ROTHIA, GEN. NOV. AN AEROBIC GENUS
OF THE FAMILY ACTINOMYCETACEAE

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SUMMARY. Rothia gen. nov. (Actinomycetaceae) is proposed as the name of a monotypic genus with the species Rothia dentocariosus comb. nov. (basionym Actinomyces dentocariosus Onisi 1949), synonyms Nocardia dentocariosus (Onisi) Roth 1957; Nocardia salivae Davis and Freer 1960. No strain of Onisi's original isolates is extant. ATCC 17931 is described and proposed as the neotype strain of Rothia dentocariosus (Onisi) Georg and Brown 1967.

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Actinomyces dentocariosus was apparently first isolated from carious dentine in humans by Onisi (1949). Onisi believed the organism belonged in the Order Actinomycetales and proposed the name Actinomyces dentocariosus because of similarities to other species of Actinomyces found in the mouth. He had studied 36 isolates at this time, and listed the following characteristics. The microorganism was Gram-positive with a variable microscopic morphology. When first isolated on solid media, it usually presented branched filaments, often with clavate ends; however, by the eighth day of cultivation, it fragmented into coccoid forms. Colonies were raised and rough-surfaced and adherent to the agar at first, but after several transfers became smooth and soft. The rough, or R type colonies, produced granular or clumped growth in broth. S type colonies produced turbid growth in broth. The variable microscopic morphology, filamentous to coccoid, reflected the R-S variation of the colonies. Onisi described the organism as "facultatively anaerobic" since he obtained growth in "Fortner's anaerobic culture." The other important characteristics which he pointed out at this time, and in a later study (Onisi and Nuckolls 1958) were production of catalase, liquefaction of gelatin,

reduction of nitrate, and fermentation of the following sugars with the production of acid: glucose, maltose, and inulin.

In 1957 Roth described several groups of organisms from carious lesions of human teeth. The organisms of her Group I, comprised of 20 strains, she considered to be identical to those isolated and described by Onisi. However, since they were "aerobic to facultatively anaerobic" she assigned them to the genus Nocardia under the name Nocardia dentocariosus. In a later publication, Roth and Thurn (1962) described this organism in greater detail, and several strains were deposited in the American Type Culture Collection under this name.

At the same time Roth was doing her studies in the United States, Davis and Freer (1960) in Great Britain described 25 strains of an "oral aerobic actinomycete," which they named Nocardia salivae.

The Actinomyces Laboratory of the CDC Mycology Unit has had an opportunity to study a number of Dr. Roth's isolates of N. dentocariosus as well as a group of isolates from the collection of Drs. Davis and Freer, which had been designated as N. salivae. We have studied also a number of similar strains isolated from human clinical materials derived from our diagnostic service.

Our studies indicate that N. dentocariosus and N. salivae are identical. However, neither Nocardia nor Actinomyces appears to be an appropriate generic name for Onisi's organism (Howell and Jordan (1963) support this view). Each of these genera, as well as the newly described genus, Odonotomyces will be discussed below with reference to this organism, which will be designated throughout this paper as N. dentocariosus, the currently generally accepted name. Table 1 summarizes the major characteristics of these genera.

A. Genus Nocardia

The genus Nocardia is inappropriate for N. dentocariosus for the following reasons:

1. Morphology

N. dentocariosus has never been shown to produce an aerial mycelium or conidia. Although all species of Nocardia do not show these characteristics readily, they are commonly observed under proper conditions. For example, Gordon and Smith (1955) studied 99 strains of Nocardia, and

TABLE 1
Comparison of Genera:
Nocardia, Actinomyces, Odontomyces, and Rothia

	Nocardia	Actinomyces	Odontomyces	Rothia
Aerial mycelium	+	0	0	0
Conidia	occasionally	0	0	0
O ₂ req.	aerobic	microaerophilic to aerobic	facultative	aerobic
CO ₂ req.	0	+	+	0
CO ₂ uptake	0	+	+	0
Catalase production	+	0	+	+
Growth on inorganic nitrogen	+	0	0	0
Growth on organic acids	+	+	+	0 to +
Acid fastness	+ or 0	0	0	0
Fermentation of glucose*	0	+	+	+
Oxidation of glucose*	+	0	0	0
Starch hydrolysis **	+ or 0	+ or 0	+ ***	0
Gelatin liquefaction	+ or 0	0	0	+ ***
Nitrate reduction	+ or 0	+ or 0	+ or 0	+
Nitrite reduction	0	0	0	+ ***
Cell wall: DAP	+	0	0	0
arabinose	+	0	0	0

* Fermentation and oxidation of glucose by method of Hugh and Liefson(1953).
 ** Hydrolysis of potato starch acc. to method of Gordon & Mihm (1957).
 *** Positive reaction may require 30 days.

with one exception demonstrated aerial mycelia which varied from rudimentary to luxuriant and which were wider and darker than the vegetative hyphae. The aerial hyphae of 18 of these strains segmented into chains of spores.

