CONTRIBUTIONS TO OUR KNOWLEDGE OF THE ACTINOMYCETALES. IV.

The Identity of Certain Species of Mycobacterium and Proactinomyces.

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(Four Text-figures.)

[Read 30th November, 1932.]

In a previous paper (Jensen, 1931) the present writer showed that two microorganisms previously classified as *Mycobacterium actinomorphum* and *Mycobacterium agreste* should correctly be placed in the new genus *Proactinomyces*. Since then, some work has been carried out on the morphology and biology of saprophytic mycobacteria and corynebacteria isolated from Australian soils, and authentic cultures of a number of related organisms have been obtained for comparison. Several of these have, on closer examination, been found, like the two organisms mentioned above, to belong to *Proactinomyces*, as previously defined (Jensen, 1931): organisms starting their life-cycle with the formation of a definite, more or less extensive vegetative mycelium which sooner or later divides, by formation of septa, into more or less bacterium-like, rod-shaped to coccoid elements, and generally producing an aerial mycelium in which no definite spores are formed. The following strains were examined: 1. *Mycobacterium agreste* Gray and Thornton (1928); 2. *M. crystallophagum* Gray and Thornton (1928); 3. *M. erythropolis* Gray and Thornton (1928); and 4. *Bacillus mycoides corallinus* Hefferan (1904),* from the National Collection of Type Cultures, Lister Institute of Preventive Medicine, London; 5. *Mycobacterium salmonicolor* den Dooren de Jong (1927); and 6. *M. opacum* den Dooren de Jong (1927), from the laboratory of Keuringsdienst van Waren, Rotterdam, Holland; and 7. *Microbacterium mesentericum* Orla-Jensen (1919), from the Biotechnical-Chemical Laboratory, Polytechnical School, Copenhagen, Denmark.

The media and the methods for cultivation and study were the same as described in the previous paper (Jensen, 1931), except that nutrient agar with 1% soluble starch was used for testing the diastatic activity.

Descriptions of the Organisms.

Proactinomyces corallinus (Hefferan), n. comb.

Synonyms: *Bacillus mycoides corallinus* Hefferan (1904).—*Serratia corallina* (Hefferan) Bergey (1923–30).—*Streptothrix corallinus* (Hefferan) Reader (1926).—*Mycobacterium agreste* Gray and Thornton (1928).—*Actinomyces agreste* (Gr. and Th.) Bergey (1930).—*Proactinomyces agrestis* (Gr. and Th.) Jensen (1931).

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* This organism was identified as a "*Streptothrix*" by Reader (1926).

Six strains were compared:

1. *Bac. corallinus* Hefferan } from the Lister Institute.
2. *Myc. agreste* Gr. and Th. }
3. AII, from garden soil, Sydney University.
4. Sc, from humus soil from Scone, N.S.W.
5. 271 } from red loam soils from the Riverina district, N.S.W.
6. 276 }

Since this comparison, as shown below, proved all the strains to be identical in all essential points, they must apparently be regarded as one single species, for which *corallinus*, on the grounds of priority, must be accepted as the valid specific name.*

Morphology.—All strains, like those previously described (Jensen, 1931), show by direct agar-microscopy according to the method of Ørskov (1923) essentially the same mode of development on dextrose-asparagine-agar: after 1–2 days small branching mycelia which after 2–3 days divide into rods of various length and arranged in angular positions, and later into quite coccoid elements. This process of division starts in the interior of the colonies and proceeds gradually towards the edges; the young colonies have a characteristic star- or burr-like appearance owing to a number of rhizoid projections which are the last to undergo division. Young mycelia show constantly a number of small refractive external granules which by high focussing are seen to rise into the air, and which doubtless represent a rudimentary aerial mycelium; they disappear again within a few days. Text-figure 1 shows the main features of the cycle of development. The rapidity with which this cycle is passed through varies considerably with the medium and the temperature (cf. Reader, 1926), and to some extent also with the strain. For instance, strains Sc and 271 appear almost exclusively as cocci on dextrose-nutrient-agar after 24 hours at 28–30° C., whereas strain 276 shows less tendency to coccus-formation, but a pronounced belt-staining. The cells of the freshly isolated strains were 1·0–1·2μ thick, as also reported by Gray and Thornton (1928); in *Myc. agreste* and *Bac. corallinus* they were somewhat thinner, probably owing to the longer period of artificial cultivation (cf. Hefferan (1904), who reports that the cells of the freshly isolated "*Bac. mycoides corallinus*" were originally as big as those of the anthrax bacillus, but that they gradually decreased somewhat in size). In the latter strain they were also somewhat more curved than in the others. None of the strains was acid-fast in nutrient or synthetic agar (as also found by Gray and Thornton), but in milk they exhibited a partial acid-fastness after 3–7 days.

Cultural characters.—The type of growth in various media is shown in Table 1. There are no very appreciable cultural differences between the strains. The intensity of the pigment varies somewhat, as was also the case with the 74 strains studied by Gray and Thornton (1928). The quantitative differences in the vigour of growth and the formation of a soluble yellow pigment in one strain (271) would hardly justify a separation into different species.

