# THE PROPIONIC ACID BACTERIA

## II. CLASSIFICATION[1]

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Systematic study of the propionic acid bacteria was initiated by the interesting observations of von Freudenreich and Orla-Jensen (1906) that the formation of the characteristic eyes in Emmental cheese is due to the production of carbon dioxide, and that the volatile acidity is to be attributed primarily to propionic and acetic acids. Moreover, these investigators isolated directly from Emmental cheese, microörganisms having the property of producing from calcium lactate relatively large quantities of propionic acid in addition to acetic acid and carbon dioxide. On the basis of cultural and morphological differences, these investigators recognized two species, one with two varieties.

1. *Bacterium acidi-propionici a*
2. *Bacterium acidi-propionici b*
3. *Bacillus acidi-propionici*

Morphologically, varieties (1) and (2) showed close resemblance to *Bacterium lactis-acidi* (*Streptococcus lactis*), being minute rods or "stretched cocci," Gram-positive and non-motile. Variety (1) was differentiated from (2) by being slightly more anaerobic and showing a tendency toward pleomorphism when grown at higher temperatures, and by the fact that it did not curdle milk, whereas the latter did, after an extended time. *Bacillus acidi-propionici* was characterized as a somewhat irregular rod-shaped organism, more anaerobic and also growing at lower temperatures than

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*Bacterium acidi-propionici a* and *b* and capable of curdling milk within two days.

Thöni and Allemann (1908) isolated bacteria from the brown and red points appearing on the cut surface of Emmental cheese. These points were found to be almost pure cultures of organisms similar to those described by von Freudenreich and Orla-Jensen, but differing in that they produced pigment. Thöni and Allemann designated their organisms *Bacterium acidi-propionici* var. *fuscum* and var. *rubrum*, respectively, for the brown and red-brown pigment produced.

Gerda Troili-Petersson (1909) reported the isolation of propionic acid bacteria from cheese. She described three relatively distinct types, two of which she believed to be identical with *Bacterium acidi-propionici a* and *b* of von Freudenreich and Orla-Jensen. The third type which she designated as *Bacterium acidi-propionici c*, was differentiated from the others by its fermentative behavior.

Sherman and his associates (1921–1923) published a series of papers relating to the significance of bacteria producing propionic acid in the production of eyes and the characteristic flavor of Swiss cheese. As a result of their investigations, *Bacterium acidi-propionici d* was described.

With the exception of Orla-Jensen's proposal of "propionibacterium" as a generic designation in 1909, no attempt had been made up to 1928 by previous investigators to establish a systematic classification and satisfactory nomenclature of the microörganisms which have been referred to as propionic acid bacteria. Such names as *Bacterium acidi-propionici a* are not in keeping with approved biological nomenclatural practice and are invalid.

Orla-Jensen (1921) later suggested "Propionicoccus" as an additional genus for the spherical forms.

Bergey (1923, 1925), in the first and second editions of his "Manual of Determinative Bacteriology," does not mention the genera Propionibacterium and Propionicoccus.

Buchanan (1925) in his "General Systematic Bacteriology" mentions that "the status of the genus (Propionibacterium) is doubtful, as no species is described or referred to" by Orla-Jensen.

van Niel (1928) published a comprehensive treatise dealing with the classification and chemism of the propionic acid bacteria. He recognized eight species as belonging in the genus Propionibacterium, seven species and one variety previously described in the literature and one *Propionibacterium technicum* as new. van Niel proposed correctly-formed names for all the species.

Bergey (1930) in the third edition of his "Manual of Determinative Bacteriology" recognizes the genus Propionibacterium, and places it in the family Bacteriaceae, tribe Propionibacterieae, as the one genus of that tribe. Bergey's characterization of the tribe Propionibacterieae as aerobic (p. 114) in differentiating it from Lactobacilleae is untenable. The propionic acid bacteria are probably to be considered more anaerobic than the lactic acid forms, and certainly not aerobic. Attention should be called to the confusion in Bergey's manual regarding *P. Thönii* for which the synonym *Bacterium acidi-propionici* var. *rubrum* is given. van Niel arbitrarily chose *Bacterium acidi* var. *fucus* as synonymous with *P. Thönii* since the work of Thönii and Allemann did not adequately differentiate these two species.

Werkman and Kendall (1931) recognized the eight species of van Niel and raised the latter's *P. Jensenii* var. *raffinosaceum* to specific rank.

Bergey (1930) presents a key to the species of the genus Propionibacterium, in which rather extensive use is made of pigment formation and the ratio of propionic to acetic acid. Such a means of differentiation seems impracticable, for Werkman and Kendall (1931) observe "that the production of the deeper colored pigment is uncertain and not sharply differentiated, and that all the species studied by the authors may be considered to produce some pigment. The color ranges from a cream to a brownish red. Any extensive use of pigment formation leads to confusion." They used pigment production to separate two species, *Propionibacterium rubrum* producing a brownish red, and *Propionibacterium raffinosaceum*, producing a cream to buff pigment. Bergey separated *P. Thönii* from *P. rubrum* by the difference in the ratios of propionic to acetic acids, *P. Thönii* showing a ratio of 5:1 and *P. rubrum* a ratio of 3:1. These two species may be

separated on the basis of dissimilation of raffinose, amygdalin, salicin and other carbohydrates.

Hitchner (1932) reported the isolation of two new species of propionic acid bacteria. To these he gave the names *P. zeae* and *P. arbinosum*.

## METHODS AND MATERIALS

The culture numbers and donors are given. The presence of more than one culture number indicates that the culture has been in the possession of the investigators whose initials follow the

TABLE 1

*Agglutination results with P. Freudenreichii antiserum*

| ANTIGEN NUMBER | SPECIES NAME | SERUM DILUTIONS | | | | | |
|---|---|---|---|---|---|---|---|
| | | 40 | 80 | 160 | 320 | 640 | 1280 |
| 1 | *P. Freudenreichii* | 4 | 4 | 4 | 4 | 3 | 0 |
| 2 | *P. Freudenreichii* | 4 | 4 | 4 | 4 | 3 | 0 |
| 44 | *P. Freudenreichii* | 4 | 4 | 4 | 4 | 2 | 0 |
| 37 | *P. Freudenreichii* | 4 | 4 | 4 | 4 | 3 | 0 |
| GROUP AGGLUTINATION | | | | | | | |
| 3 | *P. Shermanii* | 4 | 4 | 3 | 0 | 0 | 0 |
| 4 | *P. Shermanii* | 4 | 4 | 3 | 0 | 0 | 0 |
| 5 | *P. Shermanii* | 4 | 1 | 0 | 0 | 0 | 0 |
| 6 | *P. Shermanii* | 2 | 0 | 0 | 0 | 0 | 0 |
| 15 | *P. technicum* | 4 | 4 | 4 | 1 | 0 | 0 |

numeral, i.e., number 10 is the same organism which van Niel used in his investigations (15V) and was in Sherman's collection as 15S.