2. Oxygen and Nutritional Requirements

Like Nocardia species, N. dentocariosus is basically an aerobic organism and produces catalase. It is chiefly on the basis of this characteristic that Roth removed the organism from the genus Actinomyces to Nocardia. However, the nutritional requirements of N. dentocariosus differ from those of species of Nocardia. It is fastidious. It does not grow in inorganic nitrogen media, does not attack paraffin or utilize asparagine as a source of nitrogen. It grows very poorly on such media as Sabouraud dextrose agar, Czapek Dox agar, or nutrient agar, media that are commonly employed for the cultivation of N. asteroides and other species of Nocardia.

3. Biochemical Reactions

N. dentocariosus readily ferments sugars with the production of acid. N. asteroides, as well as other well defined Nocardia species, oxidizes sugars but does not ferment them. This difference in ability to ferment or oxidize sugars can be demonstrated by use of the Hugh and Leifson test (1953). According to Gordon (personal communication 1966) the inability to ferment glucose as demonstrated by the Hugh and Leifson test was characteristic of 218 strains of Nocardia species which she studied.

4. Cell-Wall Analyses

According to Davis and Baird-Parker (1959), the Nocardia-like aerobic actinomycetes isolated from the human mouth are not classifiable as members of the genus Nocardia on the evidence of cell-wall patterns. These authors, as well as Cummins (personal communication 1964), have shown that N. dentocariosus does not contain diaminopimelic acid (DAP) in its cell wall. The principal amino-acid components listed were: alanine, lysine, and glutamic acid. The only sugar detected was galactose.

Analyses of Nocardia species by these same workers (Davis and Baird-Parker 1959, and Cummins 1965) indicate that all Nocardia species tested do contain DAP. The other amino acids found are alanine and glutamic acid. The chief sugars are arabinose, galactose, and sometimes glucose.

B. The Genus Actinomyces

The genus Actinomyces is inappropriate for N. dentocariosus for the following reasons:

1. Morphology

N. dentocariosus is morphologically similar to Actinomyces species, particularly in its filamentous form. It appears, however, to be even more pleomorphic than any Actinomyces species which we have studied to date. We have never observed, for example, any Actinomyces species which can convert completely into masses of coccoid forms.

2. Oxygen and Nutritional Requirements

Like Actinomyces species, N. dentocariosus can be isolated under anaerobic or semianaerobic conditions; however, unlike Actinomyces, this organism is basically an aerobe. It grows very well aerobically and, in fact, is very difficult to maintain under anaerobic conditions.

3. Biochemical Reactions

Perhaps the most important point in differentiation between N. dentocariosus and Actinomyces species is the ability of the former to produce catalase. It is our opinion that the genus Actinomyces should include only anaerobic or microaerophilic organisms which are catalase negative. Other biochemical reactions which set N. dentocariosus apart from the genus Actinomyces are its ability to 1) liquefy gelatin, 2) peptonize milk, 3) reduce nitrate to ammonia, and 4) ferment glycerol.

4. Cell-Wall Analyses

As discussed previously by Pine and Georg (1965), N. dentocariosus is more closely related to the genus Actinomyces than Nocardia, since it lacks DAP and arabinose. Both Davis and Baird-Parker (1959) and Cummins (personal communication 1964) have indicated that the cell-wall composition of N. dentocariosus is very similar to that of A. israelii and A. bovis. In fact, Davis and Baird-Parker suggested that, on this basis, it could be placed in the genus Actinomyces. However, this would require inclusion in the genus Actinomyces, aerobic, catalase-positive organisms of this type.

C. The Genus Odontomyces

The genus Odontomyces, recently described by Howell et al. (1965) to accommodate the organism previously termed the "Hamster organism" (Howell 1963 and Howell and Jordan 1963), appears to be inappropriate for N. dentocariosus.

To date, only one species, O. viscosus, the type species, has been described as belonging to this genus. In describing their new organism Howell and Jordan compared it to a number of strains of N. dentocariosus. They concluded that the two differed sufficiently to warrant recognition of two genera. The important points of comparison between N. dentocariosus and O. viscosus are listed below.

1. Morphology

N. dentocariosus and O. viscosus have similar morphology. Both may produce mycelial, "spider" colonies on the surface of agar, and mature colonies may be heaped and rough-surfaced or entirely smooth. Colonies are soft in texture, and neither organism produces an aerial mycelium.

2. Oxygen and Nutritional Requirements

Both organisms are essentially aerobes and produce catalase. However, O. viscosus requires CO₂ for good growth. Growth under pyrogallol + CO₂ is significantly greater with O. viscosus than with N. dentocariosus.

Neither O. viscosus nor N. dentocariosus will grow in a medium containing only an inorganic nitrogen source; however, O. viscosus has the ability to utilize pyruvate, lactate, and acetate as substrates while N. dentocariosus does not.

Both organisms grow well in the presence of several amino acids or casein hydrolysate. Media such as brain heart infusion agar or trypticase soy agar are adequate.

3. Biochemical Reactions

Biochemical reactions which are useful in differentiating these two organisms are numerous. These are listed in Table 2. The most outstanding difference appears to be the proteolytic activity of N. dentocariosus, its ability to reduce nitrate to ammonia and to produce acetylmethylcarbinol.

