FOUR NEW SPECIES OF YEAST ISOLATED FROM INSECT FRASS IN BARK OF *TSUGA HETEROPHYLLA* (RAF.) SARGENT

by

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During an exploratory investigation of yeasts associated with bark beetles which attack the coast- or western hemlock (*Tsuga heterophylla*), four new species of yeast were discovered in insect frass taken from the bark or phloem of a number of specimens of killed or dying trees. The collection was made in N. W. Oregon along the bank of the Siletz River in late fall. Samples I and II were taken from young trees with dying tops and branches. In these trees identification of the causative insect was not made. Sample III was from a large tree, killed about one year previously to the collection date. Judging by the pattern of the insect galleries, the causative insect presumably was *Scolytus tsugae* Sw. Among a number of known yeasts, four new species emerged which belonged to the genera *Sporobolomyces*, *Bullera*, *Candida* and *Cryptococcus*. The new species of *Sporobolomyces* was non-pigmented and thus requires an amendment of the genus definition as given by LODDER and KREGER-VAN RIJ (1952). Neither the species of *Sporobolomyces* nor that of *Bullera* formed ballistospores on malt agar or on potato glucose agar. Spore discharge was unexpectedly found to occur on corn meal agar, which, so far, appears to be the most sensitive medium to demonstrate ballistospore formation (DO CARMO-SOUSA and PHAFF, 1962).

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Methods.

A small amount of each sample of frass was sprinkled evenly over the surface of six plates with malt agar, acidified with HCl to a pH of about 3.8 to reduce bacterial growth. The plates were incubated at room temperature (21-24°C) for 4 to 6 days, depending on the number of mold colonies which appeared with the yeasts. The relative number of the various yeasts was estimated from colony morphology and microscopic inspection. After purification, the isolates were identified by procedures given by LODDER and KREGER-VAN RIJ (1952) and by WICKERHAM (1951). Preliminary carbon assimilation studies were made by the use of a multipoint inoculating device (BEECH and CARR, 1955) with carbon compounds incorporated in yeast nitrogen base (Difco) using flat-bottomed Petri dishes. Confirmation of the assimilation reactions of the new species was done in liquid media (WICKERHAM, 1951). The pH of the media containing organic acids was adjusted to 4.5. Ballistospore formation was studied as described by DO CARMO-SOUSAl and PHAFF (1962). Growth at elevated temperatures was estimated visually after 7 days of incubation. A light inoculation was made on the surface of wort agar slants and in liquid media (0.5% yeast autolysate plus 2% glucose).

Results.

The yeasts which were isolated are listed in Table 1.

As indicated in Table 1 four new species of yeast were discovered in the samples of frass. Their descriptions are given below.

Sporobolomyces singularis nov. spec.

Description.

Growth in malt extract: After 3 days at 23°C. cells are oval to ellipsoidal, (2.6 - 3.5) \( \times \) (4.4 - 7.0) \( \mu \), single, in pairs or in short, branched chains. Cells bearing sterigmata and ballistospores did not occur. A thin ring formed gradually, but no pellicle. After one month only a ring and sediment were present.

Growth on malt agar: Cell morphology similar to that in liquid wort. After one month the streak culture was creamy-white; surface highly glossy, smooth with some transverse striations;