Received from Sherman: numbers 1 (6S), 2 (19S) (7V), 3 (1S), 5 (62S) (30V), 10 (15S) (15V), 15 (10S); from van Niel: 20 (4V), 23 (15V), 27 (1V), 32 (24V), 33 (29V); from Charlton: 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46; from Hitchner: 34 (61H), and 35 (56H).

The cultures were grown in a medium of the following composition: Difco yeast extract 10 grams, dipotassium hydrogen phos-

phate 1 gram, agar (Difco) 15 grams, distilled water 1 liter. Brom-thymol-blue was used as an indicator. The pH was adjusted to 7.0 to 7.2.

Aqueous solutions of the various carbohydrates were prepared and sterilized separately, and then added to the sterile basic medium in quantities equivalent to a concentration of 0.3 per cent of the carbohydrate. Final readings of the fermentation tests were made after seven days' incubation at 30°C. Acid production was indicated by indicator, checked by titration.

The agglutination tests were carried out macroscopically in the usual manner. Antigens were standardized by the nephelometer.

It was found that group-agglutination occurred among certain of the more closely related species of propionic acid bacteria, and in order to arrive at a more adequate serological differentiation, agglutinin-absorption tests were run.

Cultures 1, 3, 8, 11, 13, 15, 27, 32, 34, 35, and 38 were employed to immunize rabbits for the preparation of antisera.

## EXPERIMENTAL RESULTS

Generic diagnosis: *Propionibacterium*, Orla-Jensen, 1909.

*Short rods, non-motile, non-sporulating, Gram-positive, assuming involution forms in acid media or under aerobic conditions. Anaerobes, generally catalase-positive, failing to liquefy gelatin or to produce indol. Attack carbohydrates, polyalcohols, glucosides and hydroxy- and keto-acids with the production of relatively large quantities of propionic acid with lesser amounts of acetic acid and carbon dioxide. Require complex organic nitrogen compounds. Mesophilic.*

The type species is *Propionibacterium Freudenreichii.*

### *Propionibacterium Freudenreichii* van Niel 1928

*Synonym. Bacterium acidi-propionici a* von Freudenreich and Orla-Jensen 1906.

*Cultures.* 1 and 2.

*Morphology.* In yeast-glucose-phosphate medium at 30°C. very short rods, "stretched cocci," 0.5 x 0.6$\mu$, single, occasionally in chains, non-motile, Gram-positive.

*Cultural characteristics.* Growth in liquid medium distinctly turbid, ropy sediment, agar stab shows very limited surface growth and heavy stab growth. Pigment: cream to yellow, old cultures distinctly yellow.

*Biochemical characters.* Catalase-positive, nitrates reduced to nitrites, indol-negative, acetyl-methyl-carbinol not produced from glucose, gelatin not liquefied.

*Dissimilation of carbohydrates.* Acid from: adonitol, glucose, erythritol, esculin, galactose, glycerol, inositol, levulose and mannose.

No acid from: amygdalin, dextrin, dulcitol, glycogen, inulin, lactose, mannitol, maltose, melezitose, melibiose, pectin, raffinose, rhamnose, salicin, sorbitol, starch, sucrose, trehalose and xylose.

*Serological results.* Maximum titer of antiserum 1 to 640. Antiserum shows group agglutination with:

| | |
|---|---|
| *Propionibacterium Shermanii* | 1 to 160 |
| *Propionibacterium technicum* | 1 to 320 |

No agglutination with other species of propionic acid bacteria as follows: *P. Peterssonii*, culture 11; *P. Thönii*, cultures 8, 10, 23 and 39; *P. pentosaceum*, cultures 13, 20 and 36; *P. arabinosum*, culture 34; *P. zeae*, culture 35; *P. raffinosaceum*, cultures 32 and 33; *P. Jensenii*, cultures 27 and 45; *P. rubrum*, cultures 16 and 38.

Serologically *P. Freudenreichii* is related to *P. Shermanii* as indicated by cross-agglutination and in this respect agrees with the relationship expressed by the morphology and physiology of the two species. Morphologically the two species are practically identical while their abilities to dissimilate carbohydrates are likewise very similar, the characteristic difference being the inability of *P. Freudenreichii* to attack lactose. Agglutinin-absorption by the two species (table 2) serves to differentiate them satisfactorily.

Serologically *P. Shermanii*, *P. Freudenreichii* and *P. technicum* form a subgeneric group, each species showing cross-agglutination with the antisera of the others. Homologous absorption in each case removed both the homologous and heterologous group agglutinins whereas absorption by a heterologous antigen removed

only the agglutinins for the absorbing antigen and left the homologous agglutinins able to bring about agglutination of the homol-

TABLE 2

*Agglutinin-absorption results*

| ANTISERUM | ABSORBED BY | AGGLUTINATING | DILUTIONS | | | |
|---|---|---|---|---|---|---|
| | | | 40 | 80 | 160 | 320 |
| *P. Freudenreichii* | *P. Freudenreichii* (1) | *P. Freudenreichii* (1) | 0 | 0 | 0 | 0 |
| | *P. Freudenreichii* (1) | *P. Shermanii* (3) | 0 | 0 | 0 | 0 |
| | *P. Freudenreichii* (1) | *P. technicum* (15) | 0 | 0 | 0 | 0 |
| | *P. Shermanii* (3) | *P. Shermanii* (3) | 0 | 0 | 0 | 0 |
| | *P. Shermanii* (3) | *P. Freudenreichii* (1) | 4 | 4 | 3 | 0 |
| | *P. technicum* (15) | *P. technicum* (15) | 0 | 0 | 0 | 0 |
| | *P. technicum* (15) | *P. Freudenreichii* (1) | 4 | 4 | 2 | 0 |
| | *P. technicum* (15) | *P. Shermanii* (3) | 3 | 0 | 0 | 0 |
| *P. Shermanii* | *P. Shermanii* (3) | *P. Shermanii* (3) | 0 | 0 | 0 | 0 |
| | *P. Shermanii* (3) | *P. Freudenreichii* (1) | 0 | 0 | 0 | 0 |
| | *P. Shermanii* (3) | *P. technicum* (15) | 0 | 0 | 0 | 0 |
| | *P. Freudenreichii* (1) | *P. Freudenreichii* (1) | 0 | 0 | 0 | 0 |
| | *P. Freudenreichii* (1) | *P. technicum* (15) | 4 | 0 | 0 | 0 |
| | *P. Freudenreichii* (1) | *P. Shermanii* (3) | 4 | 4 | 3 | 2 |
| | *P. technicum* (15) | *P. technicum* (15) | 0 | 0 | 0 | 0 |
| | *P. technicum* (15) | *P. Freudenreichii* (1) | 0 | 0 | 0 | 0 |
| | *P. technicum* (15) | *P. Shermanii* (3) | 4 | 4 | 3 | 0 |
| *P. technicum* | *P. technicum* (15) | *P. technicum* (15) | 0 | 0 | 0 | 0 |
| | *P. technicum* (15) | *P. Freudenreichii* (1) | 0 | 0 | 0 | 0 |
| | *P. technicum* (15) | *P. Shermanii* (3) | 0 | 0 | 0 | 0 |
| | *P. Freudenreichii* (1) | *P. Freudenreichii* (1) | 4 | 0 | 0 | 0 |
| | *P. Freudenreichii* (1) | *P. technicum* (15) | 4 | 4 | 4 | 3 |
| | *P. Freudenreichii* (1) | *P. Shermanii* (3) | 2 | 0 | 0 | 0 |
| | *P. Shermanii* (3) | *P. Shermanii* (3) | 0 | 0 | 0 | 0 |
| | *P. Shermanii* (3) | *P. technicum* (15) | 4 | 4 | 2 | 0 |
| | *P. Shermanii* (3) | *P. Freudenreichii* (1) | 0 | 0 | 0 | 0 |

