

## Evidence for Bacteriophages within Gram-negative Cocci - Obligate Endoparasitic Bacteria of *Naegleria* sp.

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**Summary.** Gram-negative cocci observed as endocytobionts within the cytoplasm of a *Naegleria* strain isolated from a garden pond harboured small hexagonal particles of about 70 nm identified as bacteriophages called "Neo-Ph/2". These phages resembled the recently described phages; strain "Neo-Ph/1" observed for the first time within the *Chlamydia*-like endocytobiont *Neochlamydia hartmannellae* (Parachlamydiaceae) multiplying within *Hartmannella vermiformis* (Schmid *et al.* 2001). The possible reasons for this obvious similarity are object for discussion in this article.

**Key words:** amoeboflagellate, bacteriophages, endocytobionts, Gram-negative cocci, *Naegleria* sp., phage-heads, ultrastructure.

### INTRODUCTION

Among other species of free-living amoebae (FLA) acanthamoebae, naegleriae and hartmannellae are well known as possible hosts of pathogenic and harmless bacteria multiplying unaffected inside their host cells with the result that the host-amoebae are prone to be disintegrated and finally die releasing great numbers of the intracellular bacteria. Consequently the FLA contribute to the dispersal of bacteria as *Legionella pneumophila*, *Listeria* or *Chlamydia*-like bacteria de-

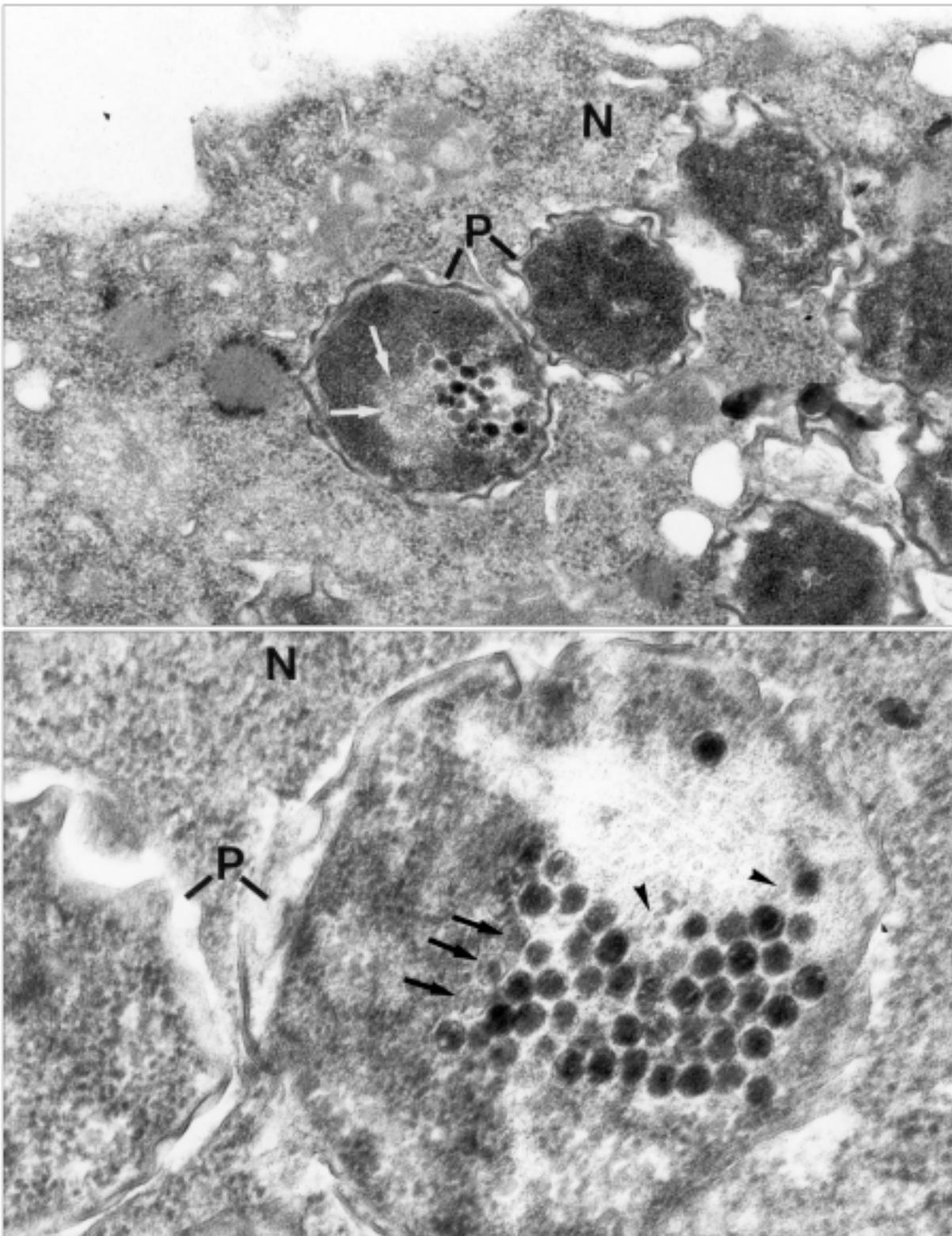
scribed recently (Rowbotham 1980, 1987; Michel *et al.* 1994; Amann *et al.* 1997; Horn *et al.* 2000)

An endoparasitic *Chlamydia*-like bacterium of *Hartmannella vermiformis* described only very recently as *Neochlamydia hartmannellae* (Horn *et al.* 2000) attracted the attention of one of us (E.N.S.) because some elementary bodies within the cytoplasm of their host appeared significantly enlarged compared to normal elementary bodies. They contained small polygonal particles of 60-70 nm identified as bacteriophages "Neo-Ph/1" described for the first time to occur within endocytobionts of FLA (Schmid *et al.* 2001).

