FURTHER INVESTIGATIONS ON DIOSZEGIA HUNGARICA ZSOLT

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The asporogenous yeast *Dioszegia hungarica* described by the author (ZSOLT 1957) was further investigated. In this article some macromorphological and micromorphological observations are published and the interpretation of the data is discussed.

Material and methods

The type strain of the yeast maintained in the collection of the Institute for Plant Physiology of the University Szeged was investigated.

According to the low optimal temperature (15—20° C) of *Dioszegia* all cultivations were on room temperature (about 18—22° C) performed.

For demonstration of capsules the cells were suspended in diluted Indian ink and the suspension was smeared out. After drying it was stained with methyl violet. The phase contrast photogrammes were on native preparates obtained.

Colour of the colonies was determined with the aid of an Ostwald-scale.

Results and discussion

I. S- and R-form of Dioszegia hungarica

Dioszegia herself is a very slow growing organism. But a more slow growing colony was observed on a malt agar plate inoculated with a dilute suspension of the original strain. The variant was inoculated on malt agar slant and the macroscopic and microscopic picture was compared with those of the original strain.

The colonies of the new strain were dull, fine warty, while those of the original strain were smooth and shining. The colonies of the original strain were low convex, while those of the new strain were definitely flat. Both of the strains had a similar colour, about 5. X. nc. according to the Ostwald-scale. The microscopic picture was the same in each of them. So in first place the oblong budding cells and the giant cells characteristic for the species were always present. The other cells had also the same form and size.

S- and R-variation of yeasts was on several occasion observed (e. g. FABIAN—MCCULLOGH 1934; NYBERG 1942, MAGER—ASCHNER 1947). The abovementioned observations on *Dioszegia* may be considered as a further example for this type of variation. Transformation of the original S-form into the R-form seems so far irreversible. No transformation in the opposite direction was observed during three years cultivation on malt agar slants.

II. Demonstration of capsules

With the aid of preparates made with the generally used „Indian ink" method and stained with methyl violet the presence of capsules was demonstrated in the original strain but not in its R-variant (Fig. 1.).

The presence of capsules appears already on unstained preparates. The neighbouring cells in microscopic preparates are never so closely packed than in the case of non-capsulated yeasts. In the latter the side by side setteled cells show polygonate contours. In contrast to this *Dioszegia* cells seem always rounded off (Plates I. and II.).

Development of a slimy capsule is characteristic on the genus *Cryptococcus* according to LODDER and KREGER VAN RIJ (1952). Capsule was demonstrated in several *Rhodotorula species* too (e. g. ULSON 1958; HASEGAWA et al. 1960). Just the presence of capsule in both abovementioned genera and also the presence of carotenoid pigments in several *Cryptococci* (e. g. NAKAYAMA et al. 1954; PETERSON et al. 1954) was the reason for supposing a closer relationship between *Cryptococcus* and *Rhodotorula* (LODDER—KREGER van RIJ 1952; HASEGAWA et al. 1960). Accordingly *Dioszegia* seems to be a member of this group. In the new system proposed for yeasts by NOVÁK and ZSOLT (1961) all the three genera are found in the same subfamily *(Cryptococcoideae)* of the family *Cryptococcaceae.*

III. The life cycle

On the Plates I. and II. microscopic pictures of 3, 10, and 60 days old cultures are shown. The microphotogrammes are produced with the aid of phase contrast system. This was necessary because the cell walls are extremely thin and the ordinary microscopic picture is therefore very contrast-less. Through the phase contrast microscope the little, oblong budding cells are dark, with few granules. One may observe on these pictures also the characteristic lateral buds arising on short sterigmata (Plate I. Figs. 1.—4.). Cells of the older cultures are for the most part greater, oval, rather hyaline with dark granules (Plate II. Figs. 1.—2.). Most of the cells of the old cultures are great, globose and hyaline except the granules which are little and very numerous or they join to a single, great central body (Plate II. Figs. 3.—4.). From the refraction in ordinary microscope may be concluded, that these granules has a lipoid nature. The wall of of the giant cells may be fragile. The preparates from old cultures are full of little, dancing granules beeing set free from disintegrated cells.

The great lipoid content of the giant cells shows that they may be considered as involutionary forms. All intermediate forms between the little, oblong budding cells and the giant cells could be observed. Examples are shown on Plate II. Figs. 1.—2. It is interesting, however, that by all of the intermediate forms and by the giant cells too arising of the little, oblong budding cells was observed if only in few cases (Plate II. Figs. 2., 4.).