ogous antigen at nearly the maximum titer. The results of the agglutinin-absorption tests separated the species nicely.

*Propionibacterium Shermanii* van Niel 1928

*Synonym.* *Bacterium acidi-propionici d* Sherman 1921.

*Culture.* 3, 4, 5, and 6.

*Morphology.* In yeast-glucose-phosphate medium at 30°C. short rods, 0.5 x 0.6$\mu$, single, non-motile, Gram-positive, metachromatic granules.

*Cultural characteristics.* In liquid medium: moderately turbid, ropy sediment. Agar stab: very slight surface growth, abundant stab growth, Pigment: slight, yellowish. Litmus milk: complete decolorization, acid, coagulation.

*Biochemical characters.* Catalase-positive, indol-negative, nitrates not reduced, acetyl-methyl-carbinol not produced from glucose, gelatin not liquefied.

*Dissimilation of carbohydrates.* Acid from adonitol, arabinose, arabitol, glucose, erythritol, esculin, galactose, glycerol, inositol, lactose, levulose and mannose.

No acid from amygdalin, dextrin, dulcitol, glycogen, inulin, mannitol, maltose, melezitose, melibiose, perseitol, pectin, raffinose, rhamnose, salicin, sorbitol, sucrose, starch, trehalose and xylose.

*Serological results.* Maximum titer of antiserum 1 to 1280. Antiserum shows group agglutination with:

| | |
|---|---|
| *P. Freudenreichii* | 1 to 320 |
| *P. technicum* | 1 to 320 |

No agglutination with other species of propionic acid bacteria, as follows: *P. Peterssonii*, culture 11; *P. pentosaceum*, cultures 13, 20 and 36; *P. Jensenii*, cultures 27 and 45; *P. arabinosum*, culture 34; *P. zeae*, culture 35; *P. raffinosaceum*, cultures 32 and 33; *P. rubrum*, cultures 16 and 38; *P. Thönii*, cultures 8, 10, 23 and 39.

The agglutinative behavior of *P. Shermanii* antiserum toward antigens of *P. Freudenreichii* and *P. technicum* agrees nicely with the behavior of *P. Freudenreichii* antiserum toward antigens of *P. Shermanii* and *P. technicum*, and lends confirmation to the suggested existence of a subgeneric grouping of these three species (table 2).

*Propionibacterium technicum* van Niel 1928

*Synonym.* None.

*Culture.* 15.

*Morphology.* In yeast-glucose-phosphate medium at 30°C. the cells appear as short rods, 0.5 x 1.1$\mu$, usually arranged in pairs, non-motile, Gram-positive, showing metachromatic granules.

*Cultural characteristics.* In liquid medium the growth is moderately turbid, the sediment extremely flocculent. In agar stab, growth is abundant, with little or no surface growth. No

TABLE 3

*Agglutination results with P. Shermanii antiserum*

| ANTIGEN NUMBER | SPECIES NAME | SERUM DILUTIONS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 40 | 80 | 160 | 320 | 640 | 1280 | 2560 |
| 3 | *P. Shermanii* | 4 | 4 | 4 | 4 | 4 | 3 | 0 |
| 4 | *P. Shermanii* | 4 | 4 | 4 | 4 | 4 | 2 | 0 |
| 5 | *P. Shermanii* | 4 | 4 | 4 | 4 | 4 | 0 | 0 |
| 6 | *P. Shermanii* | 4 | 4 | 4 | 4 | 4 | 0 | 0 |
| GROUP AGGLUTINATION | | | | | | | | |
| 1 | *P. Freudenreichii* | 4 | 4 | 4 | 3 | 0 | 0 | 0 |
| 2 | *P. Freudenreichii* | 4 | 4 | 4 | 4 | 0 | 0 | 0 |
| 15 | *P. technicum* | 4 | 4 | 4 | 4 | 0 | 0 | 0 |

growth at 37°C. Pigment creamy yellow. Litmus milk slightly decolorized, slight acidity and coagulation.

*Biochemical characters.* Catalase-positive, nitrates not reduced to nitrites, indol-negative, acetyl-methyl-carbinol not produced from glucose, gelatin not liquefied.

*Dissimilation of carbohydrates.* Acid from adonitol, amygdalin, arabitol, arabinose, glucose, dextrin, erythritol, esculin, galactose, glycerol, glycogen, lactose, levulose, mannitol, mannose, maltose, raffinose, salicin, sucrose and starch.

No acid from dulcitol, inulin, melezitose, melibiose, perseitol, pectin, rhamnose, sorbitol, trehalose and xylose.

*Serological results.* Maximum titer of antiserum 1 to 640. Antiserum shows group agglutination with:

*P. Freudenreichii* ..... 1 to 160
*P. Shermanii* ..... 1 to 160

No agglutination with other species of propionic acid bacteria, as follows: *P. Thönii*, cultures 8, 10 and 23; *P. Peterssonii*, culture 11; *P. pentosaceum*, cultures 13 and 20; *P. raffinosaceum*, cultures 32 and 33; *P. Jensenii*, cultures 27 and 45; *P. rubrum*, cultures 16 and 38; *P. arabinosum*, culture 34; *P. zeae*, culture 35.

