PHYSIOLOGICAL PROPERTIES, CONJUGATION AND TAXONOMY OF CEPHALOASCUS FRAGRANS
HANAWA 1920
(SYN: ASCOCYBE GROVESII WELLS 1954)

by

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From a piece of decayed oakwood, imported from Japan, among many other molds one species was isolated which on first sight seemed to be an ordinary Penicillium spec., but which after a closer inspection proved to be a very unusual yeast-like organism belonging to the Ascomycetes. The brushes of this mold were not formed by strings of conidia, but by asci which contained four hat-shaped ascospores each. In older cultures most of the brushes were enclosed in dew-drops. By removing the drops with a micropipet and spreading the contents of the pipet over a nutrient agar it appeared that each drop contained the astonishing large number of 300-500 ascospores.

By making single spore cultures with the aid of the Burri technique (1909) it could be proved that the ascomycete is homothallic, as from each spore a mycelium with ascospores develops. This property has led us to look for the sexual reproduction, which turned out to take place in a rather unusual way.

Initially it was found that this organism had been described earlier as Ascocybe grovesii by Wells (1954), who considered it to be the cause of the so-called “brown surface stain” on white pines in Ottawa. After thoroughly examining the literature it appeared from Ainsworth (1961) that this organism had already been described in Japan as Cephaloascus fragrans by Hanawa (1920), who remarkably enough isolated it from the external auditory canal of a student.
As we too found the yeast on wood, it seems that the presence of this yeast in a human ear must have been accidental.

G. C. AINSWORTH has informed the authors that he has come to the conclusion of synonymy after comparing the descriptions and the figures of HANAWA (1920) and WELLS (1954), to which J. W. GROVES had drawn his attention.

As both descriptions of this curious organism are still rather incomplete, we thought it worthwhile to give a more complete description.

In order to be able to compare our culture with other cultures, we asked for the original culture of *Cephaloascus fragrans* Hanawa at the Centraalbureau voor Schimmelcultures at Baarn and the type culture of *Ascocyebe grovesii* Wells at the A.T.C.C.

**Methods.**

We generally used the methods as applied by LODDER and KREGER-VAN RIJ (1952). For the assimilation of carbohydrates however we did not use the auxanographic method of BEIJERINCK, but preferred to streak on agar plates, which each contained 2% of one kind of carbohydrate. With this method we found more clear-cut results than with the auxanographic method. Growth was observed after 3–5 days' incubation at 28°C.

Nuclear staining was sometimes performed on smears, but as in these preparations conjugations are very difficult to observe, we used also other preparations. A good method was to cultivate the yeast on a sterile slide moistened with malt extract. The slide was placed in a petri dish with malt agar from which a piece of agar with the size of the slide had been cut out. When the organism had developed to the desired stage, the slides were dried, fixed and stained by a procedure of PEARSE (1954) or by a nuclear staining procedure of WINDISCH (1940) described in "Die Hefen" p. 58 (1960), which gave the best results. The technique is:

1. Fix the preparation during two minutes with chloroform.
2. Rinse one minute with aqua dest.
3. Hydrolyse at 60°C. in 1N HCl for about 5–10 minutes.
4. Rinse briefly in aqua dest.
5. Cover the object with 2% saturated alcoholic basic fuchsin solution.
6. When after some minutes the alcohol begins to evaporate, add an excess of a 15% acetic acid solution.