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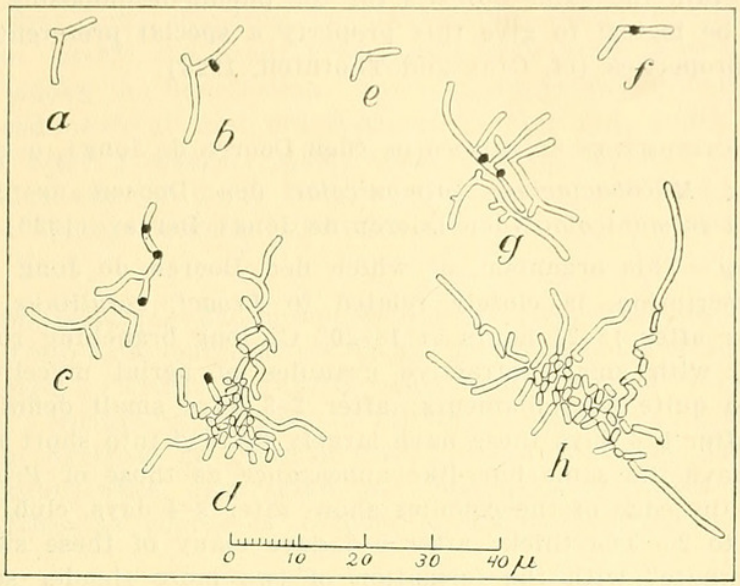
* I cannot here forgo the remark that Bergey's (1923-30) morphological description of *Serratia corallina* (syn. *Bac. mycoides corallinus* Hefferan) disagrees entirely with the descriptions by Hefferan (1904) and Reader (1926). Bergey characterizes it as a small, motile, gram-negative rod with one polar flagellum. One cannot help wondering whether the description has not been confused with that of *Bac. corallinus* Slater (1891), a non-spore-forming, motile, red-pigmented organism which would seem entitled to the name *Serratia corallina* according to Bergey's system of classification.

TABLE 1.—*Comparative Cultural Features of Strains of* Proactinomyces corallinus.

Medium.	*Mycobacterium agreste.*	*Bacillus mycoides corallinus.*	AII.	276.	Sc.	271.
Dextrose-asparagin-agar. 28–30° C.	Good, restricted, convex, myceloid edges, folded, pinkish-orange, becoming pale coral-red.	Good, restricted, convex, myceloid edges, slightly folded, pale pinkish-orange.	Abundant, convex, smooth, glistening, cream-coloured becoming pale pink.	Abundant, convex, smooth, glistening, pale pink, later greyish-orange.	Fair, restricted, myceloid edges, smooth, glistening, pale pink.	Fair, restricted, convex, becoming folded, pink, with yellow soluble pigment.
Dextrose nutrient agar. 28–30° C.	Good, restricted, convex, folded, undulate edges, pinkish cream-coloured, later deep coral-red.	Good, restricted, convex, folded, undulate edges, cream-coloured, later pale coral-red.	Similar to dextrose-asparagin-agar, still more abundant, pinkish-orange.	Similar to dextrose-asparagin-agar, still more abundant, grey pinkish-orange.	Good, convex, myceloid edges, smooth, soft, pink, later becoming coral-red.	Good, convex, myceloid edges, soft, smooth, pink, becoming deep red.
Potato. 28–30° C.	Good, spreading, granular, pink, later deep orange.	Good, spreading, raised, granular, orange.	Fair, spreading, raised, granular, dull greyish-orange.	Good, spreading, raised, granular, glistening, greyish-orange.	Good, spreading, smooth, glistening, greyish-pink.	Fair, spreading, granular, greyish-pink with yellow tinge.
Broth. 28–30° C.	Turbid, with voluminous pinkish-cream coloured sediment and fragile surface scum.	Slightly turbid with pinkish-cream-coloured surface granules and voluminous sediment.	Turbid, later becoming clear, with thick fragile cream-coloured scum and sediment.	Turbid with voluminous pinkish-cream-coloured sediment; no surface growth.	Clear with pink sediment and surface granules, later forming a pellicle.	Turbid, later becoming clear, with cream-coloured sediment, becoming pink.
Milk. 28–30° C.	Pink flakes and granules, forming a red sediment; milk slightly cleared in old cultures (6–8 weeks).	Pink granules and sediment, becoming red; milk slightly cleared in old cultures, yellowish.	Greyish-yellow to pinkisk flakes and sediment; milk semi-transparent in old cultures.	Greyish-yellow to pink flakes and sediment; milk semi-transparent in old cultures.	Pink pellicle, later red sediment; milk viscid and semi-transparent in old cultures.	Pink sediment and granules; milk very slightly cleared in old cultures.
	(Clearing of milk not due to proteolytic action; formol-titration shows no increase in amino-N.)					
Nutrient gelatin. 16–18° C.	Both strains identical; filiform, granular, yellowish growth in stab; raised, wrinkled, red surface colony; no liquefaction.		No liquefaction.	No liquefaction.	No liquefaction.	No liquefaction.



Text-fig. 1.—Development of *Proact. corallinus* on dextrose-asparagine-agar at 16-18° C. *a, Bac. mycoides corallinus,* 18 h.; *b,* same, 23 h.; *c,* same, 42 h.; *d,* same, 3 days; *e, Myc. agreste,* 18 h.; *f,* same, 23 h.; *g,* same, 42 h.; *h,* same, 3 days. × 700. Aerial mycelium heavily shaded.