For interpretation of these observations a scheme is constructed for the life cycle of *Dioszegia* (Fig. 2.). According to this the little, oblong budding

cells became gradually into giant cells and then in all likelihood they die. The possibility of rise of budding cells is, however, even from certain giant cells given.

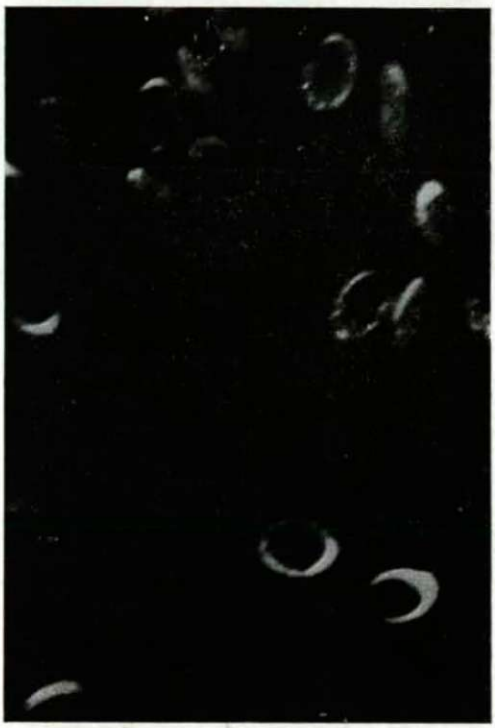

Figure 1. Capsules of *Dioszegia hungarica* cells.

The proposed life cycle is superficially similar to the life cycle of *Candida pulcherrima* supposed by VAN DER WALT (1952). But the difference between the two life cycle is great. The *„pulcherrima"* cells may be considered as chlamydospores which after a period of dormancy germinate and give rise the normal budding cells. The giant cells of *Dioszegia* are degenerated forms which mostly die and only rarely produce the normal budding form.

Summary

From the original culture of *Dioszegia hungarica* a more slow growing strain was obtained. The phenomenon may be considered as an S—R-variation. Capsules were demonstrated in the original strain by stained preparates. Phase contrast microphotogrammes are given from cultures of different age. A life cycle is proposed for interpretation of the microscopic pictures.

