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CHAPTER 3.3.46

The Genus *Nevskia*

HERIBERT CYPIONKA, HANS-DIETRICH BABENZIEN, FRANK OLIVER GLÖCKNER AND RUDOLF AMANN

Habitat

Nevskia ramosa is a conspicuous inhabitant of the air-water interface of calm freshwater bodies. Its typical habitat forms by virtue of the dipole character of water molecules and their anisotropic positioning at the surface. The water surface has a high surface tension. Therefore, hydrophobic microlayers develop which accumulate organic molecules. The interface harbors a microbial community referred to as "the neuston" (Naumann, 1915). Members of the neuston community can be subdivided into those living underneath the interface, called "hyponeuston," and those residing on the surface, the so-called "epineuston." *Nevskia ramosa* is a member of the epineuston. It forms opaque pellicles on the water surface. These are so hydrophobic that little water droplets sprayed on them (as on paraffin) keep their spherical shape (Pladdies et al., 2004). Since the air-water interface is exposed to sun radiation, an essential adaptation to life in the neuston layer is ultraviolet (UV) tolerance.

Nevskia ramosa has been discovered in the surface pellicle of aquarium water (Famintzin, 1892). Later on it was detected in various natural environments including swamps, soil, ponds, ditches, lakes and several artificial habitats (Henrici and Johnson, 1935; Babenzien, 1965; Babenzien and Schwartz, 1970; Hirsch, 1999; Babenzien and Cypionka, 2004; Pladdies et al., 2004). There are no reports on the occurrence of *Nevskia ramosa* in seawater with the exception of one observation in brackish water of the Bight of Kiel (Baltic Sea; Zimmermann, 1975).

Nevskia develops to high population densities during calm weather periods. More than 20,000 cells per cm^2 were detected on a lake after three weeks of calm summer weather. After a rainy period and deeper in the water column, the cell numbers of *Nevskia* were much lower (Pladdies et al., 2004).

Morphology

Single cells of *Nevskia ramosa* are rod-shaped, often slightly bent and stain Gram-negative. In the older literature, there are differing values for the cell size. Cells studied after phylogenetic identification (see below) in recent reports were 0.7–1.1 × 1.5–2.3 μm (Stürmeyer et al., 1998; Glöckner et al., 1998; Pladdies et al., 2004). Often the cells contain refractive globules, probably polyhydroxyalkanoates (Babenzien and Hirsch, 1974).

The most conspicuous feature of the cells is the laterally excreted slime stalk formed when the cells spread out on the water surface (Fig. 1A and B). The stalks show annual ring-like patterns that indicate growth in intervals. The exopolysaccharide of the stalks consists mainly of rhamnose and small amounts of glucose and mannose (Stürmeyer et al., 1998). It is unknown how their hydrophobicity is brought about. The characteristic stalks are, however, not always present. The cells can run through a lifecycle as described by Babenzien (1967): Young cells are motile by a polar flagellum and develop submersed, then adsorb to the water surface, lose their flagellum, and form hyaline slime stalks at the concave side of the cell. The stalks stay connected but branch when the cells multiply by binary fission. Thereby flat rosettes form that can reach a size of 80 μm in diameter (Babenzien and Cypionka, 2004). A typical rosette with 16 cells may cover an area of about 700 μm^2 (Pladdies et al., 2004). On agar plates, geometrical patterns of slime-coated cells were observed instead of the typical flat rosettes.

The development of surface pellicles depends on the availability of combined nitrogen. If ammonia, nitrate or amino acids are supplied, the cultures grow submersed (see below).

Growth Medium

Nevskia ramosa can be cultivated as surface film in a simple synthetic medium of the following composition (values in mM; Stürmeyer et al., 1998): sodium lactate, 5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.1; KH_2PO_4, 25; trace element solution SL 9 (Tschech et al., 1984), 0.5 ml · $liter^{-1}$; vitamin solution (Pfennig, 1978), 0.5 ml · $liter^{-1}$; and pH adjusted to 7.0. Cultures are incubated at