Physiological features are shown in Table 2. There is a certain amount of variation here, but not more than among the 74 strains studied by Gray and Thornton, which were obtained by a selective method (accumulation in a nutrient solution with phenol or cresol as the sole source of carbon) and therefore all capable of decomposing aromatic compounds. It might perhaps be

TABLE 2.—*Comparative Physiological Features of Strains of* Proactinomyces corallinus.

	Bacillus corallinus.	*Mycobacterium agreste.*	AII.	276.	Sc.	271.
Proteolytic action ..	−	−	−	−	−	−
Diastatic action	−	−	−	−		?
Invertase action	−	−	−	−	−	−
Decomposition of cellulose	−	−	−	−	−	−
Decomposition of phenol	−	+	?	−	−	?
Utilization of paraffin ..	+	+	+	+	+	+
Utilization of N as* :						
$NaNO_3$	2	2	3	3	2	3
$(NH_4)_2HPO_4$ ..	2	3	3	4	2	4
Asparagine ..	3	3	4	4	2	4
Peptone	3	4	4	5	4	4
Reduction of nitrate ..	+	+	−	?	+	+
Formation of indol† ..	+	+	−	+	+	+
Acid in dextrose-broth ..	−	−	−	−	−	−
Acid in glycerin-broth ..	−	−	−	−	−	−
Growth anaerobically ..	−	−	−	−	−	−

*Basic solution: Dextrose 1·0%; K_2HPO_4 0·1%; $MgSO_4$ 0·05%; NaCl 0·05%; in distilled water; N-compound 0·2%. Character for growth: 0, no growth; 1, trace or very scant; 2, scant; 3, fair; 4, good; 5, excellent.

†By Salkowski's test.

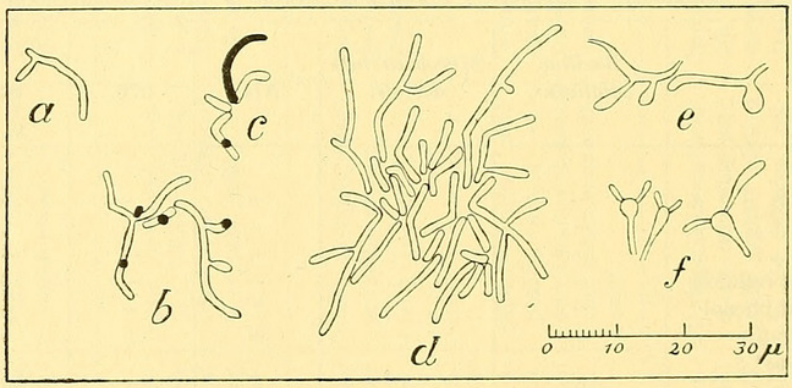
suggested to retain the name *agrestis* for the phenol-decomposing strains, but it would hardly be logical to give this property a special preference before other physiological properties (cf. Gray and Thornton, 1928).

PROACTINOMYCES SALMONICOLOR (den Dooren de Jong), n. comb.

Synonyms: *Mycobacterium salmonicolor* den Dooren de Jong (1927).—*Flavobacterium salmonicolor* (den Dooren de Jong) Bergey (1930).

Morphology.—This organism, of which den Dooren de Jong gives a rather incomplete description, is closely related to *Proact. corallinus.* On dextrose-asparagine-agar after 18–24 hours at 18–20° C., long branching rods are formed, 1·0–1·3μ thick, with small refractive granules of aerial mycelium, sometimes stretching into quite long filaments; after 2–3 days small definite mycelia are present, and after 5–6 days these have largely divided into short rods and cocci; the colonies have the same burr-like appearance as those of *Proact. corallinus.* Many cells at the edge of the colonies show, after 3–4 days, club- or pear-shaped swellings, up to 2·5–3·0μ thick; after 5–6 days many of these swollen cells are seen to "germinate" with the formation of two more slender sprouts (Ørskov (1923) gives an almost identical-looking picture of "*Streptothrix rubra*"; it is questionable, indeed, whether these two organisms are not really identical). At 28–30° C. the development is more rapid. In dextrose-asparagine solution we find, after 20 hours, long branched rods, 1·0–1·4μ thick and up to 30–35μ long, but after 2 days irregular, club- or pear-shaped rods looking like big diphtheroids. In dextrose-nutrient-agar only short rods and cocci, 1·2–1·5μ, are found after 2 days at 30° C., but in milk the long branching rods are still present after 3 days. The organism is not acid-fast in synthetic media or in young cultures on nutrient agar, but partly so in milk after 3–7 days and nutrient agar after 4–6 weeks.

The course of development is shown in Text-figure 2.



Text-fig. 2.—Development of *Proact. salmonicolor* on dextrose-asparagine-agar at 16-18° C. *a*, 18 h.; *b*, 23 h.; *c*, same, long filament of aerial mycelium; *d*, 44 h.; *e*, 3 days; *f*, 7 days. × 700. Aerial mycelium heavily shaded.