TABLE 4

*Agglutination results with P. technicum antiserum*

| ANTIGEN NUMBER | SPECIES NAME | SERUM DILUTIONS | | | | | |
|---|---|---|---|---|---|---|---|
| | | 40 | 80 | 160 | 320 | 640 | 1280 |
| 15 | *P. technicum* | 4 | 4 | 4 | 4 | 2 | 0 |
| GROUP AGGLUTINATION | | | | | | | |
| 1 | *P. Freudenreichii* | 4 | 4 | 4 | 0 | 0 | 0 |
| 2 | *P. Freudenreichii* | 4 | 4 | 3 | 0 | 0 | 0 |
| 3 | *P. Shermanii* | 4 | 4 | 3 | 0 | 0 | 0 |
| 4 | *P. Shermanii* | 4 | 4 | 2 | 0 | 0 | 0 |
| 5 | *P. Shermanii* | 4 | 4 | 2 | 0 | 0 | 0 |
| 6 | *P. Shermanii* | 4 | 4 | 2 | 0 | 0 | 0 |

A discussion of the cross-agglutination of *P. technicum* with *P. Freudenreichii* and *P. Shermanii* has been given under the description of *P. Freudenreichii*. The results in table 4 lend further confirmation to the suggested subgeneric grouping of the three species.

*Propionibacterium raffinosaceum* Werkman and Kendall 1931

*Synonyms. Bacterium acidi-propionici b* von Freudenreich and Orla-Jensen (in part), 1906. *Propionibacterium Jensenii* var. *raffinosaceum* van Niel 1928.

*Culture.* 32.

*Morphology.* In yeast-glucose-phosphate medium at 30°C. rods, single and short chains, 0.7 x 1.0–1.7$\mu$, longer than *P. Jensenii*, non-motile, Gram-positive, metachromatic granules.

*Cultural characteristics.* Growth in liquid medium: only slightly turbid, sediment flaky. Agar stab: moderate surface growth, abundant stab growth, orange-yellow pigment. No growth at 37°C. Litmus milk: complete decolorization of indicator, slight acid reaction, coagulation.

*Biochemical characters.* Catalase-positive, indol-negative, nitrates not reduced to nitrites, acetyl-methyl-carbinol not produced from glucose. No gelatin liquefaction.

*Dissimilation of carbohydrates.* Acid from adonitol, amygdalin, arabitol, glucose, erythritol, esculin, galactose, glycerol, inositol, lactose, levulose, mannitol, mannose, maltose, melezitose, raffinose, salicin, sucrose and trehalose.

No acid from arabinose, dextrin, dulcitol, glycogen, inulin, melibiose, perseitol, pectin, rhamnose, sorbitol, starch and xylose.

*Serological results.* Maximum titer of antiserum greater than 1 to 2560. Antiserum shows group agglutination with:

| | |
|---|---|
| *P. Peterssonii* | 1 to 160 |
| *P. pentosaceum* | 1 to 80 |
| *P. arabinosum* | 1 to 80 |

No agglutination with other species of propionic acid bacteria, as follows: *P. Freudenreichii*, cultures 1, 2, 37 and 44; *P. Shermanii*, cultures 3, 4, 5 and 6; *P. rubrum*, cultures 16 and 38; *P. Thönii*, cultures 8, 10, 23 and 39; *P. Jensenii*, cultures 27 and 45; *P. zeae*, culture 35; *P. technicum*, culture 15.

The antiserum of *P. raffinosaceum* shows agglutination of *P. pentosaceum*, *P. Peterssonii* and *P. arabinosum* (table 5). It is to be noted that the serum of each of the last two species in no case agglutinates *P. raffinosaceum* and that the serum of *P. raffinosaceum* does not agglutinate *P. Jensenii*, nor does the serum of *P. Jensenii* agglutinate *P. ruffinosaceum*, all of which gives weight to recognition of *P. raffinosaceum* as a species. It will be recalled that van Niel considered *P. raffinosaceum* as a variety of *P. Jensenii*.

A very weak agglutination of *P. raffinosaceum* occurs with antiserum of *P. pentosaceum*.

The results of the agglutinin-absorption tests distinctly sepa-

rate *P. raffinosaceum* and *P. Peterssonii*. The absorption of agglutinins for *P. raffinosaceum* by *P. Peterssonii* antigen is negligible (table 6).

TABLE 5

*Agglutination results with P. raffinosaceum antiserum*

| ANTIGEN NUMBER | SPECIES NAME | SERUM DILUTIONS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 40 | 80 | 160 | 320 | 640 | 1280 | 2560 |
| 32 | *P. raffinosaceum* | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 33 | *P. raffinosaceum* | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| GROUP AGGLUTINATION | | | | | | | | |
| 11 | *P. Peterssonii* | 4 | 4 | 3 | 0 | 0 | 0 | 0 |
| 13 | *P. pentosaceum* | 4 | 3 | 0 | 0 | 0 | 0 | 0 |
| 20 | *P. pentosaceum* | 4 | 3 | 0 | 0 | 0 | 0 | 0 |
| 36 | *P. pentosaceum* | 4 | 4 | 0 | 0 | 0 | 0 | 0 |
| 34 | *P. arabinosum* | 4 | 3 | 0 | 0 | 0 | 0 | 0 |

TABLE 6

*Agglutinin-absorption results*

| ANTISERUM | ABSORBED BY | AGGLUTINATING | DILUTIONS | | | |
|---|---|---|---|---|---|---|
| | | | 40 | 80 | 160 | 320 |
| *P. raffinosaceum* | *P. raffinosaceum* (32) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. raffinosaceum* (32) | *P. raffinosaceum* (33) | 0 | 0 | 0 | 0 |
| | *P. raffinosaceum* (32) | *P. raffinosaceum* (32) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. raffinosaceum* (32) | 4 | 4 | 4 | 4 |

## *Propionibacterium Jensenii* van Niel 1928

*Synonym.* *Bacterium acidi-propionici b* von Freudenreich and Orla-Jensen 1906.

*Culture.* 27.

*Morphology.* In yeast-glucose-phosphate medium at 30°C. short rods, 0.7 x 0.8–1.3$\mu$, shorter than *P. raffinosaceum*, single,

non-motile, Gram-positive. Tendency to form involution forms in acid media not as pronounced as with certain other species.

*Cultural characteristics.* Pigment: orange to yellow, distinctly yellow in old cultures. Surface growth more deeply pigmented. Slow coagulation of milk. Litmus milk reduced, acid.

*Biochemical characters.* Catalase-positive, nitrates not reduced to nitrites, no gelatin liquefaction, acetyl-methyl-carbinol produced from glucose.

