J. gen. Microbiol. (1963), **31**, 171–177
Printed in Great Britain

Rhodotorula nitens sp.nov. Isolated from the Atmosphere

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(*Received* 13 *August* 1962)

SUMMARY

A new species of the genus *Rhodotorula* isolated from the atmosphere in Edinburgh is described. It is characterized by the presence of a capsule and a predilection for low temperatures.

INTRODUCTION

During a survey of the yeast flora associated with man (Mackenzie, 1961), a single colony of a new orange-coloured yeast developed on an atmospheric settling plate exposed in the bacteriology laboratory at the Astley Ainslie Hospital, Edinburgh. Its description according to the methods and criteria defined by Lodder & Kreger-van Rij (1952) is noted below.

DESCRIPTION

Rhodotorula nitens nov. sp.

In musto maltato cellulae singulae rotundae aut subrotundae $3–8\mu \times 3{\cdot}5–9\mu$. In agaro maltato formae et dimensiones cellularium eadem sunt quae in musto maltato. Gemmae singillatim formantur in basi subgrandi. Forma capsulae. Insulae et anulus formantur. Sedimentum grave. Cultura (post unum mensem, 17°) mollis, rasilis, mucosa, nitens, convexa, patula, colore aurantiosa, margines rasiles. Pseudomycelium nullum. Fermentatio nulla. In medio minerali cum glucoso galactoso saccharo maltoso et lactoso crescit. Nitras kalicus non assimilatur. In medio minerali cum alcohole aethylico non crescit. Arbutinum finditur. Non multum amidum producitur. Ex aere separata Edinburgi in Scotia.

Typus: Dept. Microbiol. Queen's Univ. Belfast, AA8.
CBS 4256
NCYC 607

Growth in malt extract. After 3 days at 17°, cells single, rounded or subspherical, occasionally short-oval, measuring $3–8\mu \times 3{\cdot}5–9\mu$. Buds formed on a relatively broad base. Capsule present. After one month at 17°, conspicuous ring and islets; formation of a heavy flocculent sediment.

Growth on malt agar. After 6 days at 20° cells rounded or subspherical (Fig. 1). Buds formed at one point on the cell surface; prior to bud formation the cell is distinctly apiculate; subsequently, a prominent bud scar is present. Large prominent vacuole: numerous refractile cytoplasmic inclusions. Capsule present, ap-

Vol. 31, *No.* 1 *was issued* 11 *April* 1963

proximately equal to the radius of the cell. After 1 month at 17°, the streak is soft, smooth, mucoid, glistening, convex, spreading, orange-coloured, tending to accumulate at the bottom of a vertical slant. Margins entire.

Slide cultures. Pseudomycelium absent. Very rarely, two or three elongated cells may be formed.

Fermentation. Absent.

Sugar assimilation. (Liquid medium) glucose +, galactose +, sucrose +, maltose +, lactose +.

Assimilation of potassium nitrate. Absent.

Ethanol as sole source of carbon. No growth.

Splitting of arbutin. Positive.

Presence of starch. Weak reaction.

Additional carbon sources tested for assimilation in liquid media as follows:

adonitol	+	melezitose	+
aesculin	weak	melibiose	+
dl-arabinose	+	α-methylglycoside	+
cellobiose	+	raffinose	+
citric acid	−	rhamnose	−
dextrin	−	ribose	+
dulcitol	−	salicin	−
erythritol	−	sorbitol	+
glycerol	weak	l-sorbose	−
glycogen	−	starch	−
inulin	−	succinic acid	−
lactic acid	−	trehalose	weak
mannitol	+	d-xylose	+

Mrs N. J. W. Kreger-van Rij (Delft) has also examined the biochemical characteristics of *Rhodotorula nitens*, and with the exception of soluble starch (+), ribose (−), adonitol (−), α-methylglycoside (−), results of assimilation tests are similar. In addition, Mrs Kreger-van Rij found that *R. nitens* assimilates L-arabinose but not D-arabinose.

Additional observations

Relation of growth to temperature. In view of the apparent inability of the new yeast to grow at temperatures exceeding 26°, an investigation was made of the range of temperature supporting growth. Seven-day cultures on glucose nutrient agar slopes (glucose 1 % in Lemco nutrient agar) maintained at room temperature were washed off and the organisms suspended in sterile saline, the concentration being adjusted to about $2{\cdot}6 \times 10^6$ organisms/ml.