Cultural characters.—Cultivation at 28–30° C., unless otherwise stated. *Dextrose-asparagine-agar*: Good growth, restricted, rather flat, edges lobate, surface warty, glistening, first pale orange, later pure ochre-yellow; consistence crumbly. After 5–6 weeks the growth is paler with many small round raised yellow "secondary colonies"; cultures obtained by plating from these do not seem to differ from the original. *Dextrose-nutrient-agar*: Excellent growth, spreading, flat, dense, edges lobate, surface folded, glistening, yellow gradually

changing to deep orange-red. *Potato*: Good growth, raised, warty, crumbly, glistening, at first buff, changing to orange and finally to almost blood-red. *Nutrient gelatin*, 20–22° C.: Scant arborescent growth in stab; small wrinkled orange surface colony; no liquefaction. *Nutrient broth*: Fair growth; thin pellicle and granular sediment, at first cream-coloured, later red; broth clear at first, slightly turbid after 3 weeks. *Milk*: Good growth; pellicle of small cream-coloured granules after 2 days, later a thick orange sediment; milk is not coagulated, but appears slightly cleared after 5 weeks, the reaction becoming alkaline.

Physiological features.—Saccharose is not inverted, although readily utilized with sodium nitrate as a source of nitrogen. Starch is not hydrolyzed. Cellulose is not decomposed. Paraffin is readily utilized as a source of carbon. Phenol is not utilized. Nitrate is reduced to nitrite. Indol is not formed. No acid is formed from dextrose or glycerin. No growth in oxygen-free atmosphere. Nitrate, ammonium salts, asparagine and peptone are utilized almost equally well with dextrose as source of carbon, although the growth is most rapid with peptone.

The morphology of this organism shows conclusively that Bergey's (1930) classification of it as *Flavobacterium* is unjustified. This genus comprises small non-spore-forming, usually gram-negative rods, characterized by formation of a yellow pigment and by feeble powers of attacking carbohydrates, gas never being formed and acids rarely. Bergey omits to mention the tendency to branching, which den Dooren de Jong (1927) states to be present, and moreover the present organism produces a luxuriant growth on dextrose and saccharose with inorganic sources of nitrogen; this can hardly be called a "feeble power of attacking carbohydrates", since it is not fair to gauge this power by the formation of acids or gas in the case of organisms which oxidize carbohydrates completely to carbon dioxide and water (cf. Merrill, 1930).

Proactinomyces opacus (den Dooren de Jong), n. comb.

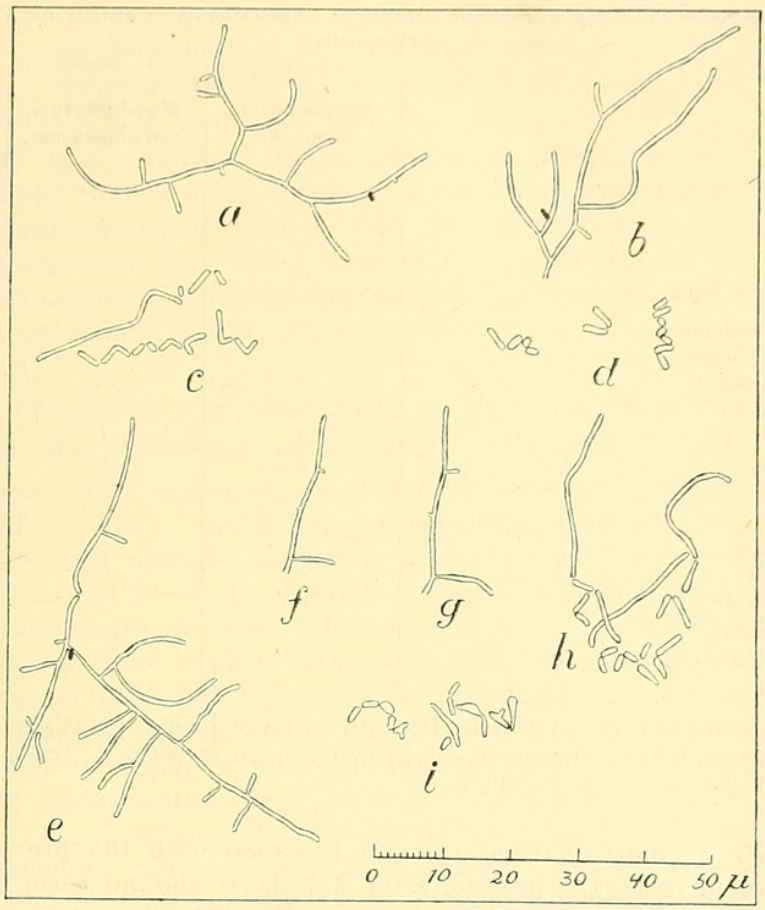
Synonyms: *Mycobacterium opacum* den Dooren de Jong (1927).—*Mycobacterium crystallophagum* Gray and Thornton (1928).—*Actinomyces crystallophagus* (Gr. and Th.) Bergey (1930).

Morphology.—The two strains *Myc. opacum* and *Myc. crystallophagum* are morphologically identical. They form in dextrose-asparagine-agar after 1–2 days at 16–18° C., quite extensive mycelia composed of richly branching hyphae, 0·6–0·9μ thick; short, simple filaments of aerial mycelium are seen, but no spores are formed in these. The mycelia are fragile and appear in stained preparations mostly as branched filaments of varying length. After 3–4 days the young colonies are round, dome-shaped, and surrounded by a flat fringe of long branching filaments which, during the following days, undergo a division giving rise to rod-shaped cells in angular arrangement and gradually growing shorter, finally quite coccoid. Text-figure 3 shows the course of development. At 28–30° C. the development is similar, but more rapid; this is also the case in nutrient agar, milk and potato. There is at the higher temperatures a tendency to formation of swollen, club-like cells, and the strain *opacum* is somewhat less prone to the formation of typical cocci than the other. Both strains exhibit a partial acid-fastness in milk, *opacum* also to a slight extent in nutrient agar.

