NOTES

Separation of Phases with Sexually Different States in Hansenula californica

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Some time ago, we isolated a Hansenula culture, Ac 12, which Wickerham identified as a strain of H. californica.

By testing the suitability of James (J. Bacteriol. 67:237, 1954) agar for detecting whether the utilization of sugars occurs after induction or constitutively, we found that Ac 12 grown on certain sugars (maltose, saccharose, galactose, or glucose) formed different colonies (Fig. 1). Upon transferring these colonies to James agar with glucose as the carbon source, cultures were obtained with permanently formed colonies of a totally different appearance (Fig. 2 and 3).

These two phases are morphologically identical; moreover, they show the same fermentation spectrum of sugars, assimilation of carbon sources, etc.

The differences between the two phases do not appear only in the colonies (on James agar, on malt-agar, or on glucose-yeast extract-agar) but show up very strikingly in the sporulation.

Ac 12 R sporulates in malt extract, glucose-yeast extract-agar, James maltose-agar, etc., from the 2nd day. When observations are made after short incubation periods (2 to 8 days), round and Saturn-shaped spores are clearly to be seen, and a predominance of ascii is formed from diploid vegetative cells (Fig. 4). On the other hand, after 20 days only spheroidal spores appear; the normal isogamous and heterogamous conjugations may be seen, as well as ascus with conjugation tubes of various lengths. The dehiscence of the ascii is rapid, and after 3 or 4 weeks of incubation only liberated spores, broken ascus, and very few vegetative cells may be seen (Fig. 6).

In contrast, in Ac 12 N, on any one of the sporulation media tested, conjugation begins after 6 to 7 days of incubation, but the formation of spores does not commence until the 20th to 25th day; no Saturn-shaped spores appear, and a greater delay in the liberation of spores is observed. The free spores agglutinate in large masses, giving the impression that they are immersed in a liquid, forming a tapioca-like aggregate (Fig. 7); the proportion of sporulation is much lower than in the R phase, and after 40 to 55 days of incubation a large number of vegetative cells are still present.

Also worthy of mention is the fact that on glucose-yeast extract-agar plates (prepared by flooding the surface of agar with a dilute suspension in sterile water, allowing to stand for a few minutes, and then inverting the plates), after 3 days of incubation, one may note in the R and N phases a strong smell of oyster and observe that the colonies are made up of vegetative cells without conjugation tubes or formation of ascus from diploid cells; after 7 days of incubation, in both cases, sporulation or conjugation, or both, are observed, and the smell of oyster disappears.

Also worthy of note is the fact that, on James saccharose- or maltose-agar, the formation of spores in Ac 12 R is similar to that produced in other sporulation media, whereas with glucose or
Fig. 2. Hansenula californica Ac 12 N. Colonies on James glucose-agar after 6 days at 25 C. X5.
Fig. 3. Hansenula californica Ac 12 R. Colonies on James glucose-agar after 6 days at 25 C. X5.
Fig. 4. Hansenula californica Ac 12 N. Colonies on James maltose-agar after 12 days at 25 C. X4.

Fig. 5. Hansenula californica Ac 12 R. Sporulation on malt extract-agar slants after 2 days at 25 C. Phase-contrast. X2,000.
Fig. 6. Hansenula californica Ac 12 R. Free spores on malt extract-agar slants after 44 days at 25 C. Phase-contrast. X2,000.
Fig. 7. Hansenula californica Ac 12 N. Sporulation on malt extract-agar slants after 44 days at 25 C. Phase-contrast. X2,000.

Galactose neither diploid asci nor asci through conjugation are formed. Evidently, this shows that, although on James glucose-agar totally uniform colonies (Fig. 2 and 3) are obtained on the purification plates, the same does not occur on James maltose- or saccharose-agar (Fig. 4).

In view of the inconsistency of characteristics now employed to define a yeast species, we consider of possible interest the above-described technique for separating different phases in yeasts, as it could be particularly interesting from the point of view of yeast genetics.