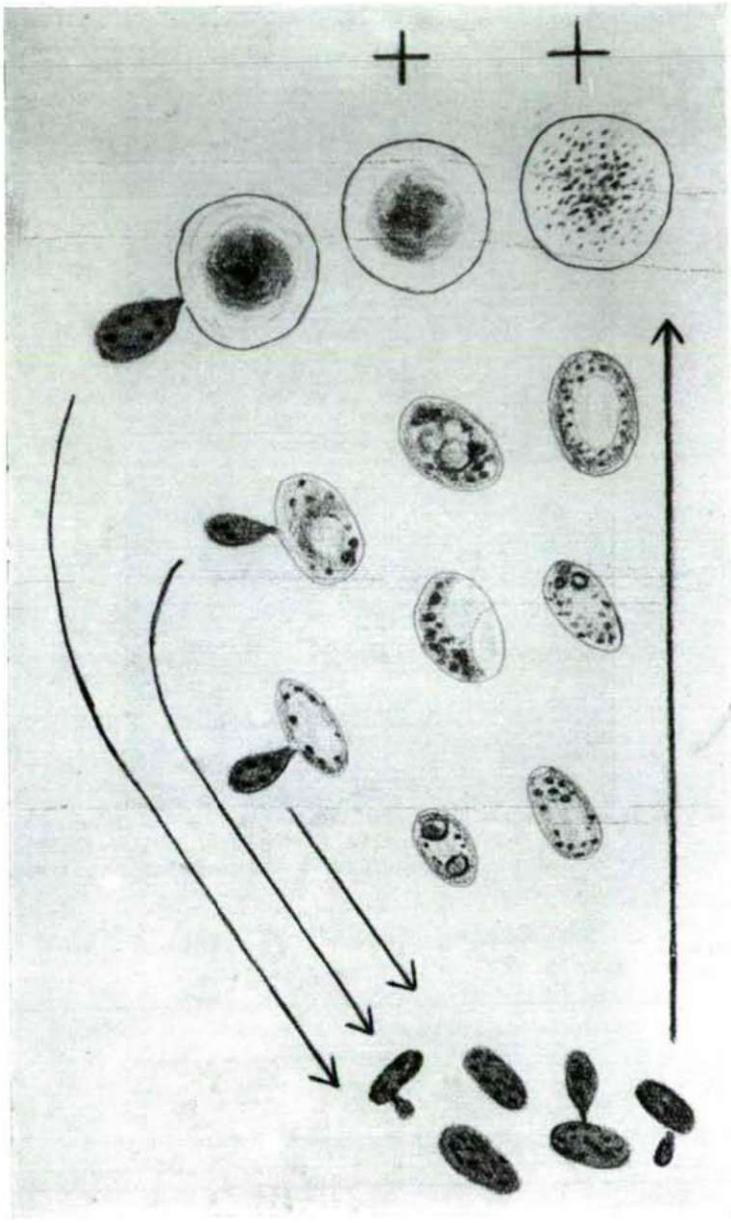

Figure 2. Life cycle of *Dioszegia hungarica*.

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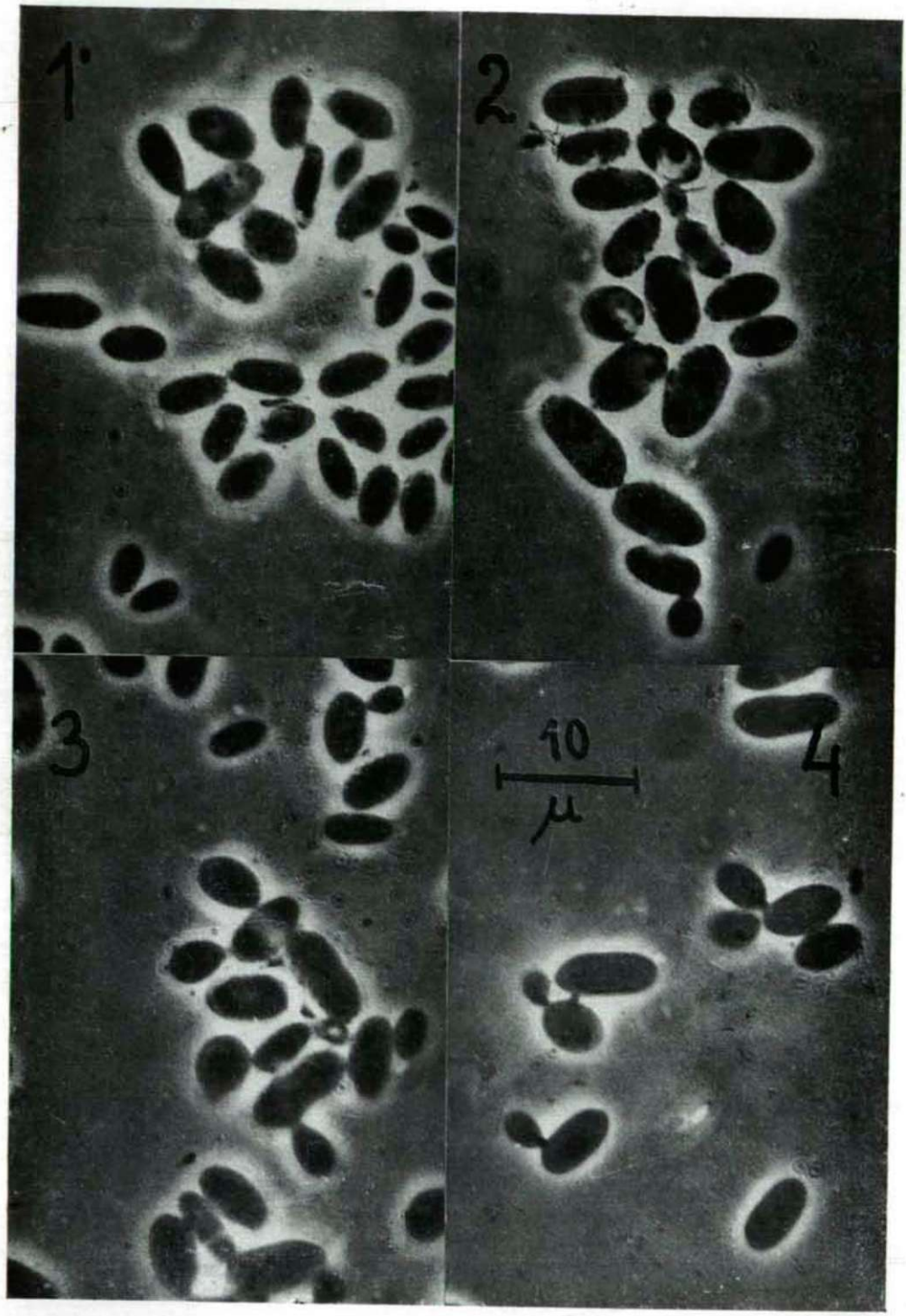
Plate I. Microphotogrammes of 3 days old malt agar cultures of *Dioszegia hungarica.*