TABLE 3.—*Comparative Cultural Features of Strains of* Proactinomyces opacus *and* Proactinomyces erythropolis.

Medium.	*Mycobacterium opacum.*	*Mycobacterium crystallophagum.*	*Mycobacterium erythropolis.*
Dextrose-asparagine-agar. 28–30° C.	Good growth, raised, spreading, edges myceloid, surface folded, pale cream-coloured changing to pale pink; consistence firm, pasty.	Abundant growth, spreading, raised, edges myceloid, surface folded, glistening, white with pale pink tinge; consistence soft, pasty.	Good growth, raised, spreading, edges lobate, surface highly folded, dull, white, becoming pinkish-cream-coloured; consistence firm, pasty.
Dextrose nutrient agar. 28–30° C.	Good growth, raised, spreading, surface folded and granulated, dull white changing to pale buff; consistence rather dry and coherent.	Abundant growth, restricted, raised, edges lobate, surface highly folded, white, glistening, changing to pale buff; consistence pasty.	Abundant growth, spreading, convex, edges entire, surface smooth, glistening, pinkish-cream-coloured; consistence soft and moist.
Potato. 28–30° C.	Good growth, raised, restricted, surface highly folded (lichnoid), dull cream-coloured, becoming greyish-pink; consistence curd-like.	Good growth, raised, spreading, surface highly folded and wrinkled, pale cream-coloured, later pink tinge; consistence curd-like.	Good growth, convex, spreading, surface slightly folded, glistening, pinkish-cream-coloured; consistence soft and moist.
Nutrient gelatin. 16–18° C.	Scant growth in stab, white, filiform, later finely arborescent; small white wrinkled surface colony; no liquefaction.	Scant growth in stab, cream-coloured, finely granulated; small raised and wrinkled pinkish-white surface colony; no liquefaction.	Scant growth in stab, cream-coloured, filiform; small raised and finely wrinkled pinkish-white surface colony; no liquefaction.
Nutrient broth. 28–30° C.	Thin, silky, white pellicle developing into a thick fragile cream-coloured scum; voluminous sediment of same colour; broth remains clear.	Thin white pellicle developing into a thick fragile cream-coloured scum; voluminous sediment of same colour; broth at first turbid, later clear.	Flaky pinkish-white sediment and surface scum, becoming cream-coloured; broth turbid, becoming clear after two weeks.
Milk. 28–30° C.	White, later pale pink to cream-coloured flakes and granules, forming a voluminous sediment; milk partially cleared in old cultures; reaction alkaline.	White to cream-coloured flakes and granules along the tube, voluminous cream-coloured sediment; milk very slowly cleared, becoming viscid in old cultures.	Pinkish-cream-coloured flakes and granules along the tube; voluminous sediment of the same colour; milk slowly and gradually cleared, becoming viscid in old cultures.

(The characteristic changes brought about in milk cultures by these strains as well as other non-proteolytic proactinomycetes do not indicate a digestion due to proteolytic action, since formol-titration does not show any increase in the content of amino-N.)



Text-fig. 3.—Development of *Proact. opacus* on dextrose-asparagine-agar at 16-18° C. *a, Myc. opacum*, 24 h.; *b*, 44 h., edge of colony; *c*, 6 days, edge of colony; *d*, 7 days, 28° C.; *e, Myc. crystallophagum*, 44 h.; *f*, same specimen, 48 h.; *g*, same specimen, 50 h.; *h*, 6 days, edge of colony; *i*, 4 days, 28° C. × 700. Aerial mycelium heavily shaded.

Cultural characters.—As Table 3 shows, these two strains are also very similar in cultural respect; *crystallophagum* is somewhat more soft and moist than *opacum* and has more tendency to produce a turbidity in liquid media, where the latter grows mostly as large discrete flakes, leaving the medium clear. Possibly the two strains represent "plane" and "perrugose" varieties of the same species.

Physiological features, listed in Table 4, show a complete identity, except for the ability of *crystallophagum* to decompose phenol. However, here as well as in the case of *Proact. corallinus*, we may doubt whether this single physiological difference is sufficient for a species differentiation. It seems, therefore, that the two strains must be regarded as a single species, the valid name of which will be *Proactinomyces opacus*.

PROACTINOMYCES ERYTHROPOLIS (Gray and Thornton), n. comb.

Synonyms: *Mycobacterium erythropolis* Gray and Thornton (1928).—*Actinomyces erythropolis* (Gr. and Th.) Bergey (1930).

Morphology.—When grown on dextrose-asparagine-agar this organism is hardly distinguishable from the previous group, apart from a somewhat more pronounced tendency to production of swollen, club-shaped cells; in certain other media its

TABLE 4.—*Comparative Physiological Features of Strains of* Proactinomyces opacus *and* Proactinomyces erythropolis.

