. Her first group resembles the organism described in this paper but differs in fermentation and method of spore formation.

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2 The writer wishes to express his appreciation to the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for the cooperation which made this study possible; to E. B. Fred and W. C. Frazier, of the University of Wisconsin, for their guidance and valuable criticism; and to C. T. Rumbold, of the Division of Forest Pathology, for her generous cooperation.
3 In cooperation with the Forest Products Laboratory, Forest Service, U. S. Department of Agriculture.
4 Reference is made by number (italic) to Literature Cited, p. 517.
Person (6), in 1931, demonstrated the concurrence of yeasts and a blue-staining fungus in beetle-attacked trees, and ascribed to the aroma of the yeast fermentation in the tree the role of attracting fresh insect attacks. No description of the yeast was given, however. Shortly after this, Rumbold (8) reported the presence of yeasts and bacteria in cultures of *Ceratostomella pini* isolated from the wood of trees attacked by *Dendroctonus frontalis* and *D. brevicomis*. The association was so intimate that dilution plates made with the ascospores from the perithecia "showed as many bacterial and yeast as fungous colonies." As stated before, many of these yeast cultures were used in the present work.

Recently Leach, Orr, and Christensen (5), in a paper on the interrelation of bark beetles and blue stain, called attention to the presence of a characteristic yeast, but gave no details as to the type of yeast. Buchner (2) has figured a yeast that forms hat-shaped ascospores, which he found occurring with a cerambycid, *Tetropium castaneum* Paky.

Grosmann's work indicated that the yeasts are not necessary to the life of the beetle as internal symbionts, but may act indirectly by making conditions in the bark more favorable for the developing brood of insects, and, as stated by Person, act as attractants through the fermentation they produce. Preliminary work on the problem, not included in this paper, has also failed to bring to light any evidence of a direct relationship between the yeast and the beetle.

**MATERIAL AND METHODS**

Cultures obtained from both eastern and western species of bark beetles over a 3-year period were studied to determine whether different species might appear from year to year or whether cultures from different species of beetles varied (table 1). The same species was isolated from year to year from different beetles. A single-cell isolation made in 1932 from a *Dendroctonus brevicomis* beetle infesting *Pinus ponderosa* Doug. in Oregon was chosen as the type culture, with which comparisons were made.

**Table 1.—Source of cultures of yeast associated with bark beetles**

<table>
<thead>
<tr>
<th>Blue-stained host</th>
<th>Beetle</th>
<th>Inoculum</th>
<th>Collected in—</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pinus ponderosa</em></td>
<td><em>Dendroctonus brevicomis</em></td>
<td>Beetles and wood</td>
<td>Oregon</td>
<td>1932</td>
</tr>
<tr>
<td>Do.</td>
<td>Do.</td>
<td>Beetles</td>
<td>do</td>
<td>1933</td>
</tr>
<tr>
<td>Do.</td>
<td>Do.</td>
<td>Wood</td>
<td>do</td>
<td>1932</td>
</tr>
<tr>
<td><em>P. echinata</em> Mill.</td>
<td><em>D. frontalis</em></td>
<td>do</td>
<td>do</td>
<td>1934</td>
</tr>
<tr>
<td>Do.</td>
<td>Do.</td>
<td>do</td>
<td>do</td>
<td>1933</td>
</tr>
<tr>
<td><em>P. strobus</em> L.</td>
<td><em>D. valens</em> Lec.</td>
<td>Beetles</td>
<td>do</td>
<td>1934</td>
</tr>
<tr>
<td><em>P. echinata</em></td>
<td>do</td>
<td>do</td>
<td>do</td>
<td>1934</td>
</tr>
<tr>
<td><em>P. ponderosa</em></td>
<td><em>Ips oregoni</em> Eich.</td>
<td>do</td>
<td>do</td>
<td>1933</td>
</tr>
<tr>
<td>Do.</td>
<td><em>I. emarginatus</em> (Lec.)</td>
<td>do</td>
<td>do</td>
<td>1934</td>
</tr>
<tr>
<td><em>P. echinata</em>, <em>P. virginiana</em> Mill.</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td>1934</td>
</tr>
<tr>
<td>Do.</td>
<td><em>I. artidus</em></td>
<td>do</td>
<td>do</td>
<td>1934</td>
</tr>
<tr>
<td><em>P. echinata</em></td>
<td><em>I. grandicollis</em></td>
<td>do</td>
<td>do</td>
<td>1934</td>
</tr>
<tr>
<td>Do.</td>
<td><em>I. calligraphus</em></td>
<td>do</td>
<td>do</td>
<td>1933</td>
</tr>
<tr>
<td><em>P. echinata</em></td>
<td><em>I. calligraphus</em></td>
<td>do</td>
<td>do</td>
<td>1933</td>
</tr>
</tbody>
</table>
A medium containing 2.5 percent of Trommer's malt extract and 1.5 percent of bacto-agar was used for all the cultural studies. Malt-extract broth of the same concentration was used for the study of growth in liquid medium. Beef-extract-peptone-starch agar was used in determining diastatic action. The fermentation work was carried out in solutions of 10-percent yeast water to which the sugars were added in approximately 2-percent concentration. No special methods were used in studying sporulation, since spore formation appears after a few days on malt-agar slants. Unless otherwise noted, all incubation was at room temperature, 24° to 26° C.