TABLE 7

*Agglutination results with P. Jensenii antiserum*

| ANTIGEN NUMBER | SPECIES NAME | SERUM DILUTIONS | | | | | |
|---|---|---|---|---|---|---|---|
| | | 40 | 80 | 160 | 320 | 640 | 1280 |
| 27 | *P. Jensenii* | 4 | 4 | 4 | 4 | 3 | 0 |
| 45 | *P. Jensenii* | 4 | 4 | 4 | 4 | 2 | 0 |
| GROUP AGGLUTINATION | | | | | | | |
| 11 | *P. Peterssonii* | 4 | 4 | 4 | 0 | 0 | 0 |
| 8 | *P. Thönii* | 4 | 4 | 3 | 0 | 0 | 0 |
| 10 | *P. Thönii* | 4 | 4 | 4 | 0 | 0 | 0 |
| 23 | *P. Thönii* | 4 | 4 | 4 | 0 | 0 | 0 |
| 13 | *P. pentosaceum* | 4 | 3 | 0 | 0 | 0 | 0 |
| 20 | *P. pentosaceum* | 4 | 2 | 0 | 0 | 0 | 0 |
| 36 | *P. pentosaceum* | 4 | 3 | 0 | 0 | 0 | 0 |

*Dissimilation of carbohydrates.* Acid from adonitol, arabitol, glucose, erythritol, esculin, galactose, glycerol, inositol, lactose, levulose, mannitol, mannose, maltose, sucrose and trehalose.

No acid from amygdalin, arabinose, cellobiose, dextrin, dulcitol, glycogen, inulin, melezitose, melibiose, perseitol, pectin, raffinose, rhamnose, salicin, sorbitol, starch and xylose.

*Serological results.* Maximum titer of antiserum 1 to 640. Antiserum shows group agglutination with:

*P. Peterssonii* ..... 1 to 160
*P. Thönii* ..... 1 to 160
*P. pentosaceum* ..... 1 to 80

No agglutination with other species of propionic acid bacteria as follows: *P. Freudenreichii*, cultures 1, 2, 37 and 44: *P. Shermanii*, culture 3, 4, 5 and 6; *P. rubrum*, cultures 16 and 38; *P. raffinosaceum*, cultures 32 and 33; *P. zeae*, culture 35; *P. arabinosum*, culture 34; and *P. technicum*, culture 15.

TABLE 8

*Agglutinin-absorption results*

| ANTISERUM | ABSORBED BY | AGGLUTINATING | DILUTIONS | | | |
|---|---|---|---|---|---|---|
| | | | 40 | 80 | 160 | 320 |
| *P. Jensenii* | *P. Jensenii* (27) | *P. Jensenii* (27) | 0 | 0 | 0 | 0 |
| | *P. Jensenii* (27) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. Jensenii* (27) | *P. Thönii* (8) | 0 | 0 | 0 | 0 |
| | *P. Thönii* (8) | *P. Thönii* (8) | 0 | 0 | 0 | 0 |
| | *P. Thönii* (8) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. Thönii* (8) | *P. Jensenii* (27) | 4 | 4 | 4 | 1 |
| | *P. Peterssonii* (11) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. Thönii* (8) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. Jensenii* (27) | 4 | 4 | 3 | 0 |
| *P. Thönii* | *P. Thönii* (8) | *P. Thönii* (8) | 0 | 0 | 0 | 0 |
| | *P. Thönii* (8) | *P. Peterssonii* (11) | 3 | 0 | 0 | 0 |
| | *P. Thönii* (8) | *P. Jensenii* (27) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. Thönii* (8) | 4 | 4 | 4 | 3 |
| | *P. Peterssonii* (11) | *P. Jensenii* (27) | 4 | 4 | 2 | 0 |
| | *P. Jensenii* (27) | *P. Jensenii* (27) | 0 | 0 | 0 | 0 |
| | *P. Jensenii* (27) | *P. Peterssonii* (11) | 4 | 3 | 0 | 0 |
| | *P. Jensenii* (27) | *P. Thönii* (8) | 4 | 4 | 4 | 2 |

*P. Jensenii* is easily differentiated from *P. Peterssonii* and *P. Thönii* which its serum cross agglutinates, by the results of agglutinin-absorption (table 8). The cross agglutination of *P. Jensenii* serum and antigen of *P. pentosaceum* is weak and there is no difficulty in separating the species.

### *Propionibacterium Peterssonnii* van Niel 1928

*Synonym.* *Bacterium acidi-propionici c* Troili-Petersson 1909.

*Culture.* 11, 12, 24 and 25.

*Morphology.* In yeast-glucose-phosphate medium at 30°C. short rods, 0.7 x 1.4$\mu$, single and in pairs, non-motile, Gram-positive, metachromatic granules.

*Cultural characteristics.* Growth in liquid medium: slightly turbid, flocculent. Agar stab: moderate surface growth, abundant stab growth with yellow pigment. Litmus milk slightly decolorized, acid and slightly coagulated.

*Biochemical characters.* Catalase-positive, nitrates not reduced to nitrites, indol-negative, acetyl-methyl-carbinol not (?) produced from glucose. No gelatin liquefaction.

TABLE 9

*Agglutination results with P. Peterssonii antiserum*

| ANTIGEN NUMBER | SPECIES NAME | SERUM DILUTIONS | | | | | |
|---|---|---|---|---|---|---|---|
| | | 40 | 80 | 160 | 320 | 640 | 1280 |
| 11 | *P. Peterssonii* | 4 | 4 | 4 | 4 | 4 | 0 |
| GROUP AGGLUTINATION | | | | | | | |
| 13 | *P. pentosaceum* | 3 | 1 | 0 | 0 | 0 | 0 |
| 20 | *P. pentosaceum* | 3 | 1 | 0 | 0 | 0 | 0 |
| 34 | *P. arabinosum* | 4 | 2 | 0 | 0 | 0 | 0 |

*Dissimilation of carbohydrates.* Acid from adonitol, amygdalin, arabitol, glucose, erythritol, esculin, galactose, glycerol, inositol, lactose, levulose, mannitol, mannose, maltose, melezitose, salicin, sucrose and trehalose.

No acid from arabinose, cellobiose, dextrin, dulcitol, glycogen, inulin, melibiose, perseitol, pectin, raffinose, rhamnose, sorbitol, starch and xylose.

*Serological results.* Maximum titer of antiserum 1 to 640. Antiserum shows group agglutination with:

*P. pentosaceum*..... 1 to 80
*P. arabinosum*..... 1 to 80

No agglutination with other species of propionic acid bacteria, as follows: *P. Freudenreichii*, cultures 1, 2, 37 and 44; *P. Thönii*,

cultures 8, 10 and 23; *P. Shermanii*, cultures 3, 4, 5 and 6; *P. raffinosaceum*, cultures 32 and 33; *P. zeae*, culture 35; *P. technicum*, culture 15; *P. Jensenii*, cultures 27 and 45; *P. rubrum*, cultures 16 and 38.

