INTERNATIONAL JOURNAL OF
SYSTEMATIC BACTERIOLOGY

Vol. 19, No. 3

July 1969

pp. 267-272

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RECLASSIFICATION OF ACTINOMYCES PROPIONICUS

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ABSTRACT. Classification of the organism Actinomyces propionicus (Buchanan and Pine 1962) has been reconsidered in light of its production of propionic acid and the presence of diaminopimelic acid in its cell wall. By these characteristics it differs from all other species of the genus Actinomyces. It also differs significantly from members of the genera Bacterionema and Propionibacterium. A. propionicus is placed in the new genus Arachnia under the name of Arachnia propionica comb. nov.

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In 1959 Pine and Hardin isolated a facultative actinomycete from a case of lacrimal canaliculitis. Although it resembled Actinomyces israelii morphologically, it produced acetic and propionic acids as its major products of glucose fermentation whereas A. israelii produces lactic and succinic acids as the major products of glucose fermentation. Subsequently, Buchanan and Pine (1962) compared the characteristics of this isolate with those of A. israelii, A. bovis, A. naeslundii and species of Propionibacterium. Although the organism had characteristics of species of both genera, it was designated as a new species of Actinomyces—A. propionicus—on the basis of its colonial and cellular morphology, absence of catalase, its inability to ferment glycerol or lactic acid, and its pathogenicity. The single isolate was placed in the American Type Culture Collection as ATCC 14157. Recently, Gerencser and Slack (1967) described three additional isolates of A. propionicus which emphasized the pathogenic nature of the organism; two of the isolates had caused typical cases of actinomycosis, whereas the third was isolated from lacrimal canaliculitis. Pine and Georg

(1965) emphasized the need for a reevaluation of the taxonomic position of A. propionicus, since it alone, of all the species of Actinomyces, contained diaminopimelic acid in its cell wall and formed propionic acid from glucose. As a result of the recommendation of the "Subgroup on Taxonomy of Microaerophilic Actinomyces"* to retain within the genus only those isolates having lysine as the major dibasic amino acid, A. propionicus is placed in a new genus—Arachnia (from Greek noun, Arachnion meaning "cobweb")—as Arachnia propionica. The genus is placed in the family Actinomycetaceae for reasons listed below:

The characteristics of A. propionica are given in Table 1.

A consideration of these characteristics suggests two possible families wherein A. propionica could be placed—Actinomycetaceae and Propionibacteriaceae. A. propionica resembled members of the genus Propionibacterium in its general physiology, the presence of diaminopimelic acid in its cell wall, and by the production of propionic acid from glucose. It differs from the Propionibacterium species by the presence of aspartic acid in its cell wall. Although the fermentation balances themselves are similar to those of Propionibacterium species, much less glucose is fermented (Buchanan and Pine 1962).

In addition, A. propionica differs from members of the genus Propionibacterium by the absence of catalase, its failure to ferment lactate, pyruvate or glycerol, its formation of mycelial microcolonies when grown on agar, its pathogenicity in animals, and its production of filamentous and mycelial elements in pathological material.

Within the family Actinomycetaceae, three genera have been accepted by the Subgroup on the Taxonomy of Microaerophilic Actinomyces—Actinomyces, Rothia, and Bacterionema. Bifidobacterium has also been proposed for this family, but is subject to further consideration. A. propionica differs from all members of the genus Actinomyces (A. naeslundii, A. bovis, A. israelii, and A. viscosus) by the presence of diaminopimelic acid in its cell wall and because propionic acid is produced as a major product of glucose fermentation; these same characteristics, plus the presence of aldolase (Buchanan 1962; Buchanan and Pine 1967), mycelial colony formation and pathogenicity clearly distinguish

* A subgroup of the International Committee on Nomenclature of Bacteria of the International Association of Microbiological Societies.