Cultures from wood were obtained according to the method described by Rumbold (7). Beetles were cultured by removing them from freshly exposed galleries in the tree, with either a sterile brush or needle, to malt-agar slants. When fungi were present along with the yeast they could be eliminated by making transfers every 24 hours for several days. If bacteria occurred mixed with the yeasts, a few transfers on malt agar acidified to pH 2.8 to 3.0 would yield a culture of yeast only. Final purification was accomplished by dilution plating, followed by single-cell isolations according to the method of Wright and McCoy (10).

As previously mentioned, all cultural observations were made on malt agar or malt-extract broth. Optimum temperature was determined by inoculating 10-cc amounts of malt-extract broth with equal volumes of a suspension of young cells, counts being made after 24 and 48 hours with a Neubauer blood-counting chamber. Cell size and shape were determined in water mounts. Measurements were made with a filar micrometer.

The action of the yeast on various sugars was tested qualitatively by the Durham tube method, and quantitative determinations of the amount of sugar destroyed were made by the method of Stiles, Peterson, and Fred (9). The Einhorn or Smith tube was also tried initially, but the results were not so reliable as those obtained with the Durham tube.

**DESCRIPTION OF ZYGOSACCHAROMYCES PINI, N. SP.**

A review of the literature has revealed no named organism with which the one here described can be identified. *Zygosaccharomyces pastori*, first described by Guilliermond (4), bears some resemblance to it in that hat-shaped spores are produced, but the differences in cell size, cultural characters, and sexual mechanism preclude the possibility that the two are identical or even closely related. The name *Zygosaccharomyces pini* is therefore proposed.5

**DIAGNOSIS**

*Zygosaccharomyces pini*, sp. nov.

Cellulis globosis, ellipsoideis vel ovatis, 2.0–6.4 × 2.5–7μ (in culturis liquidis 2.9–5.5 × 3.6–7μ), gemmantibus; ascosporis 4, post copulationem heterogamicam oriundis, pileiformibus costulatis, sine costula 1.6–2.0 × 2.3–3μ.

In ligno *Pini* ssp. cum *Dentrocrono* et *Ipse* ssp. consociatus, U. S. A. Indueit fermentationem in glucosio, fructosio, et mannosio, nec allii saccharis vulgaribus.

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5 Type cultures have been deposited at Centraalbureau voor Schimmelcultures at Baarn, the Netherlands, and at the Division of Forest Pathology, Forest Products Laboratory, Madison, Wis.
MORPHOLOGY

In 3-day-old malt-extract agar slants or malt-extract broth, cells round, ellipsoidal, or egg-shaped; cells on slant 2.0μ to 6.4μ by 2.5μ to 7.3μ; in broth, 2.9μ to 5.5μ by 3.6μ to 7.0μ. Asexual reproduction by budding (fig. 1, A).

Ascospores formed after heterogamie conjugation. Sporulation occurs readily after several days on malt-extract slants. Asci contain four hat-shaped ascospores measuring, without the brim (fig. 1, B), 1.6μ to 2.0μ by 2.3μ to 3.0μ. Spores germinate without fusion, commonly by budding, occasionally by means of germ tube.

