deep orange wurde papierchromatographisch festgestellt. In allen geprüften Stadien von *dor/dor-* und *ClB/dor-*Weibchen ist mehr Isoxanthopterin nachweisbar als bei +-Weibchen. Bei Männchen der Mutante findet sich in Puppenstadien weniger Isoxanthopterin als bei +-Männchen; nur bei frisch geschlüpften Fliegen entsprechen sich normale und *dor*-Männchen in ihrem Isoxanthopterin-Gehalt. Bei *dor/dor-* und *dor/Y*-Tieren sind ausserdem noch andere Pterine betroffen. So zeigen die Köpfe der *dor*-Mutante weniger rote Pigmente, sowie mehr «Xanthopterin» und «Sepia-Pterin» als Fliegen des Wildtyps, dagegen kommen HB_1 und HB_2 in normaler Menge vor. Im Kopf der heterozygoten Weibchen werden für alle diese Pterine Werte gemessen, die sogar den Wildtypus beträchtlich übertreffen. Bei *dor/dor* und *dor/Y* findet sich in den Malpighischen Gefässen eine stark erhöhte Konzentration an fluoreszierenden Stoffen.

Taxonomical Implications of the Properties of a Riboflavin-producing Mutant Yeast

Introduction.—SUBRAMANIAM and RANGANATHAN[1] isolated a top yeast, BY 2, after treatment of a brewery bottom yeast with acenaphthene for 90 days. Studies on the biochemical properties of the mutant revealed[2] that the strain is capable of synthesizing and excreting into the medium a considerable amount of riboflavin under simple cultural conditions. The remarkable stability of this property of the mutant has been varified through continued observation and study[3] during the past 10 years.

Certain yeasts[4] and yeast-like organisms[5] are known to be endowed with this property of producing large amounts of riboflavin. The question was posed whether the artificial alteration of the chromosomal constitution[1] of the parent strain had resulted in the creation of one of these organisms having acknowledged property of riboflavin production. An alternative hypothesis would be that the mutant yeast, BY 2, is a new species of yeast created by an induction of mutation. In order to elucidate this point, the properties of the mutant yeast were analysed with regard to the major characteristics having taxonomical implications.

Observations.—I. Cultural characteristics. Typical giant colony of the mutant in barley malt agar is rough and has a wavy outline with a distinct ring around the periphery. The young colonies are white and dry; they become moist, slimy and brown with age.

In *still* liquid culture, the strain forms distinct pellicles which are white, dull and wavy in character. They appear as islets and subsequently unite to form a distinct surface film; the rest of the medium remains clear. On prolonged incubation, the film gains in thickness and finally settles down forming a flocculant sediment and leaves a distinct ring around the surface. A homogeneous growth is obtained in *agitated* liquid media. Deep yellow colour due to the excretion of riboflavin is evident in appropriate liquid media on prolonged incubation.

II. Morphological characters.—The cells of the mutant show a wide degree of polymorphism. The cell shape varies from bulb-shape through long oval to fairly elongated ones. Polymorphism is less evident in young culture in agitated liquid media where the range of size of the cells is usually (5–9) $\mu \times (2{\cdot}5\text{–}3{\cdot}5)\mu$. The reproduction of the cells is by multilateral budding and the cells have a very distinct tendency to become pseudomycelial. Sporulation is absent.

III. Physiological characteristics

(1) *Sugar fermentation.*—Fermentation tests in Einhorn tubes were carried out with media composed of 1% peptone and 4% of the individual sugars, *viz.*, glucose, galactose, maltose, sucrose and lactose. No gas formation was observed in any of the tubes even on prolonged incubation for 15 days. The mutant yeast is a purely non-fermentative organism.

(2) *Sugar utilization.*—Experiments on the assimilation of sugar in Laurent's medium enriched with the vitamin supplement solution show that the mutant yeast can utilize glucose, galactose, maltose and sucrose. No growth is observed in medium containing lactose as the sole source of carbon.

(3) *Assimilation of nitrogen.*—Lodder's medium with the vitamin supplements was used for these tests. Nitrogen was supplied at a level of 21 mg/ml in the form of peptone, urea, asparagine hydrate, ammonium sulphate and potassium nitrate in the individual media tested. The results of these experiments show that very good growth of the mutant is obtained in medium containing peptone as the sole source of nitrogen. Ammonium sulphate, asparagine hydrate and urea can also serve independently as suitable sources of nitrogen for the growth of the mutant. The mutant is incapable of utilizing potassium nitrate as a source of nitrogen.

(4) *Utilization of ethanol.*—A simple inorganic medium containing $MgSO_4$, $7H_2O$; KH_2PO_4; $(NH_4)_2SO_4$ and enriched with biotin was used for these tests. A positive growth is obtained when ethanol is incorporated at a level of 2% by volume in this medium. The mutant is capable of utilizing ethanol as the sole source of carbon in the medium.

(5) *Effect on litmus milk.*—Tubes containing sterile litmus milk with and without calcium lactate were inoculated with the mutant yeast and incubated at 30°C. Results of observation after 3 weeks of incubation show that: *a*) the colour of litmus milk changes to blue, and *b*) in tubes containing added calcium lactate, there is coagulation along with the changing of the colour to blue.

Discussion. An analysis of the properties of the mutant yeast, BY 2, detailed above shows that the strain resembles in a general way *Candida scotii* DIDDENS et LODDER[6] but differs from it in the following characters:

[1] M. K. SUBRAMANIAM and B. RANGANATHAN, Nature *157*, 49 (1946).

[2] K. K. MITRA, J. sci. industr. Res. *8B*, 236 (1949); *11B*, 109 (1952).