*P. Peterssonii*, *P. pentosaceum* and *P. arabinosum* appear to form a subgeneric group. The antiserum of each shows cross agglutination of the other two antigens. The agglutinin-absorption tests differentiated the species nicely (table 10).

TABLE 10

*Agglutinin-absorption results*

| ANTISERUM | ABSORBED BY | AGGLUTINATING | DILUTIONS | | | |
|---|---|---|---|---|---|---|
| | | | 40 | 80 | 160 | 320 |
| *P. Peterssonii* | *P. Peterssonii* (11) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. pentosaceum* (13) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. arabinosum* (34) | 0 | 0 | 0 | 0 |
| | *P. arabinosum* (34) | *P. arabinosum* (34) | 0 | 0 | 0 | 0 |
| | *P. arabinosum* (34) | *P. pentosaceum* (13) | 0 | 0 | 0 | 0 |
| | *P. arabinosum* (34) | *P. Peterssonii* (11) | 4 | 4 | 3 | 0 |
| | *P. pentosaceum* (13) | *P. pentosaceum* (13) | 0 | 0 | 0 | 0 |
| | *P. pentosaceum* (13) | *P. arabinosum* (34) | 4 | 0 | 0 | 0 |
| | *P. pentosaceum* (13) | *P. Peterssonii* (13) | 4 | 4 | 3 | 2 |

*Propionibacterium pentosaceum* van Niel 1928

*Synonym. Bacillus acidi-propionici* von Freudenreich and Orla-Jensen 1906.

*Culture.* 13, 20 and 31.

*Morphology.* In yeast-glucose-phosphate medium at 30°C. rods, 0.9 x 1.6$\mu$, single, in pairs and short chains, cells may be swollen, branched, curved. Non-motile, Gram-positive, with metachromatic granules.

*Cultural characteristics.* Agar stab: moderate surface growth, abundant in stab with cream-colored pigment. Litmus milk decolorized, acid and slightly coagulated.

*Biochemical characters.* Catalase-positive (weak), nitrates re-

duced to nitrites, indol-negative, acetyl-methyl-carbinol not produced from glucose, no gelatin liquefaction.

*Dissimilation of carbohydrates.* Acid from adonitol, amygdalin, arabinose, arabitol, cellobiose, glucose, erythritol, esculin, galactose, glycerol, inositol, lactose, levulose, mannitol, mannose, maltose, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose and xylose.

No acid from dextrin, dulcitol, glycogen, inulin, perseitol, pectin. Starch is very slightly attacked.

TABLE 11

*Agglutination results with P. pentosaceum antiserum*

| ANTIGEN NUMBER | SPECIES NAME | SERUM DILUTIONS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 40 | 80 | 160 | 320 | 640 | 1280 | 2560 |
| 13 | *P. pentosaceum* | 4 | 4 | 4 | 4 | 4 | 4 | 3 |
| 20 | *P. pentosaceum* | 4 | 4 | 4 | 4 | 4 | 3 | 0 |
| 36 | *P. pentosaceum* | 4 | 4 | 4 | 4 | 4 | 3 | 0 |
| GROUP AGGLUTINATION | | | | | | | | |
| 11 | *P. Peterssonii* | 4 | 4 | 4 | 3 | 2 | 0 | 0 |
| 34 | *P. arabinosum* | 4 | 3 | 0 | 0 | 0 | 0 | 0 |
| 32 | *P. raffinosaceum* | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | *P. raffinosaceum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*Serological results.* Maximum titer of antiserum 1 to 2560. Antiserum shows group agglutination with:

*P. Peterssonii*..... 1 to 640
*P. arabinosum*..... 1 to 80
*P. raffinosaceum*..... 1 to 40

No agglutination with other species of propionic acid bacteria as follows: *P. rubrum*, cultures 16 and 38; *P. Freudenreichii*, cultures 1, 2 and 37; *P. Shermanii*, cultures 3, 4, 5 and 6; *P. Thönii* cultures 8, 10 and 23; *P. zeae*, culture 35; *P. technicum*, culture 15; *P. Jensenii*, cultures 27 and 45.

A slight agglutination of *P. raffinosaceum* at 1:40 occurred but

in view of the relatively low dilution of the serum, it was felt unnecessary to run absorption tests. Table 11 shows the grouping of *P. Peterssonii*, *P. pentosaceum* and *P. arabinosum* and table 12 again shows the clear separation of the three species on the basis of agglutinin-absorption.

TABLE 12

*Agglutinin-absorption results*

| ANTISERUM | ABSORBED BY | AGGLUTINATING | DILUTIONS | | | |
|---|---|---|---|---|---|---|
| | | | 40 | 80 | 160 | 320 |
| *P. arabinosum* | *P. arabinosum* (34) | *P. arabinosum* (34) | 0 | 0 | 0 | 0 |
| | *P. arabinosum* (34) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. arabinosum* (34) | *P. pentosaceum* (13) | 0 | 0 | 0 | 0 |
| | *P. pentosaceum* (13) | *P. pentosaceum* (13) | 0 | 0 | 0 | 0 |
| | *P. pentosaceum* (13) | *P. Peterssonii* (11) | 4 | 1 | 0 | 0 |
| | *P. pentosaceum* (13) | *P. arabinosum* (34) | 4 | 4 | 4 | 4 |
| | *P. Peterssonii* (11) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. pentosaceum* (13) | 4 | 3 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. arabinosum* (34) | 4 | 4 | 4 | 2 |
| *P. pentosaceum* | *P. pentosaceum* (13) | *P. pentosaceum* (13) | 4 | 4 | 2 | 0 |
| | *P. pentosaceum* (13) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. pentosaceum* (13) | *P. arabinosum* (34) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. Peterssonii* (11) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. arabinosum* (34) | 0 | 0 | 0 | 0 |
| | *P. Peterssonii* (11) | *P. pentosaceum* (13) | 4 | 4 | 4 | 3 |
| | *P. arabinosum* (34) | *P. arabinosum* (34) | 0 | 0 | 0 | 0 |
| | *P. arabinosum* (34) | *P. Peterssonii* (11) | 4 | 4 | 2 | 0 |
| | *P. arabinosum* (34) | *P. pentosaceum* (13) | 4 | 4 | 4 | 4 |

*Propionibacterium arabinosum* Hitchner 1931

*Synonym.* None.

*Culture.* 34.