Twenty ml. quantities of glucose nutrient agar in 4 oz. (113·7 ml.) medicine bottles stoppered with cotton wool were inoculated with 0·7 ml. of the standardized suspension of the new yeast and allowed to stand for 1 hr. at room temperature. After removal of excess liquid the bottle was placed upright in a thermostatically controlled water bath. The low temperatures required were obtained by placing the water bath in a cold room at +4°. Incubations were made for 14 days each at 4°, 8°, 12°, 16°, 20°, 24° and 26°. Three bottles were inoculated and incubated at each temperature. After 14 days the yeast was washed off with sterile saline, filtered and the dry weight determined. Combined results of two trials are shown in Fig. 2.

The results show that *Rhodotorula nitens* is capable of growth between 4° and 20° inclusive, and that the optimum is about 14°. The new yeast is remarkably intolerant of temperatures above 24°. Three-day cultures in 2 % malt broth are killed when placed at 37° for 8 to 10 hr. No growth occurs at 26°, but the organism remains viable at this temperature.

Relation of growth to pH value. Growth was studied over a range of values from pH 2·2 to pH 8, at intervals of 0·2 units. The pH values of samples of the basal medium [$(NH_4)_2SO_4$, 5 g.; KH_2PO_4, 1·0 g.; $MgSO_4.7H_2O$, 0·5 g.; $CaCl_2.6H_2O$, 0·1 g; NaCl, 0·1 g.; glucose, 5·0 g.; concentrated vitamin solution (Lodder & Kreger-van

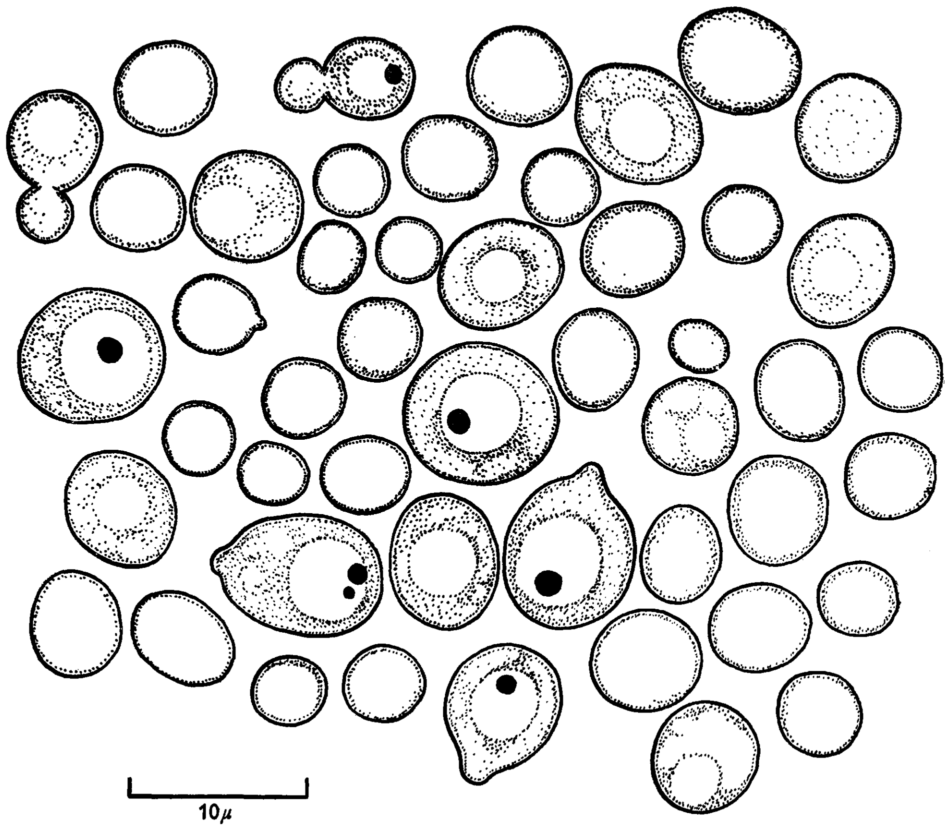

Fig. 1. *Rhodotorula nitens* nov.sp. Camera lucida drawing of cells from 3-day culture on 2·5 % malt agar.

Rij, 1952), 0·5 ml.; distilled water, 1000 ml.] were adjusted by 0·2 M–Na_2HPO_4 or 0·1 M-citric acid (McIlvaine, 1921). The presence of SO_4'', Cl' and PO_4''' ions in the nutrient solution appreciably altered the calculated pH values, particularly in the higher pH range, and pH values were accordingly adjusted by an electric pH meter. Two tubes were prepared for each pH value; the experiment was later duplicated. The pH stability was generally satisfactory over the 14-day period of incubation. Inoculated tubes showed a maximum drift of 0·25 pH units after incubation for 20 days. Potassium acid phthalate + sodium hydroxide, and potassium acid phthalate + hydrochloric acid buffer solutions (Clark & Lubs, 1915) were also used but were unsatisfactory because of toxicity and pH drift. The phosphate and citric acid components of the buffer solution finally used in determination of growth at different pH values were non-toxic. No assimilation of either substance was noted in liquid assimilation tests.