Similar particles have now been detected within Gram-negative cocci parasitizing *Naegleria* sp. (N-DMLG), isolated from a small garden pond. Unusu-

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**Fig. 1.** A number of endocytobionts (P) can be observed randomly distributed within the cytoplasm of the host amoeba *Naegleria* sp. strain N-DMLG (N). One organism appears slightly enlarged harbouring phage particles. Arrows - electron lucent DNA region with faint empty phage envelopes; x 43500

**Fig. 2.** Higher magnification of an infected endoparasitic bacterium (P) showing details of numerous particles within the bacterial cell: N - cytoplasm of the host amoeba *Naegleria* sp. Arrows - partly filled or empty phages; arrowheads - faintly visible phage-tails; x 85000

ally the infected trophozoites of this *Naegleria* species harboured at least two different populations of endocytobiotic bacteria - one within the nucleus the other one within the cytoplasm (Michel *et al.* 1999).

Only the latter population produced and released particles resembling those described recently from *Neochlamydia hartmannellae* and are subject to the present description.

## MATERIALS AND METHODS

The *Naegleria* strain (N-DMLG) serving as natural host strain of obligate intracellular Gram-negative cocci was isolated from a small private garden pond with ornamental fishes, on non-nutrient-agar according to Page (1988). Parasite population were harvested from 3-5 days-old axenic SCGYE-cultures and after centrifugation at 1800 rpm they were fixed in cacodylate buffered 3% glutaraldehyde. After a period of 1 h at 4°C they were centrifuged at 2500 rpm and resuspended in 0.1 M cacodylate buffer, postfixed in 1% OsO<sub>4</sub>, and stained with uranyl acetate and lead citrate. Subsequently, specimens were dehydrated in alcohol and embedded in epoxy resin according to Spurr. Ultrathin sections were examined in a LEO 910 transmission electron microscope (Leo, Oberkochen).

## RESULTS

The infected *Naegleria* strain could be detected by its inability to form cysts and led therefore to the assumption of being infected by some kind of endoparasitic bacteria. Investigation of this particular strain by light and electron microscopy revealed a simultaneous infection of the amoeba by at least two different populations of Gram-negative cocci: an intracytoplasmic population and another one multiplying exclusively within the nucleus of its host (Michel *et al.* 1999). Already during the first inspection of electron micrographs of these host-parasite relations some of the intracellular bacteria appeared enlarged markedly and somehow damaged.

We first supposed that some of the endocytobionts might be susceptible to digestion by the host and show therefore signs of disintegration. But in the course of extended search and inspection of those stages we discovered that these changes were the result of infection with virus-like particles we are going to describe herewith.

At lower magnification (Fig.1) one of the endocytobiotic cells shows a few particles of attracting density with adjacent structures of less density in an irregular arrangement. Within the electron lucent DNA-region (arrows) the nucleoid of the prokaryotic

endocytobiont, faint empty phage envelopes are discernible. In Fig. 2 a more paracrystalline array of particles is found, which is typical for the arrangement of bacteriophages. Within this cell, most of the phage-heads are filled, only a few phages (arrows) are filled partly or are empty. The dimension of the hexagonal mature phage-heads is about 70 nm. Phage-tails are hardly discernible (arrowheads).

Novel phage strain was named "Neo-Ph2", as it resembles the phages "Neo-Ph1" from *Neochlamydia*.

## DISCUSSION

The bacteriophages described so far were found only in the cytoplasmic fraction of the endoparasites - never within the more polymorphic stages replicating inside the nucleus attached to the surface of the karyosome.

They have not been found within the similar endocytobionts KNic described previously within another isolate of *Naegleria* sp. from an aquarium (Michel *et al.* 2000). The size of the heads is comparable to the phage described recently from *Neochlamydia hartmannellae* (Schmid *et al.* 2001).

Although few striated filaments of about 60 nm are discernible in less electron dense parts of the phage-producing endocytobionts, further studies with negatively stained phages are necessary to allow distinct descriptions of existing flexible, rigid or contractible phage-tails.

With respect to the dimension of the phage-heads the great similarity between the phage Neo-Ph/1 from *Neochlamydia* and the phage Neo-Ph/2 from cocci multiplying within *Naegleria* is remarkable, because they have been found within taxonomically nearly unrelated species of endocytobiotic bacteria. On the other hand these dimensions are in the range of a great number of phages and only additional information of tail structures and DNA composition will allow a more profound comparison.

One aim of future investigations will be the test of infectivity of these particles for nearly related species of bacteria - and attempts to identify the two phage types by DNA sequencing methods.

What had been stressed in our recent article about the meaning of the finding of a phage within *Neochlamydia* for the host-parasite relation is also due for the present case: the phages are expected to exert multiple influences on the host-parasite interaction - all the more as

the infected host bacterium is condemned to disintegration and death so it now might be easily digested by the host amoeba.

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