*Morphology.* In yeast-glucose-phosphate medium, rods elongated, curved, swollen, thread like, single, pairs and long chains, 3.0–8.0 x 0.8–1.4$\mu$, Gram-positive, non-motile. In a neutral medium relatively short rods.

*Cultural characteristics.* In agar stab, moderate surface growth, abundant stab growth, light orange pigment.

*Biochemical characters.* Catalase-negative, nitrates slowly reduced, milk coagulated in three to four weeks. Gelatin not liquefied. Acetyl-methyl-carbinol not produced from glucose.

*Dissimilation of carbohydrates.* Acid from adonitol, amygdalin, arabinose, arabitol, glucose, dextrin, erythritol, esculin, galactose, glycerol, glycogen, inositol, lactose, levulose, mannitol, mannose, maltose, melezitose, rhamnose, salicin, sucrose, starch and trehalose.

No acid from cellobiose, dulcitol, inulin, melibiose, pectin, raffinose and xylose.

*Serological results.* Maximum titer of antiserum 1 to 2560. Antiserum shows group agglutination with:

| | |
|---|---|
| *P. pentosaceum* | 1 to 320 |
| *P. Peterssonii* | 1 to 160 |

No agglutination with other species of propionic acid bacteria, as follows: *P. Freudenreichii,* cultures 1 and 2; *P. Shermanii,* cultures 3, 4, 5 and 6; *P. Thönii,* cultures 8, 10 and 23; *P. technicum,* culture 15; *P. rubrum,* cultures 16 and 38; *P. raffinosaceum,* cultures 32 and 33; *P. Jensenii,* cultures 27 and 45; *P. zeae,* culture 35.

The same grouping is again apparent, placing *P. arabinosum, P. Pentosaceum* and *P. Peterssonii* together (table 13). Separation of species is again accomplished easily by agglutinin-absorption tests (table 12).

### *Propionibacterium zeae* Hitchner 1931

*Synonym.* None.

*Culture.* 35.

*Morphology.* In yeast-glucose-phosphate medium at 30°C. rods, somewhat swollen, stains irregularly, size 2.5–4.0 x 1.2–1.4$\mu$, Gram-positive, non-motile.

*Cultural characteristics.* Growth in agar stab, luxuriant, moderate surface growth, pigment orange. In liquid medium pronounced turbidity, ropy sediment. Milk is not coagulated.

*Biochemical characters.* Catalase-positive, nitrates not reduced. Indol-negative. Acetyl-methyl-carbinol not produced from glucose.

*Dissimilation of carbohydrates.* Acid from adonitol, arabitol, glucose, dextrin, erythritol, esculin, galactose, glycerol, glycogen, inositol, levulose, mannitol, mannose, maltose, melezitose, melibiose, raffinose, salicin, sucrose, starch and trehalose.

No acid from amygdalin, arabinose, cellobiose, dulcitol, inulin, lactose, pectin, rhamnose and xylose.

*Serological results.* Maximum titer of antiserum 1 to 1280. Antiserum does not agglutinate other species of propionic acid bacteria, as follows: *P. Shermanii*, cultures 3, 4, 5 and 6; *P. Freudenreichii*, cultures 1 and 2; *P. Thönii*, cultures 8, 10 and 23; *P. technicum*, culture 15; *P. raffinosaceum*, culture 32; *P. rubrum*, culture 38; *P. pentosaceum*, culture 13; *P. Peterssonii*, culture 11; *P. arabinosum*, culture 34; *P. Jensenii*, culture 27.

TABLE 13

*Agglutination results with P. arabinosum antiserum*

| ANTIGEN NUMBER | SPECIES NAME | SERUM DILUTIONS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 40 | 80 | 160 | 320 | 640 | 1280 | 2560 |
| 34 | *P. arabinosum* | 4 | 4 | 4 | 4 | 4 | 4 | 3 |
| GROUP AGGLUTINATION | | | | | | | | |
| 13 | *P. pentosaceum* | 4 | 4 | 4 | 3 | 0 | 0 | 0 |
| 20 | *P. pentosaceum* | 4 | 4 | 4 | 3 | 0 | 0 | 0 |
| 11 | *P. Peterssonii* | 4 | 4 | 3 | 0 | 0 | 0 | 0 |

*P. zeae* is unique among the species of Propionibacterium in that its antiserum does not agglutinate the antigen of any other of the species and in turn antigen of *P. zeae* is not agglutinated by the antiserum of any other species.

## *Propionibacterium Thönii* van Niel 1928

*Synonym.* *Bacterium acidi-propionici* var. *rubrum* Thönii and Allemann 1908.

*Cultures.* 8, 10, 22, 23 and 30.

*Morphology.* In yeast-glucose-phosphate medium at 30°C. short rods, 1.0 x 1.5$\mu$, short chains, non-motile, Gram-positive, metachromatic granules.

*Cultural characteristics.* Growth in liquid medium moderately turbid, ropy sediment. Abundant growth in agar stab with slight surface growth. Pigment, dark red-orange. Litmus milk slightly decolorized, slight acidity and coagulation.

TABLE 14

*Agglutination results with P. Thönii antiserum*

| ANTIGEN NUMBER | SPECIES NAME | SERUM DILUTIONS | | | | | |
|---|---|---|---|---|---|---|---|
| | | 40 | 80 | 160 | 320 | 640 | 1280 |
| 8 | *P. Thönii* | 4 | 4 | 4 | 4 | 3 | 0 |
| 10 | *P. Thönii* | 4 | 4 | 4 | 3 | 2 | 0 |
| 23 | *P. Thönii* | 4 | 4 | 4 | 3 | 2 | 0 |
| 39 | *P. Thönii* | 4 | 4 | 4 | 4 | 3 | 0 |
| GROUP AGGLUTINATION | | | | | | | |
| 27 | *P. Jensenii* | 4 | 4 | 3 | 0 | 0 | 0 |
| 11 | *P. Peterssonii* | 4 | 3 | 0 | 0 | 0 | 0 |

*Biochemical characters.* Catalase-positive, indol-negative, nitrates not reduced to nitrites, acetyl-methyl-carbinol produced from glucose. No gelatin liquefaction.

*Dissimilation of carbohydrates.* Acid from adonitol, arabitol, glucose, erythritol, esculin, galactose, glycerol, lactose, levulose, mannose, maltose, salicin, sorbitol, sucrose and trehalose.

No acid from amygdalin, arabinose, dextrin, dulcitol, glycogen, inulin, mannitol, melezitose, melibiose, perseitol, pectin, raffinose, rhamnose, starch and xylose.

*Serological results.* Maximum titer of antiserum 1 to 640.