Table 1. Characteristics of *Arachnia propionica*.*

<u>Cellular morphology.</u>	<i>A. propionica</i> grows as a short branching rod but, dependent upon media and conditions, also forms long, branched filamentous cells. As the culture ages, but dependent upon the sugar used as a substrate, spherical cells or osmotically stable spheroplasts may be formed.
<u>Microcolony.</u>	Microcolonies are definitely mycelial with filamentous hyphal elements often emanating from a common center to give a spider-like or "cobweb-like" appearance. Branching occurs irregularly on long hyphal elements.
<u>Relative pathogenicity.</u>	<i>A. propionica</i> is pathogenic for the white mouse; of four strains studied all caused multiple abscesses with mycelial elements when inoculated intraperitoneally into white mice. Seven isolates have been obtained from different infections of man—two cases of lacrimal canaliculitis and five from typical cases of both localized and disseminated actinomycosis. Two isolates have apparently been obtained from the oral cavity of man (Rasmussen et al. 1966). Although these latter isolates were not identified, the characteristics described by these workers clearly fit those of <i>A. propionica</i> .
<u>Physiological characteristics.</u>	The organism is Gram +, catalase -, facultative, having no requirement for carbon dioxide when grown either aerobically or anaerobically. It produces H ₂ S; nitrate is reduced to nitrite; gelatin is liquefied very slowly if at all, indole is not produced, and starch is hydrolyzed only very slightly.
<u>Fermentation balances.</u>	Anaerobically in media containing 0.5% glucose, only 16 micromoles of glucose were fermented; approximately 54.2 micromoles of CO ₂ , 54.5 micromoles of acetic acid, 113 micromoles of propionic acid, 2.5 micromoles of lactic acid and 3.0 micromoles of succinic acid were formed per 100 micromoles of glucose fermented. Aerobically, glucose is fermented stoichiometrically to acetic acid and carbon dioxide (Buchanan 1962). Enzymatic analysis (Buchanan 1962) shows the organism contains aldolase and is homofermentative as described by Buyze, Van den Hamer, and deHaan (1957).
<u>Fermentation of carbohydrates.</u>	<i>A. propionica</i> does not ferment xylose, arabinose, cellobiose, cellulose, salicin, lactic acid, pyruvic acid, glycogen or rhamnose; it does ferment glucose, mannose, dihydroxyacetone, galactose, lactose, maltose, sucrose and mannitol with the production of acid but no gas in 7 days. Glycerol may or may not be weakly fermented within this time.
<u>Lactic acid.</u>	DL-lactic acid is formed.
<u>Cell wall composition.</u>	The type culture (ATCC 14157) was shown to have glutamic acid, alanine, aspartic acid, and diaminopimelic acid as its major amino acid cell wall constituents; glucose and galactose were the major sugars present. The presence of diaminopimelic acid has been verified in ten isolates.
<u>Serological characteristics.</u>	Fluorescent antibody techniques have shown that <i>A. propionica</i> does not react with specific fluorescent conjugates for <i>A. israelii</i> , <i>A. bovis</i> , <i>A. naeslundii</i> , <i>A. eriksonii</i> , or <i>A. odontolyticus</i> .

* The characteristics are those described by Buchanan (1962), Buchanan and Pine (1962), Gerencser and Slack (1967), Buchanan and Pine (1967), and Georg (unpublished results, 1968).

it from members of the genus Bifidobacterium (deVries and Stouthamer 1967). Its facultative physiology, absence of catalase, pathogenicity, formation of glucose with the production of propionic acid and the presence of diaminopimelic acid in the cell wall suffice to distinguish it from Rothia dentocariosa.

Of the genera in the family Actinomycetaceae, Bacterionema has many physiological characteristics in common with Arachnia (Gilmour and Beck 1961; Howell and Pine 1961). Both may be facultative, but, in general, the isolates of Bacterionema are aerobes while A. propionica is a facultative anaerobe. Both contain diaminopimelic acid in their cell walls, and both ferment glucose producing propionic acid and DL-lactic acid. But A. propionica is anaerobic in its physiology, the products of glucose accumulate in its medium, whereas the products of Bacterionema are oxidized, presumably to CO₂ and water (Howell and Pine 1961). In addition, A. propionica is catalase negative, is pathogenic, and does not have the definitive cellular morphology of the Bacterionema species or their characteristic manner of cellular and hyphal growth (Gilmour 1961).

Characteristics of all of the members of the family Actinomycetaceae is the branching filamentous or budding cellular morphology of cells at some specific phase of growth. This manner of cellular reproduction results in the formation of a definite mycelium usually observed in the microcolony. Mycelial elements are invariably found in lesions caused by these pathogenic species. A. propionica not only demonstrates such mycelium in culture and in tissue, but has proved to be the causal agent of typical localized and disseminated actinomycosis, producing clubbed granules. Infections of this type have been classically ascribed to various species of Actinomyces. Placing the genus Arachnia within the family of Actinomycetaceae for the taxonomical reasons cited, is thus supported by the historical and the medical aspects of the disease it produces (Pine and Hardin 1959; Pine et al. 1960).

CONCLUSION

A new generic name, Arachnia, is proposed for the organism previously named Actinomyces propionicus. It is proposed that this genus be placed in the family Actinomycetaceae. The genus is defined as follows:

Arachnia gen. nov. (Gr. noun Arachnion, a "cobweb.")

Microcolonies are definitely mycelial, composed of branched septate and nonseptate filamentous elements often originating from a common center. Undergoes fragmentation to produce short irregularly shaped rod-like cells which may or may not be branched. Dependent upon media, large spherical cells may be formed. No aerial mycelium or spores are formed. Gram-positive, non acid-fast, facultative but best growth obtained anaerobically. Catalase negative, glucose fermented primarily with production of propionic and acetic acids and small amounts of succinic acid and lactic acid. Contains diaminopimelic acid as a major cell wall amino acid.

The characteristics of the single species Arachnia propionica are listed in Table 1. As the genus as proposed is monotypic, A. propionica is the type species.

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