Rediscovery of Bertrand's Sorbose Bacterium (*Acetobacter aceti* subsp. *xylinum*): Proposal to Designate NCIB 11664 in Place of NCIB 4112 (ATCC 23767) as the Type Strain of *Acetobacter aceti* subsp. *xylinum*

Request for an Opinion

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None of three culture collection strains (NCIB 4112, ATCC 23767, and CIP 57.14) presumed to be Bertrand's sorbose bacterium (type strain of *Acetobacter aceti* subsp. *xylinum*) was found to be a member of the genus *Acetobacter*. These three strains were identical, displayed considerable phenotypic differences compared with descriptions of the original sorbose bacterium published before 1959, and belonged to *Gluconobacter oxydans*. However, a 1949 Sordelli-dried culture of strain NCIB 4112 was revived at the National Collection of Industrial Bacteria and was found to belong to the genus *Acetobacter*. This culture showed similarities to other *A. aceti* subsp. *xylinum* strains and therefore is most similar to the culture originally deposited in 1933 by Bertrand in the National Collection of Type Cultures. The new accession number of the revived sorbose bacterium is NCIB 11664, and we request an opinion to designate this strain as the type strain of *A. aceti* subsp. *xylinum* in place of strain NCIB 4112.

In 1896, Bertrand (1, 2) isolated and described a bacterium capable of producing sorbose from sorbitol, and consequently he named this bacterium "la bactérie du sorbose." Because of its morphological, cultural, and biochemical properties, this sorbose bacterium was identified as "*Acetobacter xylinum*" (Brown 1886) Holland 1920 (3, 4, 6). The most distinctive characteristics of this organism are overoxidation of acetic acid, a strong ketogenic activity from mannitol, sorbitol, and glycerol, and formation of a thick leathery pellicle (3). The importance of this strain is that it led to the discovery of ketogenesis (3) and later to the Bertrand-Hudson rule (8). This strain was deposited by G. Bertrand in the National Collection of Type Cultures, London, England, in 1933 as strain NCTC 4112 and came from there to the National Collection of Industrial Bacteria (NCIB), Aberdeen, Scotland, in 1949 (NCIB 4112). The typical properties of this organism were confirmed by Tošić in 1942 (personal communication) and by Kulka and co-workers (9, 10), who drew attention to phenotypic similarities between strain NCIB 4112 and "*A. xylinum*" NCIB 5346. In *Bergey's Manual of Determinative Bacteriology*, 8th ed. (5), "*Acetobacter xylinum*" was renamed *Acetobac-*

ter aceti subsp. *xylinum*, and according to the Approved Lists of Bacterial Names (12) strain NCIB 4112 is the type strain of this subspecies. The NCIB supplied strain NCIB 4112 in 1957 to the Collection of the Institut Pasteur, Paris, France and in 1968 to the American Type Culture Collection Rockville, Md. (designations, CIP 57.14 and ATCC 23767, respectively).

We prepared protein electropherograms of strains NCIB 4112, ATCC 23767, and CIP 57.14 by using previously described techniques (13) and found that these electropherograms were identical, as expected (Fig. 1). However, deoxyribonucleic acid-ribosomal ribonucleic acid (rRNA) hybridizations and phenotypical analyses indicated that in contrast to other *A. aceti* subsp. *xylinum* strains, these strains do not belong in the genus *Acetobacter*, but are definitely members of *Gluconobacter oxydans* (7). For example, strain NCIB 4112 does not overoxidize acetic acid, does not form a leathery pellicle, and shows no ketogenic activity or very weak ketogenic activity on mannitol, sorbitol, and glycerol. The vigorous oxidation of these polyols to ketoses by the authentic sorbose bacterium (3) was in fact the origin of the discovery of ketogenesis. Figure 1 shows that

FIG. 1. Protein electropherograms of "*Acetobacter aceti* subsp. *xylinum*" strains NCIB 4112 (= NCIB 11301), ATCC 23767, and CIP 57.14, *G. oxydans* NCIB 9013^T and revived Bertrand's sorbose bacterium (*A. aceti* subsp. *xylinum*) strain NCIB 4112B (= NCIB 11664).

the protein pattern of *G. oxydans* subsp. *oxydans* NCIB 9013^T (= ATCC 19357^T [type strain of *G. oxydans*]) is very similar to the patterns on the electropherograms of NCIB 4112 and its descendants. Thus, strain NCIB 4112 does not now correspond to the original sorbose bacterium, which must have been interchanged with a strain of *G. oxydans* between 1933 and 1957. Comparisons with the protein electropherograms of about 100 *Gluconobacter* strains (Kerstens, unpublished data) suggest that the culture of the authentic sorbose bacterium may have been interchanged inadvertently with a culture of NCIB 3734, NCIB 4943, NCIB 6426, or NCIB 8131; all of these strains have almost identical protein profiles.

As a consequence of these results, workers at the NCIB revived a Sordelli-dried (surface growth mixed with horse serum in a cotton-plugged inner tube, with phosphorus pentaoxide

in an outer tube that is sealed under a vacuum) culture of the sorbose bacterium prepared in 1949 either at the National Collection of Type Cultures or at the NCIB. This subculture, which we designated NCIB 4112B, was found to be pure and to be capable of producing a thick pellicle. This strain was examined by the methods (7, 13) used for strain NCIB 4112.

With ¹⁴C-labeled rRNA from *G. oxydans* subsp. *oxydans* NCIB 9013^T, strain NCIB 4112B forms a deoxyribonucleic acid-rRNA hybrid with a thermal denaturation temperature of 76.3°C and an rRNA binding percentage of 0.20%, proving that strain NCIB 4112B belongs definitely in *Acetobacter* (thermal denaturation temperature, 74.5 to 78.3°C; rRNA binding, 0.10 to 0.28% [7]). The protein pattern of NCIB 4112B (Fig. 1) is completely different from that of strain NCIB 4112 and differs also from the patterns of approximately 100 *Gluconobacter* strains.

Phenotypically, strain NCIB 4112B corresponds to the original description of the bactérie du sorbose. It does overoxidize acetic acid and lactate, it does form a thick leathery pellicle, and it does effect strong ketogenesis from mannitol, sorbitol, and glycerol. In colony morphology and in cultural aspects this strain is very similar to other *A. aceti* subsp. *xylinum* strains, such as strains NCIB 613 and NCIB 5346 (Gosselé and Swings, unpublished data). It should be noted that the *A. aceti* subsp. *xylinum* strains do not form a phenotypically and electrophoretically homogeneous group of bacteria (F. Gosselé and P. Pauwels, unpublished data). The results of an extensive phenotypic analysis of *Acetobacter* will be published elsewhere.

Our conclusion is that strain NCIB 4112B is most probably a descendant of the culture originally deposited by Bertrand in the National Collection of Type Cultures in 1933.

The NCIB has decided to keep both strains and to use the following designations: (i) NCIB 11301 (= NCIB 4112), now known to belong to *G. oxydans*; and (ii) NCIB 11664 (= NCIB 4112B), the authentic Bertrand strain belonging to *A. aceti* subsp. *xylinum*. According to the Approved Lists of Bacterial Names (12), strain NCIB 4112 (= ATCC 23767 = NCIB 11301 = CIP 57.14) is the type strain of *Acetobacter aceti* subsp. *xylinum*; however, we have shown here that this strain should be replaced by NCIB 11664 (= NCIB 4112B) according to Rules 18g and 18i of the *International Code of Nomenclature of Bacteria* (11).

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