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BUDDING NITRIFYING BACTERIA: THE NOMENCLATURAL STATUS
OF NITROMICROBIUM GERMINANS STUTZER AND HARTLEB 1899
AND NITROBACTER WINOGRADSKYI WINSLOW ET AL. 1917*

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ABSTRACT. Budding as a mode of replication of nitrite oxidizing bacteria was observed and illustrated by Winogradsky in his work on Nitrobacter, and, shortly thereafter, by Stutzer and Hartleb, studying Nitromicrobium germinans. The identity of this bacterium with the Nitrobacter cultures of Winogradsky was established by Stutzer. Nitromicrobium is thus a later synonym to Nitrobacter. The species name germinans would have had priority over winogradskyi but it was overlooked. Opinions 20 and 23 of the Judicial Commission clearly validate Nitrobacter winogradskyi Winslow et al. 1917, and thus Nitromicrobium germinans is to be placed among the synonyms of N. winogradskyi.

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The isolation, purification and naming of nitrite-oxidizing bacteria were first reported by Winogradsky (1892). The organisms were described and depicted as short rods, which often were wedge-shaped or coccoid. Unfortunately, Winogradsky did not name a type species for his proposed new genus, Nitrobacter. This situation was subsequently discussed by Winslow et al. (1917) and a type species was named (N. winogradskyi), which generally has been accepted up to this date. However, the Index Bergeyana (Buchanan, Holt and Lessel 1966) claim that this name is illegitimate.

A publication pertinent to this problem (Stutzer and Hartleb 1899) was discovered during a literature search for the occurrence and distribution of budding as a means of reproduction among bacteria. The paper contained a detailed description of a budding and nitrite-oxidizing bacterium, which was named Nitromicrobium germinans n. spec. The authors were familiar with Winogradsky's work. Although unable to obtain cultures at that time, they compared the properties of Nitrobacter published by Winogradsky (1899, 1896) with those of their new organism. Illustrations and descriptions found in the two works show a surprising similarity:

Nitrobacter is described as " ... a very small rod, 1 μ long, or slightly more, about 0.5 μ in thickness. But if one examines it closely, one finds one aspect sufficiently characteristic: the shape is triangular, a wedge, and the sharp part forms a sort of little

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beak, staining much more weakly than the rest. One also finds in microscopic pictures a large number of individuals simply elongating or growing round ..." Winogradski, 1890, 1933, translated).

Nitromicrobium is described as follows: "... besides oval cells of even shape, some were found with a tapering pole on one side, and others with the tapering pole enlarged as with budding yeast. On still other organisms we saw the above mentioned bud so far developed that a constriction could be observed between it and the mother body. If one follows such a cell in a hanging drop for a long time, one sees that the young bud enlarges, that the connection with the mother cell becomes narrower, and that eventually it is bound off..." (Stutzer and Hartleb 1899, p. 205), translated).

Stutzer and Hartleb also pointed cautiously to some minor inconsistencies, which are summarized in Table 1.

Table 1.

Property	<u>Nitromicrobium</u>	<u>Nitrobacter</u>
Cell surface:	capsule absent	presence of a capsule ("Schleimhülle"), stainable with gentian violet in saline.
Cell shape:	rod, only one end tapering	rod, one or both ends tapering
Cell size:	0.6-1.0 x 1.0-1.5 μ	0.3-0.4 x 1.0 μ (According to Winogradsky [1892, 1933] the correct size is 0.5 x 1.0 μ)

Budding as the mode of replication of nitrite-oxidizing bacteria has been observed by several later authors (Gibbs 1919; Sack 1924; Zavarzin and Legunkova 1959; Pope, Hoare and Smith 1969). Several authors stressed repeatedly that the classical enrichment procedures employed by Winogradsky always rendered only one type of nitrite-oxidizer (Winogradsky 1892, 1933; Stutzer 1901; Engel and Skallau 1938; Zavarzin and Legunkova 1959). Moreover, the validity of other, newly described Nitrobacter species was often questioned. Isolates supposedly with the ability to oxidize nitrite were later found to be incapable of performing this process and hence were heterotrophic.

Apparently, contact was established between Stutzer and Winogradsky soon after the publication of the description of Nitromicrobium. A renewed study of nitrite-oxidizing bacteria was then conducted by Stutzer (1901), who included Nitrobacter cultures obtained from Winogradsky. The results led Stutzer to reconsider the aforementioned minor differences: "... under identical nutritional conditions the (two) forms were always alike ..." (Stutzer 1901, translated).

Considering all of these facts, it appears highly probable that both Winogradsky and Stutzer dealt with the same organism: a budding, nitrite-oxidizing, chemolithotrophic bacterium. The authors were aware of each other's work, although Winogradsky probably did not refer to the similarity with Nitromicrobium because his description preceded that of Stutzer and Hartleb. Stutzer (1901) stated, however: "... therefore, I

would not like to name the nitrate-former 'Nitrobacter' as was done by Winogradsky, especially also since the mode of propagation seems to be different from that of the bacteria. I believe that the nitrate-former belongs to a special group of organisms and consider the name 'Nitromikrobium' as more correct. Evidently, there exists only one species of Nitromikrobium, and the microbes isolated by me from Silesian and East Prussian soil proved to be identical with those which Professor Winogradsky was so kind to send me..." (Stutzer, 1901, translated).

Unfortunately, the original cultures are no longer available, and thus the problem of correct naming has become one of synonymy. The spelling by Stutzer in 1901 of "Nitromikrobium" was obviously a typographical error since the original description contained the validly published name Nitromicrobium." Clearly, the validly published and legitimate name "Nitrobacter" Winogradsky (1892) has priority over Nitromicrobium. Opinion 23 (part 5) of the Judicial Commission reads as follows:

"The generic name Nitrobacter Winogradsky 1892 is placed in the list of nomina generum conservanda with the species described by Winogradsky and later named Nitrobacter winogradskyi Winslow et al. 1917 as the nomenclatural species."

The species name N. winogradsky Winslow et al. 1917 was illegitimate when published since the work of Stutzer had been overlooked at that time. However, it became legitimate in 1958 by approval of Opinion 20 by the Judicial Commission, which reads in part 3:

"Name of a new genus with a described species which is neither named nor identified with a previously named species. A new generic name published in a combined description of a genus and species, without the species being named, without citation of a previously and effectively published description of the species, and without subsequent acceptance of the generic name and naming of the species by a later author, should be regarded as not validly published. Such a generic name may be placed in the list of nomina rejicienda."

"However, if a later author has recognized the generic name and has used it with a specific epithet in naming the species described by the first author, particularly if there has been later general acceptance of the name, there may be validation of the generic name as proposed by its author, with the name of the species ascribed to the later author who gave it. Proposals for such validations of names should be made to the Judicial Commission for appropriate action."

The name Nitrobacter winogradsky Winslow et al. 1917 is therefore validated and Nitromicrobium germinans is to be regarded as a synonym of it.

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