[3] K. V. GIRI and P. R. KRISHNASWAMI, J. Bact. *67*, 309 (1954). – K. K. MITRA, J. sci. industr. Res. *14C*, 21 (1955); *15C*, 257 (1956). – K. V. GIRI and P. R. KRISHNASWAMI, J. sci. industr. Res. *13A*, 106 (1954).

[4] P. R. BURKHOLDER, Arch. Biochem. *3*, 121 (1943).

[5] F. W. TANNER jr. and J. M. VAN LANEN, J. Bact. *54*, 38 (1947). – A. GUILLIERMOND *et al.*, C. r. Acad. Sci. *201*, 1077 (1935).

[6] J. LODDER and N. J. W. KREGER-VAN RIJ, *The yeasts – a taxonomic study* (Amsterdam 1952).

Properties	*C. scotii*	BY 2
1. Growth in malt extract	Sediment	Pellicle
2. Assimilation of potassium nitrate	Positive	Negative
3. Utilization of ethanol	Occasionally some growth.	Positive
4. Riboflavin excretion	Nil	Positive

The question arises as to how far these differences in properties are of taxonomic importance. Pellicle formation has been used as the criterion for differentiating certain species of *Candida* (e.g. *C. mycoderma* from *C. zeylanoides*)[6] capable of assimilating only glucose. Similarly, the ability to utilize potassium nitrate separates *C. pelliculosa* from *C. albicans* and *C. parapsilosis*. Finally, while the mutant yeast, BY 2, excretes into the medium an appreciable quantity of riboflavin, there is no record of a similar behaviour in *C. scotii*.

Therefore the riboflavin producing mutant yeast, BY 2, has to be considered as a new species, and the name[7] *Candida ghoshii* n. sp. is proposed for it.

Its position in the key to the species of the genus *Candida* given by LODDER *et al.*[6] is indicated below: No sugar fermentation. Glucose, galactose, saccharose and maltose assimilated.

a) Early pellicle formation; nitrate not assimilated; riboflavin excreted ... *C. ghoshii* n. sp.

b) No pellicle; nitrate assimilated; no riboflavin excretion ... *C. scotii*.

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K. K. MITRA

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Résumé

Pour une détermination taxonomique, nous avons étudié les caractères morphologiques, physiologiques et culturels d'une levure mutante produisant la lactoflavine. Tout d'abord cette souche mutante est produite en traitant à l'acenaphthène et levure de bière à fermentation basse. Cette souche est asporogène et présente des formes pseudomycéliques. Elle est incapable de fermenter le glucose et n'utilise pas le KNO_3 comme source d'azote. Elle n'assimile pas le lactose. Après une étude approfondie sur les différents caractères de cette souche, nous estimons qu'elle peut être considérée comme une nouvelle espèce de levure du genre *Candida*. Nous l'avons appelée *C. ghoshii* n. sp.

[7] The species is named after Sir J. C. GHOSH, who gave a fillip to these investigations on yeast at the Indian Institute of Science, Bangalore.

Glycolitic Enzymes in the Human Amniotic Fluid

Very few enzymatic activities have so far been described in the amniotic fluid. Only scanty data exist on cholinesterase[1], phosphatase[2], and lysozima[3].

As it is well known that glucose, fructose and, probably, other reducing sugars are present in the amniotic fluid[4], it was a matter of interest to investigate whether glycolytic activities could be detected in this liquid.

The following enzymatic activities have been examined: phosphohexose-isomerase, ribose-5-phosphate-isomerase, aldolase and lactic-dehydrogenase.

Determinations have been made of the total protein content of the amniotic fluids under examination.

Experimental.—The samples of amniotic fluid (of about 15 ml each) were collected from pregnant women at term by transabdominal puncture, *intrapartum* by vaginal puncture of amniotic sac or during Caesarean section.

The samples were filtered and stored at 0° for no longer than 24–48 h. Samples contamined by blood or meconium were discarded.

The protein content was determined by the GOA's micromethod[5].

Phosphohexoso-isomerase.—The activity of this enzyme was determined according to BODANSKY[6]: in a centrifuge tube 0·3 ml veronal buffer pH 7·8 0·1 *M*, 0·3 ml glucose-6-phosphate sodium salt 0·045 *M*, 0·2 ml amniotic fluid were pipetted. The mixture was incubated at 37° for 1 h and the reaction stopped by addition of 3 ml of 20% trichloroacetic acid (TCA). After centrifugation, fructose was determined on 2 ml of the supernatant according to ROE[7].

Aldolase.—Determinations were carried out in a centrifuge tube mixing 1 ml of TRIS-HCl buffer pH 7·4 0·1 *M*, 0·25 ml hydrazine 0·56 *M* pH 7·4, 0·25 ml iodoacetate 0·002 *M*, 0·25 ml distilled water and 1 ml of amniotic fluid and 0·25 ml hexose 1-6-diphosphate Na salt 0·06 *M*[8].

The mixture was incubated for 1 h at 37°, and successively deproteinized by 3 ml of 10% TCA. After centrifugation, triosephosphate was determined on 1 ml of the supernatant according to SIBLEY and LEHNINGER[9]. For standardization of method, in some samples alkali-labile phosphorus was determined[10] in duplicate.

Ribose-5-phosphate-isomerase.—This activity was tested according to AXELROD[11]: 0·5 ml of TRIS-HCl buffer pH 7·4 0·1 *M*, containing 0·5 mg Ribose-5-phosphate (Ba salt) were mixed with 0·5 ml of amniotic fluid; after incubation at 37° for 30 min ribulose-5-phosphate

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