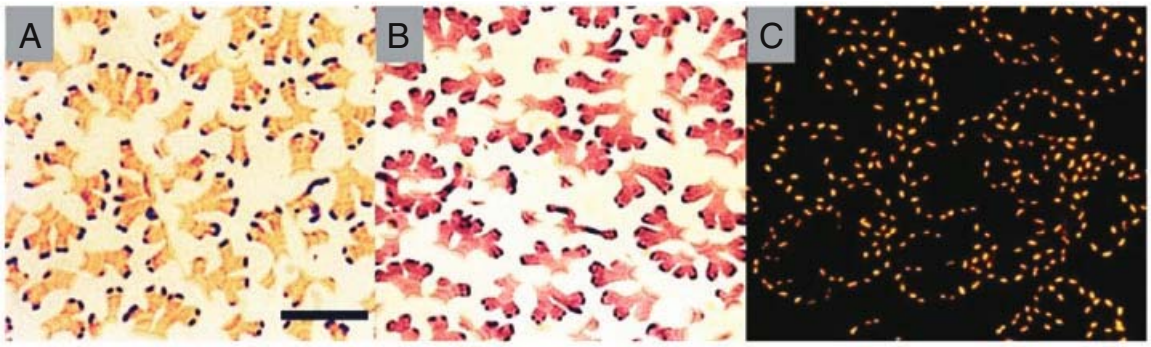

Fig. 1. *Nevskia ramosa* microcolonies stained with safranin (A), toluidine blue (B), and cells identified by means of the species-specific rRNA probe NEV656 (C). Bar, 25 μm.

room temperature (20–25°C) without shaking. The special feature of this medium, which induces growth in the surface film, is the lack of nitrogen compounds. If submersed growth of a culture is desired, the medium is supplemented with 5 mM NH_4Cl.

Identification

Different from most other bacteria, *Nevskia* can be identified microscopically because of its typical habitat and microcolony form. If a pellicle from a freshwater aquatic environment contains the typical flat, dichotomously branched and hydrophobic microcolonies with single cells in the tips, one can be quite sure to have *Nevskia ramosa*. However, in the neuston layer there might be other types of stalk-forming bacteria with three-dimensional microcolonies. These belong to the hyponeuston and are not *Nevskia ramosa* (Pladdies et al., 2004).

The polysaccharides of the stalks can be stained with toluidine blue (0.1% in distilled water) or safranin (0.1–0.5% in distilled water). Staining is performed on slides covered with gelatin (0.1%) and $CrKSO_4$ (0.01%). After spreading of the biofilm, the slide is dried under air, then stained for 30 min, and washed with distilled water (Stürmeyer et al., 1998).

A reliable identification is possible by fluorescence *in situ* hybridization (Fig. 1C). Glöckner et al. (1998) have designed and evaluated two probes, NEV656 (CGCCTCCCTCTACCGTTT, binding to the positions 656–674 of the small subunit [SSU] ribosomal RNA) and NEV177 (GCTCTTGCGAGATCATGC, binding to the positions 177–195 of the SSU ribosomal RNA), which turned out to be specific for *Nevskia ramosa*.

Ecophysiology

Nevskia ramosa has a strictly aerobic metabolism and grows with broad range of organic compounds including monomeric sugars, sucrose, starch, cellulose, organic acids, ethanol, amino acids, benzoate and Tween 20 or Tween 80 (Stürmeyer et al., 1998). It shows weak catalase, but no cytochrome oxidase activity. Cytochromes of the *b*- and *c*-type are present. Nitrogenase activity was not detected.

As mentioned above, rosette formation depends on the availability of nitrogen compounds. The cells do not form rosettes during growth with amino acids, or when ammonia is added to the growth medium. It is well known that under nitrogen limitation and carbon surplus, bacteria form reserve material like polyhydroxyalkanoate globules and extracellular polysaccharide slime. Once spread over the surface in the biofilm, *Nevskia* cells have a good chance to trap ammonia from the air. Thus, rosette formation appears to be caused by nitrogen deficiency, but at the same time represents a mechanism to overcome it.

Growth in hydrophobic films stabilizes the positioning of the rosettes. Furthermore, the bacteria might be protected against grazers that cannot permeate the interface and are unable to take up cells by phagocytosis.

The open exposure to sunlight might cause DNA damage. However, *Nevskia ramosa* was found to have a very effective photorepair mechanism. The number of cells surviving UV radiation of 254 nm increased by seven orders of magnitude if the cells were exposed to light of 350 nm after the UV treatment (Stürmeyer et al., 1998). Since under natural conditions UV radiation is always combined with white light, *Nevskia* appears to be well prepared to survive sun radi-

ation. However, no positive enrichments were obtained from the site with the highest UV exposure (i.e., an alpine lake located at 2413 m above sea level; Pladdies et al., 2004).

Enrichment and Isolation of Pure Cultures

Nevskia can easily be enriched from freshwater surface pellicles on the medium described above or most simply on sterilized water from the habitat supplied with 5 mM lactate. Samples for inoculation are collected on sterile glass slides or loops. The cells have doubling times of 2–4 days and require 2–4 weeks for development of the typical rosettes of dichotomously branched slime stalks with single cells in the tip. Growth can also be monitored in a hanging drop (Stürmeyer et al., 1998).