The amount of growth, represented by the turbidity produced after 14 days of incubation at 20° was measured by an EEL (Evans Electroselenium Ltd.) absorptiometer. The results in Fig. 3 show that growth of *Rhodotorula nitens* at 20° occurs between pH 2·2 and pH 8 (opt. about pH 5). In further trials with 500 ml. quantities of unbuffered glucose nutrient broth (original pH 6·8), incubation for 4 weeks at room temperature resulted in a final pH value of 4·6.

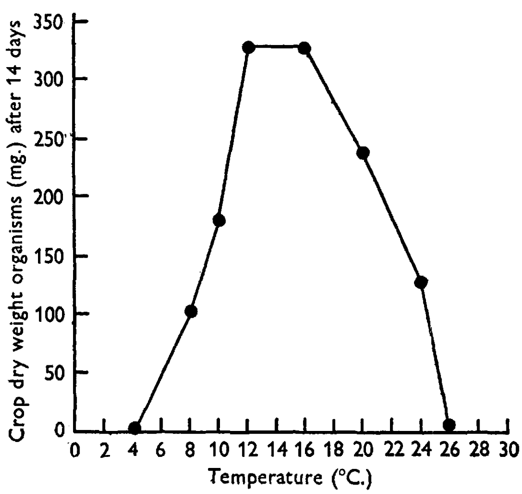

Fig. 2

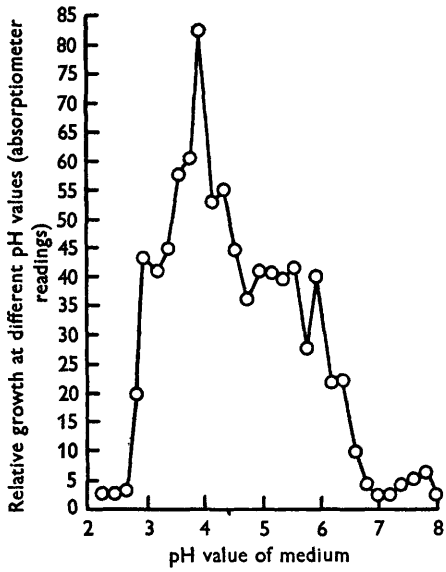

Fig. 3

Fig. 2. Effect of temperature on growth of *Rhodotorula nitens*, sp. nov.

Fig. 3. Effect of pH value of medium on growth of *Rhodotorula nitens*, sp.nov. Readings were made after 14 days of incubation at 20°. Relative amounts of growth shown as absorptiometer reading.

Pigment production. Rhodotorula nitens was incubated in glucose nutrient broth for 14 days at room temperature, centrifuged, washed with water and dried in air. The dried yeast was ground to a powder in a mortar and the pigments extracted by prolonged mechanical agitation in a mixture of 20 % methanolic KOH and benzene (Bonner, Sandoval, Tang & Zechmeister, 1946); after centrifugation the pigments were localized in the benzene layer. Separation of the pigments was achieved chromatographically on a small (1 × 10 cm.) column of a 4+1 mixture of calcium hydroxide and acid-washed alumina. Elution with light petroleum ether (b.p. 40–60°) containing 7·5 % (v/v) acetone, and finally with ethanol, gave three pigmented and three colourless fractions. Examination of the absorption spectra of each fraction, dissolved in light petroleum and subsequently in carbon disulphide, with a Unicam spectrophotometer and/or an Optika recording spectrophotometer, showed that none of the colourless fractions gave distinguishable peaks between 420 and 600 mμ. Details of absorption maxima are noted in Table 1.

One (yellow) fraction corresponded closely to figures obtained with authentic β-carotene; another (yellow) appeared to be γ-carotene. The composition (possibly complex) of fraction 6 was not determined.

The absorption maximum of a crude extract of pigment derived from cells grown in a potato+yeast medium (Hasegawa, Banno & Yamauchi, 1960) was 453 mμ.

Vitamins. The vitamin requirements of *Rhodotorula nitens* were determined for eight vitamins in liquid media incubated for 13 days at room temperature. The basal medium was identical with that used in determining the pH range for growth. Individual vitamins were tested both singly and as the only deficiency in a solution containing the other seven. Each tube was triplicated. The results (Table 2) showed that *R. nitens* required thiamine; partial requirement was noted for Ca-pantothenate.