	Mycobacterium opacum.	*Mycobacterium crystallophagum.*	*Mycobacterium erythropolis.*
Proteolytic action	−	−	−
Diastatic action	−	−	−
Invertase action	−	−	−
Decomposition of cellulose	−	−	−
Decomposition of phenol	−	+	+
Utilization of paraffin	+	+	+
Utilization of N as*:			
$NaNO_3$	4	4	3
$(NH_4)_2HPO_4$	4	4	3
Asparagine	4	4	3
Peptone	5	4	4
Reduction of nitrate	+	+	−
Formation of indol	−	−	−
Acid in dextrose-broth	−	−	−
Acid in glycerin-broth	−	−	−
Growth anaerobically	−	−	−

* Basic solution: Dextrose 1·0%; K_2HPO_4 0·1%; $MgSO_4$ 0·05%; NaCl 0·05%; in distilled water; N-compound 0·2%. Character for growth: 0, no growth; 1, trace or very scant; 2, scant; 3, fair; 4, good; 5, excellent.

mycelial growth is more marked than is the case with the previous: in milk, long branching mycelia are present after 3–7 days, and no cocci are formed in broth (cf. Gray and Thornton). It is not acid-fast in synthetic or nutrient agar, but somewhat acid-fast in milk after 3 days.

Cultural and physiological features (see Tables 3 and 4) are similar to those of *Proact. opacus*, apart from the absence of nitrate reduction and a characteristic semi-transparent, watery growth on sugar-free nutrient agar (cf. Gray and Thornton, 1928).

The last three strains (*opacum*, *crystallophagum*, and *erythropolis*) show by direct microscopical examination of the young agar colonies a very clear picture of the formation of mycelial branches (cf. Jensen, 1931): minute granules appear outside the hyphae, grow into a small pear-shaped bud attached to the main stem by a thin stalk, and stretch into a lateral branch (see Text-figure 3, *e–g*).* One cannot help being struck by the resemblance of this phenomenon to the formation of what are described as "reproductive bodies" by Löhnis (1921); the pictures of *Myc. tuberculosis* according to Meirowsky and of *Bact. coli* according to Hort, as reproduced by Löhnis, are particularly instructive, as well as the more recent observations by Cunningham (1931) on "reproductive bodies" in *Bac. saccharobutyricus* and by Stoughton (1929) on "stalked gonidia" in *Bact. malvacearum*. Löhnis (1921) describes the phenomenon in the following words:

"If the gonidia are not liberated by the partial or complete dissolution of the cell wall, but remain confined within the cell, they develop into either buds or branches" (p. 127). . . . "Two to four or more gonidia may be produced within

* Reader (1926) gives a microphotograph showing exactly the same phenomenon in a young culture of *Proact. corallinus*.

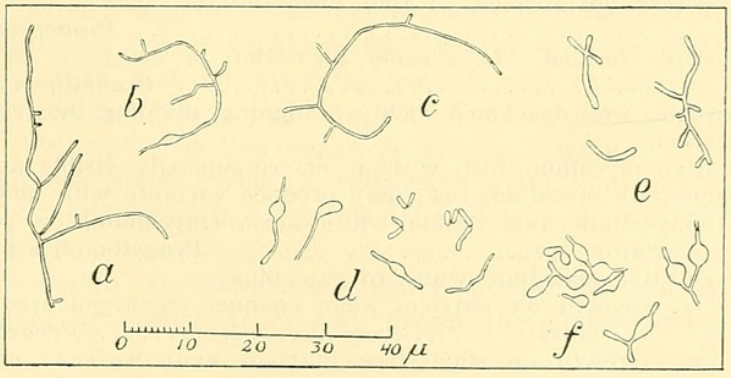
each cell; . . . They may start growing while still within the parent cell, forming buds and branches or directly new vegetative cells within the cell membrane. . . Sometimes they remain temporarily attached to the parent cell by a comparatively long stem" (p. 163).

While the final judgment on the nature of these phenomena may still be held in suspense, there can be no doubt as to their objective existence, and the conclusion might not be unjustified, that this formation of alleged reproductive bodies and gonidia in the "true" bacteria is a phenomenon homologous to the formation of branches in *Proactinomyces* and *Actinomyces*, and the spore formation in *Micromonospora*.

PROACTINOMYCES MESENTERICUS (Orla-Jensen), n. comb.

Synonym: *Microbacterium mesentericum* Orla-Jensen (1919).

Morphology.—This organism, of which Orla-Jensen gives a morphologically and culturally rather incomplete description, proved to be a typical *Proactinomyces*. On dextrose-asparagine-agar, nutrient agar, Saboureaud's agar* and broth it grows at 16–18° C. as extensive mycelia composed of richly branching hyphae of a somewhat variable thickness, 0·4–0·8μ; no aerial hyphae are seen. With increasing age the hyphae divide into fragments of varying size and shape, partly diphtheroid rods, but no real cocci. There is, particularly in the richer media, a tendency to formation of large, swollen, fusiform to almost spherical cells, up to 3·5μ in diameter and staining intensely with carbol fuchsin; when transferred to fresh media, they germinate and produce a new mycelium. On nutrient agar at 28–30° C. the organism appears after 1 day exclusively as irregular, branching rods of varying thickness (cf. Orla-Jensen, 1919, Pl. L). After 2 days and in older cultures the microscopical picture is entirely dominated by big lemon-shaped to spherical swollen cells. In milk, long rods and even definite mycelia are still seen at 30° C. after 10 days. The various cell types are reproduced in Text-figure 4. There is no acid-fastness in any medium. It is characteristic of this as well as of other non-acid-fast proactinomycetes (*Proact. actinomorphus* and *flavescens*, Jensen, 1931), that their cells, when examined by the direct agar-microscopy method of Ørskov (1923), are much less refractive to the light than those of previously described partially acid-fast organisms.