CULTURAL CHARACTERS

Slant cultures.—On 7-day-old slant cultures (pl. 1, A) growth moderate, filiform, raised, smooth, white, glistening, opaque, butyrous; medium not discolored. In older cultures growth spreading, edge finely lobate, surface may become papillate, color changes from white to fawn (pl. 1, B), often with white sectors remaining, perpendicular to line of inoculation.

Broth cultures.—Moderate turbidity, with slight ring present at surface after several days, no pellicle; finely granular sediment.

Giant colony.—Moderate growth, spreading, surface at first smooth, may later become papillate, irregular to lobate edge, color at first white, later fawn-colored, white radial sectors often persisting (pl. 1, C).

PHYSIOLOGY

Glucose, fructose, and mannose fermented with production of acid and gas; xylose, arabinose, maltose, galactose, sucrose, lactose, and raffinose not fermented. No hydrolysis of starch. Gelatin not liquefied. No change in litmus milk after 7 weeks.

Maximum temperature for growth on malt agar, 37° to 38° C.; optimum, near 30°. Maximum temperature for sporulation, 34° to 35°; growth and sporulation at 4°.

DISCUSSION

The description given for morphology and cultural characters is that of a recently isolated, freely sporulating culture. When the cultures are carried for a period of from several months to a year or more, anascosporous strains commonly begin to appear, as evidenced by the frequency of white sectors. By repeated transfer from the white sectors it is usually possible to obtain cultures that no longer produce ascospores. Even without such selection certain cultures...
ultimately lose the sexual stage and a distinct change in morphology takes place, by which such cultures may be recognized; that is, large numbers of relatively larger round to slightly oval cells 4μ to 6μ in diameter, exhibiting multiple budding (fig. 1, C), appear. In fact, the appearance of these cells may be taken as a forecast of incipient anascosporogeny. No change in physiology has been found to occur when a culture loses its ascospore-forming property.

There may be a variation, even with freshly isolated cultures, in the rapidity and extent of sporulation. With some cultures ascospores have not been observed until the culture is 2 to 3 weeks old, while in others after only 5 days approximately 95 percent of the cells present were ascospores. Cell size, cultural character, and fermentation properties of these various cultures were found to be in agreement, and no separation into strains appears to be justifiable on the basis of vigor of sporulation alone.

Cultures of *Zygosaccharomyces pini* possess only weak fermentative power. The yeast grows readily on glucose, fructose, and mannose, producing acid and gas in Durham tubes at room temperature, but in many cases gas does not appear until the eighth or ninth day even upon shaking, and from then on the amount increases slowly, reaching a maximum after several weeks. The fact that no acid or gas is produced cannot be taken as evidence that the sugar is not used by the organism. For example, when xylose, arabinose, maltose, galactose, sucrose, lactose, or raffinose yeast-water broths were inoculated with *Z. pini*, neither gas nor acid has produced, yet by quantitative analyses it was found that both xylose and galactose had been utilized, 25 percent of the xylose and 60 percent of the galactose having disappeared after 37 days. The remaining five sugar solutions still contained the original amount of sugars after inoculation and prolonged incubation, although the yeast grew in the solutions. It is of interest to note that the five sugars which the organism can break down, that is, glucose, fructose, mannose, xylose, and galactose, all are known to occur in coniferous woods but not as simple sugars.

**SUMMARY AND CONCLUSIONS**

A yeast has been found generally associated with the bark beetles *Dendroctonus brevicomis*, *D. frontalis*, *D. valens*, *Ips oregoni*, *I. emarginatus*, *I. avulsus*, *I. grandicollis*, and *I. calligraphus*. A determinative study of the organism showed it to belong to the genus *Zygosaccharomyces*, as ascospore formation is preceded by a sexual process. The formation of hat-shaped ascospores, together with the fact that of the common sugars only glucose, fructose, and mannose are fermented, make it necessary to consider this yeast as a new species. The name *Zygosaccharomyces pini*, n. sp., is therefore proposed, and a description of the cultural, morphological, and physiological characters of the species is given.

**LITERATURE CITED**