4. Fermentation Products

Fermentation products of O. viscosus have been listed by Howell and Jordan (1963) as: formic, acetic, lactic, and

TABLE 2*

Differential Biochemical Reactions of *O. viscosus* and *N. dentocariosus*

Biochemical Reactions	<i>O. viscosus</i>	<i>N. dentocariosus</i>
Gelatin liquefaction	0	+
Litmus milk	acid, or acid clot	No reaction or slow peptonization
Starch hydrolysis**	+	0
Nitrate reduction	+ or 0	+
Nitrite reduction	0	+
Voges-Proskauer test	0	+
4% salt tolerance	0	+
Sugar fermentations		
Lactose	+	0
Inositol	+	0
Salicin	+	0
Raffinose	+	0
Galactose	+ or 0	0
Glycerol	0	+ (weak)
Organic acids produced		
Formic	+	Usually 0
Acetic	+	+
Lactic	+	+
Succinic	+	trace
Growth in organic acids		
Pyruvate	+	0
Lactate	±	0
Succinate	±	0
Citrate	0	0
Acetate	±	0

* Majority of data from Howell and Jordan, 1963. Tests were read at 30 days incubation, at 37° C.

** Utilizing potato starch by the Method of Gordon and Mihm, 1957.

succinic acids. They also reported similar products from N. dentocariosus with the marked exception that this organism produces only traces of succinate or none, while O. viscosus produces succinate in large amounts.

5. Cell-Wall Analyses

Cell-wall analyses of these two organisms by Cummins (personal communication 1964) indicated that both organisms show a similar pattern of amino acids: alanine, glutamic acid, lysine, and possibly ornithine. No DAP was found in strains of either. A clear distinction can be made, however, in the sugar constituents. The cell walls of O. viscosus contain glucose, mannose, and a 5 methyl-pentose (probably rhamnose). Only galactose has been detected in N. dentocariosus.

CONCLUSIONS

A new genus, Rothia, is proposed in the family Actinomycetaceae for the organism previously known as Nocardia dentocariosus. The type species is Rothia dentocariosus (Onisi) comb. nov.

ROTHIA gen. nov.

(Named in honor of Dr. Genevieve D. Roth, who has done important basic studies with organisms of this genus.)

Microcolonies on agar surface composed of filamentous branched elements (1 μ or less in diameter) which rapidly undergo septation and fragment into bacillary or coccoid forms. No aerial mycelium or spore formation, nonacid fast, nonmotile. Good growth under aerobic or microaerophilic conditions, but poor or no growth anaerobically. Organic nitrogen required for growth. Catalase positive. Organisms weakly proteolytic. Sugars fermented with production of acid only. Lactic acid is the major product formed from the fermentation of glucose with no demonstrable uptake of CO₂. Type Species: Rothia dentocariosus (Onisi) comb. nov.

This organism has been adequately described by a number of workers (Onisi 1949; Roth 1957; Onisi and Nuckolls 1958; Davis and Freer 1960; Roth and Thurn 1962; Howell and Jordan 1964). However, it is felt that a summary of these findings should be included here.

Mycelium composed of branched filaments, often with clavate ends ($1\ \mu$ or less indiameter) which fragment readily into bacillary and coccoid forms. Occasionally cultures are isolated in a diplococcoid or coccoid form; however filaments can be demonstrated in subcultures either on the surface of agar plates or in liquid medium. Microcolonies on agar surfaces may be completely filamentous or may present a filamentous border only with the center of the colony a mass of rods and cocci. Mature colonies may be raised and highly convoluted, reflecting a filamentous micromorphology, or may be round, convex and smooth-surfaced reflecting a bacillary or coccoid micromorphology. All mature colonies whether rough or smooth (R or S) are creamy white, entire, and of soft texture. No aerial hyphae or spores are produced. In broth there is usually a mixture of soft, turbid, and finely granular or floccose growth. Requires organic nitrogen for growth. Poor growth on pyruvate, lactate, or acetate. Gram-positive, nonacid fast, nonmotile. Aerobic to microaerophilic. Very poor growth or none anaerobically. CO_2 not required for growth. Cell walls composed of galactose, alanine, glutamic acid, lysine, and possibly ornithine. Ferments fructose, glucose, maltose, sucrose and glycerol. Lactic acid is the major product produced from glucose, together with acetic acid and a small amount of succinic acid. Voges-Proskauer test positive. Gelatin liquefied. Starch, albumin, and casein not hydrolyzed (by methods of Gordon and Mihm 1957). Milk unchanged, or slowly peptonized. Nitrate and nitrite reduced. Indole not produced. Nonhemolytic on human and sheep blood, variable on rabbit blood. Catalase positive.

Habitat: Oral cavity of man. Pathogenicity not demonstrated in experimental animals.

None of the original strains isolated by Onisi is extant and available as a type strain. A neotype strain is designated and deposited in the American Type Culture Collection as ATCC 17931.

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