Text-fig. 4.—*Proact. mesentericus*. *a*, dextrose-asparagine-agar, 20 h. 16° C.; *b*, same, 4 days 16° C.; *c*, Saboureaud's agar, 24 h. 16° C.; *d*, same, 4 days 16° C.; *e*, dextrose-nutrient-agar, 20 h. 28° C.; *f*, same, 3 days 28° C. × 700.

* Milk is boiled for 5 min. with 0·2% HCl, and filtrated; the filtrate is neutralized, and added 1% peptone, 1% dextrose, 0·3% urea, and 1·5% agar.

Cultural characters.—This organism grows decidedly better at 16–18 than at 28–30° C.; the following description, therefore, refers to the former temperature unless otherwise stated.—*Dextrose-asparagine-agar*: Fair growth, narrow, raised, granular, very pale yellow, glistening; condensation water clear, with small granules. At 30° C. only scant growth consisting of small irregular white granules, growing deeply down into the agar.—*Dextrose-nutrient-agar*: Good growth, restricted, with undulate edges, surface with high transversal folds, cream-coloured; the consistence is firm and cartilaginous after 2 days, later more loose and brittle. Growth at 28–30° C. rather scant; smooth, soft, glistening, cream-coloured smear.—*Saboureaud's agar*: Excellent growth, spreading, at first flat and smooth, pale straw-yellow, perfectly hard and cartilaginous, later raised and strongly folded, of a loose, curd-like consistence, bright lemon-yellow. Growth at 28–30° C. only fair, restricted, folded, cream-coloured, soon becoming soft and smeary.—*Potato*: Scant growth; restricted, soft, cream-coloured smear.—*Nutrient gelatin*: Good growth; finely arborescent, cream-coloured growth in the stab; raised, folded, pale yellow surface colony. No liquefaction.—*Broth*: Good growth; voluminous, flaky, whitish sediment; broth clear.—*Milk*: 28–30° C. Small cream-coloured granules along the tube; the milk undergoes no visible changes within 4 weeks. No proteolytic action.

Physiological features.—Saccharose is inverted. Starch is hydrolyzed. Cellulose is not decomposed. Nitrate is reduced to nitrite. Indol is not formed. No growth in oxygen-free atmosphere. N is utilized as sodium nitrate, ammonium phosphate, and asparagine, although these are inferior to peptone as sources of N. The fermentative properties of this organism were studied in detail by Orla-Jensen.

Key to Identification of Species of Proactinomyces.

The species of *Proactinomyces* described here and in a previous paper (Jensen, 1931) may be classified according to the following key:

I. Partially acid-fast organisms with strongly refractive cells. Non-proteolytic and generally non-diastatic; constantly capable of utilizing paraffin.

A. Initial mycelia very small, rapidly dividing into rods and cocci. (Transition to *Mycobacterium.*)

1. Slowly growing organism; cells 0·5-0·7μ in diameter ..... *Proactinomyces minimus*

2. Rapidly growing organisms; cells 1·0-1·2μ in diameter.

a. Cystites* not formed. Rapid formation of cocci ..... *Proactinomyces corallinus*

b. Cystites formed. Less rapid formation of cocci ..... *Proactinomyces salmonicolor*

B. Initial mycelia well developed, richly branching, dividing into rods and generally into cocci.

1. Vegetative mycelium soft, without macroscopically visible aerial mycelium.

a. Vegetative mycelium red; may produce variants with undivided vegetative mycelium and visible white aerial mycelium, or yellow and white variants ..... *Proactinomyces polychromogenes*

b. Vegetative mycelium white to pale pink.

x. Growth on nutrient agar opaque, cream-coloured; cocci in broth culture ..... *Proactinomyces opacus*

xx. Growth on sugar-free nutrient agar watery; no cocci in broth culture ..... *Proactinomyces erythropolis*

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* In the present writer's opinion, the term "cystites" (Enderlein, 1925) may conveniently be used as a collective term for the swollen cells which characterize many of these organisms as well as the corynebacteria, without it being necessary to commit oneself to Enderlein's definition of them as cells with a "polydynamic elemental nucleus" (polydynamen Mych.).

2. Vegetative mycelium hard, yellow, with white aerial mycelium; hyphae divide into chains of acid-fast cocci ..... *Proactinomyces paraffinae*

II. Non-acid-fast organisms with weakly refractive cells; no distinct formation of cocci. Constantly diastatic.

A. Non-proteolytic. No aerial mycelium; marked formation of cystites .... *Proactinomyces mesentericus*

B. Proteolytic organisms.

1. Growth on nutrient agar with rapid formation of unbranched diphtheroid-like rods; no typical cystites; broth turbid .... *Proactinomyces actinomorphus*

2. Growth on nutrient agar with extensive mycelia; simple unbranched rods not formed; cystites present. Broth clear .... *Proactinomyces flavescens* (*Transition to Actinomyces.*)

The pathogenic *Act.* (*Proact.*) *asteroides*, *caprae*, and *farcinicus* obviously belong to Group IB. The same is doubtless the case with numerous other acid-fast, non-proteolytic actinomycetes isolated from and possibly etiologically connected with actinomycotic affections. Such organisms have been described by Cornwall and Lafrenais (1922), Pijper and Pullinger (1927), Kulikowska (1930), and numerous earlier authors summarized by Henrici and Gardner (1921). The fact that similar organisms occur as widespread saprophytic forms suggests that they might easily be encountered as secondary infections in morbid affections. In Group IA we would probably have to place the organisms studied by Vierling (1921; cf. Haag (1927) who recognized as actinomycetes a number of paraffin-decomposing, weakly acid-fast, mycobacterium-like organisms similar to those studied by Vierling).