Pure cultures can be obtained by repeated streaking on plates with the same medium and agar (1%, w/v). On agar, *Nevskia* does not develop the characteristic rosettes. Instead, geometrical patterns of slime-coated cells are typical (Stürmeyer et al., 1998). However, pure cultures form the characteristic microcolonies if brought back to ammonia-free liquid medium.

Phylogeny

The DNA base composition of the DNA was 67.8 ± 0.1 mol% G+C for *Nevskia ramosa* strain Soe1 and 69.0 ± 0.2 mol% G+C for strain OL1 (Stürmeyer et al., 1998).

The phylogenetic analysis of the 16S rRNA indicates that *Nevskia ramosa* has no near relatives known so far. Currently, this species is the sole representative of a deep branch of the Gammaproteobacteria (Fig. 2). It is not closely related to other stalk-forming bacteria like the Alphaproteobacterium *Caulobacter crescentus* (83% similarity) or the Betaproteobacterium *Gallionella ferruginea* (85% similarity).

So far the genus has only one species, *Nevskia ramosa*. Two strains, *N. ramosa* Soe1 (DSM 11499^T) and strain OL1 (DSM 11500) were deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. Different isolates (Stürmeyer et al., 1998; Pladdies et al., 2004) and clones with the

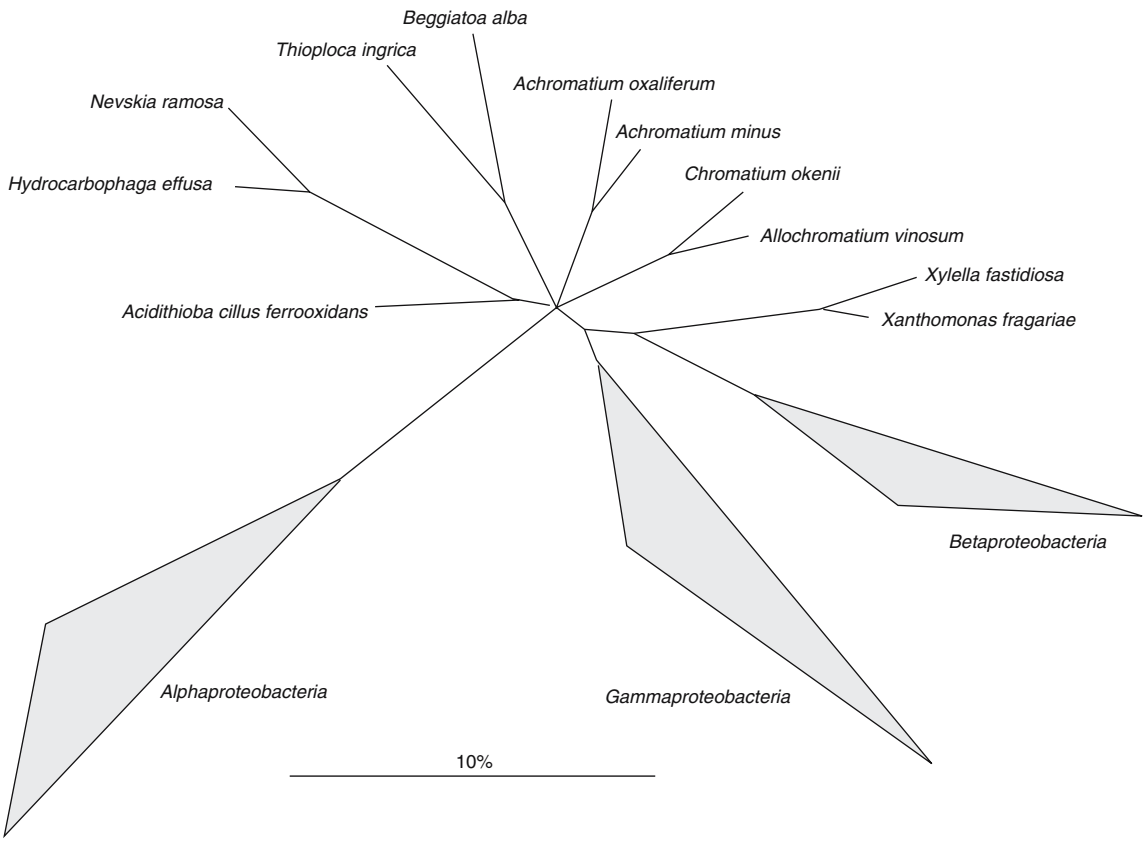

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing *Nevskia ramosa* branching deeply at the gamma-subclass of the Proteobacteria.

16S rRNA gene (Glöckner et al., 1998) of *Nevskia ramosa* showed very similar sequences (at least 98% similarity). However, genomic fingerprints generated by enterobacterial repetitive intergenic consensus sequence–polymerase chain reaction (ERIC PCR) showed different banding patterns with all isolates, thus indicating some genetic diversity within the species.

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