Bacillus pantothenticus (n.sp.)

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SUMMARY: A new mesophilic species of the genus Bacillus was isolated from soil. Within the genus it most nearly resembles B. circulans but is clearly distinct in that it produces no motile colonies and no gas from glucose in the presence of an inorganic nitrogen source, hydrolyses casein, liquefies inspissated serum and gives a restricted egg-yolk reaction. It also differs from all other Bacillus spp. tested in that its growth is stimulated by 4% NaCl, and that pantothenic acid satisfies a nutritional requirement. The name Bacillus pantothenticus is proposed.

During the isolation from soil of mesophilic species of the genus Bacillus (Knight & Proom, 1950) an apparently new organism was repeatedly obtained by incubating soil suspensions in 4% NaCl broth, followed by plating on nutrient agar and incubation at 37°. This organism is considered to be a new species; the following description is based on a study of eleven strains isolated from eleven different specimens of soil from widely separated localities in southern England. The methods and media used were similar to those previously described (Smith, Gordon & Clark, 1946; Knight & Proom, 1950).

CHARACTERS

Morphology. Vegetative rods of average size 0.5 x 4.0 µ., slightly curved, usually occurring singly or in bundles consisting of a few organisms. The rods are motile. After 24 hr. growth the organisms are frankly Gram-positive; after further incubation the Gram-staining reaction is variable. The sporangium is swollen and the organism almost drumstick in appearance, the spore being thin-walled, nearly spherical or sometimes spherical. In wet preparations the spores appear spherical more often than in stained preparations. Average spore size in Gram-stained preparations, 1.0 µ. x 1.0 to 1.5 µ.

Nutrient agar. After 48 hr. at 37° colonies greyish white, opaque with granular surface and irregular edge, 1.5-2.0 mm. diameter.

Nutrient agar slope. After 24 hr. at 37° moderate slightly moist greyish white growth.

Nutrient agar slope (pH 6.0). Growth.

Glucose nitrate agar. No growth.

Semi-solid glucose agar + iron. Gas.

Potato. No growth.

Loeffler blood serum. After 24 hr. at 37° moderate growth with some digestion; marked liquefaction within 7 days.

Nutrient broth. After 24 hr. at 37° faint uniform turbidity with slightropy deposit. Growth markedly stimulated and improved by addition of 4% NaCl; will give good growth in nutrient broth containing 10% NaCl.

Metabolism. Aerobe, facultative anaerobe. Growth at 37° better than at 28° and usually slightly better than at 45°.
**Fermentation tests.** Neither acid nor gas from glucose, arabinose or xylose with inorganic basal medium. Acid but no gas from glucose, arabinose and xylose with organic basal medium.

**Voges-Proskauer reaction.** Negative.

**Nitrate reduction to nitrite.** Variable.

**Urease.** Negative.

**Catalase.** Positive.

**Starch hydrolysis.** Positive.

**Casein hydrolysis.** Positive.

**Gelatin hydrolysis.** Weak positive, the plate-test being positive in 4–7 days; some liquefaction of gelatin-stab within 20 days at 37°.

**Egg-yolk reaction.** Positive, restricted (Knight & Proom, 1950).

**Nutrition.** Will grow in a medium containing salts, acid-hydrolysed casein (Knight & Proom, 1950) with aneurin, biotin and pantothenic acid, but not when any one of these three essential metabolites is omitted. Growth is stimulated by the addition of 4% NaCl. Nine of eleven strains required the intact pantothenic acid molecule, and two strains grew in the presence of β-alanine instead of pantothenic acid.

**DISCUSSION**

Smith *et al.* (1946) divide the mesophilic species of the genus *Bacillus* into three morphological groups. The new species produces a nearly spherical or spherical spore which appreciably swells the sporangium and therefore belongs to morphological group 3 (see Pl. 1, figs. 3, 4). Comparison of the new species with the named species of the genus *Bacillus* shows that apart from the morphological differences it is more closely related to *B. circulans* than to any other species in the genus. Besides the morphological differences the new species can be distinguished from *B. circulans* by the following characters.

Cultures on nutrient agar after 24 hr. at 37° are frankly Gram-positive and the growth on nutrient agar after 48 hr. is moderate, greyish white and slightly moist. The colonial appearance on nutrient agar is characteristic and motile colonies have not been observed (see Pl. 1, figs. 1, 2). The growth after 24 hr. (37°) in nutrient broth is faint with a slight ropy deposit. This growth is stimulated and improved by the addition of 4% NaCl and the species will grow well in nutrient broth containing 10% NaCl. The growth of *B. circulans* in broth is not stimulated by the addition of 4% NaCl and although an occasional strain of *B. circulans* grows in nutrient broth containing 6% NaCl and one of our strains grew in nutrient broth containing 8% NaCl, none of the strains we have examined grew in nutrient broth containing 10% NaCl.

The new species does not produce acid from glucose with basal medium containing only inorganic nitrogen. The new species, in contradistinction to *B. circulans*, hydrolyses casein, liquefies inspissated serum and gives a positive restricted egg-yolk reaction.

The nutritional requirements of the new species are different from those of *B. circulans*, and from any of the other species of this genus which we have examined (Knight & Proom, 1950), in that the initial growth is stimulated by
H. Proom & B. C. J. G. Knight—*Bacillus pantothenticus* (n.sp.). Plate 1
Bacillus pantothenicus (n.sp.)

the addition of 4% NaCl and that pantothenic acid satisfies a nutritional requirement, the intact pantothenic acid molecule being required for most strains.

We propose Bacillus pantothenicus as the name of this new species, derived from pantothenic acid which is a characteristic nutrient for this organism. Six strains of B. pantothenicus have been deposited at the National Collection of Type Cultures, of which one (CN 8028) is the type strain, now N.C.T.C. no. 8162.

We are indebted to Mr E. Harris for valuable technical assistance and to Mr E. A. Jones for the photographs.

REFERENCES


EXPLANATION OF PLATE

Fig. 1. Colonies of B. pantothenicus, strain CN 3028, on nutrient agar after 48 hr. at 37°. Seen by reflected light. Magnification × 2.

Fig. 2. Colonies of B. pantothenicus, strain CN 3028, on nutrient agar after 48 hr. at 37°. Seen by transmitted light. Magnification × 8.

Fig. 3. Photomicrograph of Gram-stained film of B. pantothenicus, strain CN 3028. Magnification × 2000.

Fig. 4. Photomicrograph of Gram-stained film of B. circulans. Magnification × 2000.

(Received 28 March 1950)