Summary.

A number of organisms previously described as species of *Mycobacterium* were found, on account of their definite mycelial growth in the initial stages of their life cycles, to have their proper place in the genus *Proactinomyces*.—*Myc. agreste* Gray and Thornton and *Bac. mycoides corallinus* Hefferan were found to be so similar that they must be regarded as one species, *Proact. corallinus*.—*Myc. salmonicolor* den Dooren de Jong is closely related to this and should be called *Proact. salmonicolor*.—*Myc. opacum* den Dooren de Jong and *Myc. crystallophagum* Gray and Thornton proved to be identical; this species should be called *Proact. opacus*.—*Myc. erythropolis* is closely related to this; its proper name should be *Proact. erythropolis*.—*Microbacterium mesentericum* Orla-Jensen showed a very distinct mycelial growth and should be called *Proact. mesentericus*.—These organisms, together with some species, previously described by the present writer, form two separate groups. Group I consists of non-proteolytic organisms with strongly refractive cells showing a partial acid-fastness in milk and sometimes in other media, and constantly capable of decomposing paraffin; some species of this group form a transition to *Mycobacterium*. Group II comprises mostly proteolytic forms with weakly refractive, non-acid-fast cells; from this group there is a close transition to *Actinomyces*.

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References.

BERGEY, D. H., et al., 1930.—Manual of Determinative Bacteriology, 3rd Ed., Baltimore (earlier editions, 1923 and 1925).

CORNWALL, J. W., and LAFRENAIS, H. M., 1922.—A New Variety of Streptothrix Cultivated from Mycetoma of the Leg. *Ind. Journ. Med. Res.*, 10, i, 239-248.

CUNNINGHAM, A., 1931.—The Life Cycle of *B. saccharobutyricus* v. Klecki. *Cent. f. Bakt.*, ii, 83, 1-21.

den DOOREN de JONG, L. E., 1927.—Über protaminophage Bakterien. *Cent. f. Bakt.*, ii, 71, 193-232.

ENDERLEIN, G., 1925.—Bakterien-Cyclogenie (W. de Gruyter, Berlin).

GRAY, P. H. H., and THORNTON, H. G., 1928.—Soil Bacteria that Decompose Certain Aromatic Compounds. *Cent. f. Bakt.*, ii, 73, 74-96.

HAAG, F. E., 1927.—Die saprophytischen Mykobakterien. *Cent. f. Bakt.*, ii, 71, 1-45.

HEFFERAN, M., 1904.—A Comparative and Experimental Study of Bacilli Producing Red Pigment. *Cent. f. Bakt.*, ii, 11, 456-475, 520-536.

HENRICI, A. T., and GARDNER, E. L., 1921.—The Acidfast Actinomycetes (etc.). *Journ. Inf. Dis.*, 28, 232-248.

JENSEN, H. L., 1931.—Contributions to Our Knowledge of the Actinomycetales. II. PROC. LINN. SOC. N.S.W., 56, 345-370.

KULIKOWSKA, H., 1930.—Ein Pilz der Gruppe "Trichomycetes" in einem Falle von Lungenmykose. *Cent. f. Bakt.*, i Or., 117, 90-98.

LÖHNIS, F., 1921.—Studies upon the Life Cycles of the Bacteria. Part I. *Mem. Nat. Acad. Sci. Washington*, 16, No. 2.

MERRILL, M. H., 1930.—Carbohydrate Metabolism of Organisms of the Genus *Mycobacterium*. *Journ. Bact.*, 20, 235-286.

ORLA-JENSEN, S., 1919.—The Lactic Acid Bacteria. *Kgl. Dsk. Vidensk. Selsk. Skr., Mat.-Naturv. Afd.*, 8, Rk., 2, 81-197.

ØRSKOV, J., 1923.—Investigations into the Morphology of the Ray Fungi (Thesis, Copenhagen).

PIJPER, A., and PULLINGER, B. D., 1927.—South African Nocardiases. *Journ. Trop. Med. and Hyg.*, 30, 153-156.

READER, V. B., 1926.—The Identification of the So-called *Bacillus mycoides corallinus* as a Streptothrix. *Journ. Path. and Bact.*, 29, 1-4.

SLATER, C., 1891.—On a Red Pigment-forming Organism, *B. corallinus* (?). *Quart. Journ. Micr. Sci.*, N.S., 32, 409-416.

STOUGHTON, R. H., 1929.—The Morphology and Cytology of *Bacterium malvacearum* E.F.S. *Proc. Roy. Soc. London*, B, 105, 469-485.

VIERLING, K., 1921.—Morphologische und physiologische Untersuchungen über bodenbewohnende Mykobakterien. *Cent. f. Bakt.*, ii, 52, 193-214.