Table 1. *Absorption maxima (mμ) of pigments isolated from* Rhodotorula nitens

Solvent	Fraction 2	Commercial β-carotene	Bonner *et al.* (1946) β-carotene	Fraction 4	Fraction 6
Light	424	425		435	450
petroleum	450	449	453	459	470
	476	478	480	489	503
Carbon	451	460	452	466	474
disulphide	482	490	484	494	508
	510	520	519	528	540

Table 2. *Vitamin requirements of* Rhodotorula nitens

Vitamin	Amount (μg./10 ml. medium)	Growth* As sole vitamin	As sole deficiency in mixture of remaining vitamins
Biotin	2	±	++
Ca-pantothenate	400	+	++
Inositol	2000	±	++
Nicotinic acid	400	±	++
p-Aminobenzoic acid	200	±	++
Pyridoxine HCl	400	±	++
Thiamine HCl	400	++	—
Riboflavin	200	−	++
Control 1. Vitamins absent		−	.
Control 2. All vitamins present		++	.

* −, No growth; ±, trace of growth, faint turbidity; +, tube opalescent; ++, heavy growth, coalescent surface growth.

DISCUSSION

The new yeast is placed in the genus *Rhodotorula* Harrison rather than the genus *Cryptococcus* Kützung emend. Vuillemin on the basis of its conspicuous carotenoid pigmentation and absence of a strongly positive starch reaction. It most closely resembles *Rhodotorula flava* (Saito) Lodder in sugar and nitrate assimilation patterns, inability to utilize ethanol and ability to split arbutin. Although the presence of a capsule is not generally associated with the red-pigmented yeasts, Hasegawa *et al.* (1960) record its existence in several species of Rhodotorula and observe that it is not unknown in *R. flava*. *Rhodotorula nitens* differs from *R. flava* in cell morphology, appearance of the colony and in having an orange rather than a yellow colour. Characteristic features of the new yeast include the capsule and a marked intolerance of a temperature of 37°; there is a slight psychrophilic tendency, with optimum growth occurring between 12 and 16°.

The dependence on pigmentation for generic characterization of Rhodotorula has been criticized by several workers (Wickerham, 1952; Peterson, Bell, Etchells & Smart, 1954; Nakayama, Mackinney & Phaff, 1954). It has been shown that carotenoid pigments may occur in Cryptococcus (Nakayama *et al.* 1954) and because of this, Lodder & Kreger-van Rij (1955) have conceded that red pigmentation is not an entirely satisfactory criterion for the classification of Rhodotorula. Nakayama *et al.* (1954) suggest that separation of Cryptococcus and Rhodotorula is best achieved on the basis of starch formation, and although this may be of value in classifying doubtful cases, starch production (like red pigmentation) is not in itself an absolute character. There are marked inconsistencies in the characters (presence of capsules, starch and carotenoid pigments) used to distinguish the three genera *Cryptococcus*, *Rhodotorula* and *Torulopsis*, and these have yet to be resolved. The 'subgenus' *Flavotorula* created by Hasegawa *et al.* (1960) includes asporogenous, non-fermenting, capsulated, starch-forming, budding yeasts, producing yellow to pale orange colonies and having an absorption maximum of 450 mμ in light petroleum when grown in potato + yeast extract. In the latter respect *Rhodotorula nitens* resembles 'Flavotorula', apparently lacking the specific pigment or pigments giving the peak of 480 mμ which is characteristic of 'Rhodotorula'. In *Rhodotorula nitens* the absorption peak of the crude pigment extract is due to a high proportion of β-carotene. This was produced in abundance when the organism was grown in a potato + yeast extract medium, but the crude extract is a mixture of pigments, and changes in their relative proportions would alter the absorption maximum. If the qualitative aspects of carotenoid pigmentation are to be used as taxonomic criteria, it is essential that the organisms should be grown in a chemically defined medium.

The original isolate AA 8 is maintained at the Mycological Laboratory, Department of Microbiology, Queen's University of Belfast, Northern Ireland. Subcultures (isotypes) have been deposited at the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, Netherlands and the National Collection of Yeast Cultures, Brewing Industry Research Foundation, Nutfield, Surrey, England.

Acknowledgements are made of the assistance received from Mrs N. J. W. Kreger-van Rij and Dr Takezi Hasegawa in examining the new yeast, Professor M. J. Boyd for the Latin diagnosis, and the technical assistance of Miss Hilary Bell and Miss Lesley Rusk.

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