Plate II. 1. and 2. Microphotogrammes of 10 days old malt agar cultures of *Dioszegia hungarica.* 3. and 4. Microphotogrammes of 60 days old malt agar cultures of *Dioszegia hungarica.*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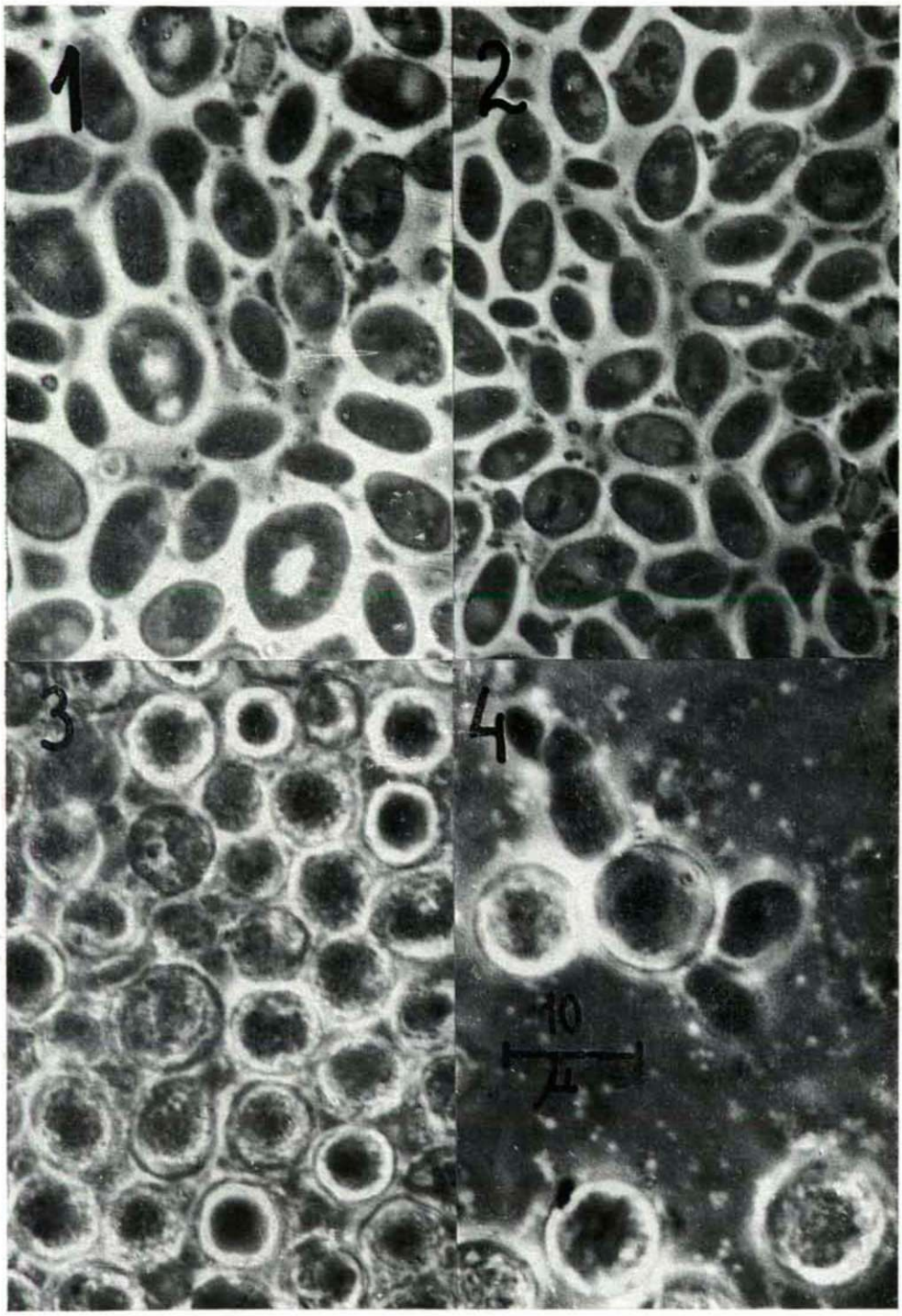
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