ISOLATION AND CHARACTERISTICS OF A NEW SPECIES OF RHODOCOCCUS.

(*R. nuciferii*)

By A. V. Varadaraja Iyengar.

The author's attention was recently drawn to the development of pink colour on the exposed kernel of cocoanut (*Cocos nucifera*) which is extensively used in Indian household for culinary purposes. The tint was traced to the occurrence of a coloured organism which thrives on the sugars present in the kernel. No growth was visible on fresh kernel but the colour developed rapidly on exposure to air for even a few hours, so it has to be inferred that the organism is a contamination from the air. It is found in various localities and in all seasons of the year. The *Rhodococcus* has been found to grow on the kernel from tender or mature cocoanut. It can also be grown on copra.

The organism possesses all the characteristics of the genus *Rhodococcus* as described by Bergey *et al* (*Manual of Determinative Bacteriology*, 3rd Edition, 1930). It differs however from the generic type of *R. rhodocrous* Zopf. in its action on gelatin and milk. The pigment was isolated from a 20 per cent. gelatin liquefied culture by extraction with alcohol and after concentrating the extract, crystals deposited which were collected and found to melt at 140°–145°. Alcoholic solution of the pigment changes to yellow on the addition of alkali and regains its original tint on the addition of excess of acid. The characteristics of the organism are described below according to the scheme of American Association of Bacteriologists (*J. Bact.*, 1925, 10, 315). Nitrate reduction was tested in the manner recommended by Conn and others (*J. Bact.*, 1922, 7, 524). There was a small amount of ammonia in commercial peptone but a control with uninoculated tube compensated for the error.

The name *R. nuciferii* has been proposed since it was isolated from the kernel of cocoanut.

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Brief Characterisation.

Microscopic features.	*Remarks.*
Form (3) Micrococci ..	The organism occurs generally single but frequently in pairs, in fours and in chains.
Endospores (0)—Nil.	
Flagella (0)—Nil. ..	The organism is non-motile.
Gram stain (1)—positive.	

	Remarks.
Microscopic features.	
Biochemical reactions.	
Biologic relationship (not tested) .—(5 ?)	The organism is a saphrophyte since it is found to thrive on dead tissues such as cocoanut kernel.
Relation to oxygen (1) strict ærobe.	It does not develop in the absence of oxygen.
Gelatin liquefaction (1) positive.	It liquefies gelatin in strata proceeding to the sides from the point of inoculation. The liquefaction is quite vigorous, even 20 per cent. gelatin being acted on in the course of a week.
In nitrate media (2) nitrite present.	Reduction of nitrate proceeds at a very rapid pace, nitrites being formed after even half-an-hour. No reduction to ammonia stage. No gas could be detected.
Chromogenesis (7) or (9) positive.	The colour of the organism depends on the medium employed.
Carbohydrate reactions.	
Diastatic action—(1) feeble.	
Glucose (1)—acid and gas	High acidity developed even in the course of 24 hours. Gas production is definite and easily distinguishable. pH value of 4.8 is reached in the course of 72 hours and the reaction of the medium becomes subsequently nearly neutral (pH 6.8). The organism loses colour at the acid stage, but does not regain it, under any condition.
Lactose (2)—acid without gas.	Acidity is first noticed only after 48 hours but increases rapidly in the subsequent stages.
Sucrose (2)—acid (gas not tested).	Highly acid as in the case of glucose. The organism loses colour when the medium turns acidic.
Vegetative cells.	
Diameter between 0.6 and 0.8μ	
Length (= diameter)	
Chains (present)	
Capsules (absent)	
Cultural features.	
Agar stroke	Agar stroke was filiform, raised and translucent. An odour resembling that of cocoanut kernel was noticed.
Growth (scanty).	
Lustre (glistening).	
Surface (smooth).	
Agar colonies—Circular	The colonies were raised, glistening, translucent and red.
Milk	Peptonization was noticed in the course of 48 hours. Litmus was reduced. Acidity was noticed even in 24 hours.
Acid (sufficient for curdling).	
Rennet (curd) (absent).	
Peptonization (present).	
Indole production (negative).	

In addition to the above, the following observations were also made:—

Carbohydrate reactions.—Lævulose.—High acidity was noticed as in the case of glucose but with no gas production. After a week, the reaction of the medium became nearly neutral reaching a pH value of 6.8.

Maltose.—Growth abundant. The medium became highly acidic.

Polysaccharides.—In the case of inulin, slight acidity was observed, while with starch the reaction remained unaltered, although in both cases, growth was excellent.

Sugar alcohols.—Glycerol, mannitol, and dulcitol were found very favourable for the growth of this organism. High acidity was noticed only in the case of glycerol and mannitol.

Temperature.—The optimum temperature for the development of this organism was found to be in the neighbourhood of 22°. At 37°C. there was inhibition of growth attended by loss of colour.

Pigmentation.—Different colours were noticed in different media. Thus, pink was observed in gelatin, red in nutrient agar, brick red in potato and scarlet in milk, crimson red in a mixture of peptone and gelatin.

Relation to oxygen.—The organism is a strict ærobe and does not develop in the absence of air. Even a well developed culture loses colour under anærobic conditions.

Reaction of the medium in relation to growth.—Growth was found to be optimum at pH 6.8; when the medium was more acid, the organism made poor growth. It did not also develop colour. Thus, at pH 6.2 or 5.2 development was scanty and sub-cultures from these growths to normal media did not bring back the colour.

This organism differs from other gelatin liquefiers such as *R. roseus, R. rosaceus, R. corallinus* and *R. prometheus* (Corbet, *Centralblatt. Bakt.*, 1933, Abt. II, **88**, 475) in several respects—in rendering litmus milk highly acidic with a pink sediment, in the strong reduction of nitrates and heavy liquefaction of gelatin. Unlike *R. prometheus,* this organism does not show any evidence of rennet curd. It further differs from Corbet's organism in producing high acidity with sucrose and lactose.

SUMMARY.

(1) A new organism belonging to the genus *Rhodococcus* has been isolated and its cultural and physiological characters are described. It is provisionally termed *R. nuciferii* as it was first isolated from the cocoanut kernel.

(2) The organism is a heavy gelatin liquefier and produces high acidity from most of the sugars that were studied. It is also a strict ærobe.

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Department of Biochemistry,
Indian Institute of Science.
Bangalore.

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