Antiserum shows group agglutination with:

*P. Jensenii*..... 1 to 160
*P. Peterssonii*..... 1 to 80

No agglutination with other species of propionic acid bacteria as follows: *P. Freudenreichii*, cultures 1 and 2; *P. Shermanii*, cultures 3, 4, 5 and 6; *P. pentosaceum*, cultures 13 and 20; *P. raffinosaceum*, cultures 32 and 33; *P. zeae*, culture 35; *P. arabinosum*, culture 34; *P. rubrum*, cultures 16 and 38; *P. technicum*, culture 15.

It is interesting to note that *P. Thönii* shows no cross agglutination with *P. rubrum*, two species which were separated and recognized by van Niel (1928) only after careful consideration of their physiological behavior. *P. rubrum* attacked raffinose and mannitol, whereas *P. Thönii* did not; also the latter produced greater quantities of acid from glucose. Serologically the species are distinct. *P. Thönii* antiserum shows cross agglutination of *P. Jensenii* and *P. Peterssonnii* (table 14) but separation is accomplished by agglutinin-absorption (table 8).

*Propionibacterium rubrum* van Niel 1928

*Synonym.* *Bacterium acidi-propionici* var. *rubrum* Thönii and Allemann 1908.

*Culture.* 38, 9, 16 and 19.

*Morphology.* In yeast-glucose-phosphate medium at 30°C. short rods, singly or in pairs, 0.8 x 1.2$\mu$, non-motile, Gram-positive, metachromatic granules.

*Cultural characteristics.* In agar stab, growth luxuriant, moderate surface growth. Red-orange pigment. In liquid medium, slight turbidity, sediment finely flocculent, showing reddish orange pigment. Litmus milk, slight decolorization, acid and slow coagulation.

*Biochemical characters.* Catalase-positive, indol-negative, nitrates not reduced to nitrites, no gelatin liquefaction, and no acetyl-methyl-carbinol produced from glucose.

*Dissimilation of carbohydrates.* Acid from adonitol, amygdalin, arabitol, glucose, erythritol, esculin, galactose, glycerol, lactose, levulose, mannitol, mannose, maltose, melezitose, raffinose, salicin, sucrose and trehalose.

No acid from arabinose, dextrin, dulcitol, glycogen, inositol, inulin, melibiose, perseitol, pectin, rhamnose, starch and xylose.

*Serological results.* Maximum titer of antiserum 1 to 640. Antiserum shows group agglutination with:

*P. Peterssonii*..... 1 to 40
*P. pentosaceum*..... 1 to 40
*P. arabinosum*..... 1 to 40

No agglutination with other species of propionic acid bacteria as follows: *P. Freudenreichii*, culture 1; *P. Shermanii*, cultures 3 and 4; *P. technicum*, culture 15; *P. Thönii*, culture 8; *P. Jensenii*, culture 27; *P. raffinosaceum*, cultures 32 and 33; *P. zeae*, culture 35.

TABLE 15

*Agglutination results with P. rubrum antiserum*

| ANTIGEN NUMBER | SPECIES NAME | SERUM DILUTIONS | | | | | |
|---|---|---|---|---|---|---|---|
| | | 40 | 80 | 160 | 320 | 640 | 1280 |
| 38 | *P. rubrum* | 4 | 4 | 4 | 4 | 3 | 0 |
| 16 | *P. rubrum* | 4 | 4 | 4 | 2 | 0 | 0 |
| GROUP AGGLUTINATION | | | | | | | |
| 11 | *P. Peterssonii* | 4 | 0 | 0 | 0 | 0 | 0 |
| 13 | *P. pentosaceum* | 4 | 0 | 0 | 0 | 0 | 0 |
| 20 | *P. pentosaceum* | 4 | 0 | 0 | 0 | 0 | 0 |
| 34 | *P. arabinosum* | 4 | 0 | 0 | 0 | 0 | 0 |

*P. rubrum* antiserum showed agglutination at a dilution of 1:40 of all three members of the last subgroup (table 15) although the sera of none of the group agglutinated *P. rubrum*. *P. rubrum* is unusual in that it is not agglutinated by the antiserum of another species at the serum dilutions used.

KEY TO THE SPECIES OF THE GENUS PROPIONIBACTERIUM

A. Attacking sucrose and maltose
  B. Attacking the polysaccharides (starch, dextrin, glycogen)
    C. Attacking lactose and arabinose
      D. Attacking rhamnose and trehalose, not attacking raffinose, catalase-negative
        —*Propionibacterium arabinosum*

DD. Not attacking rhamnose and trehalose, attacking raffinose, catalase-positive
—*Propionibacterium technicum*
CC. Not attacking lactose and arabinose
—*Propionibacterium zeae*
BB. Not attacking polysaccharides
C. Attacking xylose and arabinose, nitrates reduced
—*Propionibacterium pentosaceum*
CC. Not attacking xylose and arabinose, nitrates not reduced
D. Attacking raffinose
E. Pigment yellow
—*Propionibacterium raffinosaceum*
EE. Pigment red-brown
—*Propionibacterium rubrum*
DD. Not attacking raffinose
E. Attacking mannitol, not attacking sorbitol
F. Attacking amygdalin and salicin
—*Propionibacterium Peterssonii*
FF. Not attacking amygdalin and salicin
—*Propionibacterium Jensenii*
EE. Not attacking mannitol, attacking sorbitol
—*Propionibacterium Thönii*
AA. Not attacking sucrose and maltose
B. Attacking lactose, nitrates not reduced
—*Propionibacterium Shermanii*
BB. Not attacking lactose, nitrates reduced
—*Propionibacterium Freudenreichii*

## SUMMARY AND CONCLUSIONS

Morphological and physiological (including serological) study of 30 strains of propionic-acid producing bacteria belonging to the genus Propionibacterium Orla-Jensen, 1909 leads to a clear-cut recognition of eleven species. The general agreement among morphological, physiological and serological classifications is unusual, in that recognition of a species by one method is clearly confirmed by the other methods.

The propionic acid bacteria constitute a natural group which should be recognized as a genus, Propionibacterium Orla-Jensen 1909. In the present state of knowledge it is doubtful whether a more natural genus is known. We may accept the production of substantial quantities of propionic acid in a true fermentation at mesophilic temperatures as characteristic of members of the genus.

Serological differentiation led to three subgeneric groups. *P.*

*zeae* and *P. rubrum* showed relatively little relationship to any of the three subgroups.

Eleven species have been clearly differentiated on the basis of physiological, serological and morphological behavior.

A key has been presented based upon characters which should be of practical use in identifying species.

It is of interest to note that *P. arabinosum* is catalase-negative. This behavior necessitates modification of the generic diagnosis to include the species.

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