The obligately intracellular coccoid bacterium UWE25, a symbiont of *Acanthamoeba* spp., was previously identified as being related to chlamydiae based upon the presence of a chlamydia-like developmental cycle and its 16S rRNA gene sequence. Analysis of its complete genome sequence demonstrated that UWE25 shows many characteristic features of chlamydiae, including dependency on host-derived metabolites, composition of the cell envelope and the ability to thrive as an energy parasite within the cells of its eukaryotic host. Phylogenetic analysis of 44 ribosomal proteins further confirmed the affiliation of UWE25 to the 'Chlamydiae'. Within this phylum, UWE25 could be assigned to the family *Parachlamydiaceae* based on comparative analyses of the 16S rRNA, 23S rRNA and endoribonuclease P RNA genes. The distinct dissimilarities from its closest relative, *Parachlamydia acanthamoebae* Bn9T (7-1, 9-7 and 28-8 %, respectively), observed in this analysis justify its classification in a new genus. Therefore, the name 'Candidatus Protochlamydia amoebophila' is proposed for the designation of the *Acanthamoeba* sp. symbiont UWE25 (＝ATCC PRA-7).

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The symbiont UWE25 was originally found intracellularly within an *Acanthamoeba* sp. isolated from a soil sample in western Washington State, USA (Fritsche et al., 1993, 2000). All attempts to cultivate UWE25 in host-cell-free media have failed, suggesting that these bacteria are obligately intracellular symbionts. UWE25 is able to multiply in various *Acanthamoeba* hosts and even in the distantly related amoeba *Dictyostelium discoideum* (Fritsche et al., 1998; Skriwan et al., 2002). UWE25, however, had a cytoytic effect on its original host amoeba, and was therefore transferred to another host, *Acanthamoeba* sp. UWl (Fritsche et al., 1998; Gautom & Fritsche, 1995), which was axenically cultivated in trypticase/soy/yeast extract broth (Visvesvar, 1999) at 20–24 °C.

In a previous study, UWE25 was identified as a Gram-negative chlamydia-like organism belonging to the family *Parachlamydiaceae* (Fritsche et al., 2000). This family was introduced by Everett et al. (1999) and comprises obligately intracellular symbionts of free-living amoebae that are closely related to the medically important *Chlamydiaceae* (Everett et al., 1999; Mahoney et al., 2003). Within the *Parachlamydiaceae*, two genera have been described, represented by *Parachlamydia acanthamoebae* Bn9T (Amann et al., 1997) and *Neochlamydia hartmannellae* A1HspT (Horn et al., 2000).

Fluorescence in situ hybridization and electron microscopy demonstrated that UWE25 resides inside its host cells within small inclusions (containing only one or few bacteria) dispersed in the cytoplasm (Fig. 1), differentiating UWE25 from other members of the *Parachlamydiaceae*, which form large inclusions inside their host cells or seem to reside directly in the cytoplasm (Amann et al., 1997; Fritsche et al., 2000; Horn et al., 2000). Occasionally, however, small clusters or morulae of UWE25 cells could be found inside the host cells (Fig. 1a). In contrast to *N. hartmannellae*, UWE25 does not prevent encystation of its amoeba host and can be found in *Acanthamoeba* trophozoites and cysts (Fritsche et al., 2000; Horn et al., 2000). UWE25 showed a chlamydia-like developmental cycle including morphological stages resembling chlamydial elementary bodies (0.5–0.8 μm in diameter; Fig. 1b) and reticulate bodies (0.7–1.0 μm in diameter; Fig. 1c) (Fritsche et al., 2000).

Complete genome sequencing of UWE25 (Horn et al., 2004) revealed that its genome is about twice as large as those of all members of the family *Chlamydiaceae* sequenced to date (2 414 465 bp versus 1–1.2 Mb; Kalman et al., 1999; Read et al., 2000, 2003; Shirai et al., 2000; Stephens et al., 1998), and has an overall G+C content that is much lower than those of genomes of *Chlamydiaceae* (35-8 versus 39-2–41-3 %). Like members of the family *Chlamydiaceae,*
UWE25 has only limited biosynthetic capabilities and relies on the import of several host-derived metabolites. In particular, UWE25 is not able to synthesize nucleotides or the electron carrier NAD\(^+\) de novo but uses specific transporters to import these compounds from its host (Haferkamp et al., 2004; Schmitz-Esser et al., 2004), and produces only a limited number of amino acids (glycine, alanine, serine, aspartic acid, glutamine, glutamic acid and proline) and cofactors (riboflavin, haem, folate and menaquinone), illustrating its obligately intracellular lifestyle. The outer membrane of UWE25 contains cysteine-rich proteins characteristic of members of the Chlamydiaceae although no homologue for the major outer-membrane protein (MOMP), a primary antigen of known chlamydiae, has been identified so far. This is consistent with the failure of polyclonal antibodies directed against the MOMP of members of the Chlamydiaceae to bind to elementary bodies of UWE25 in micro-immunofluorescence tests (data not shown). In addition, monoclonal antibodies (CF2) directed against the Chlamydiaceae-specific lipopolysaccharide epitope did not react with UWE25 (data not shown), further illustrating differences in cell wall composition between UWE25 and the Chlamydiaceae.

The taxonomic affiliation of UWE25 was examined by phylogenetic analyses of the 16S rRNA, 23S rRNA and endoribonuclease P (RNase P) RNA genes, and a concatenated dataset of 44 ribosomal proteins, by using the ARB software package (Ludwig et al., 2004). Phylogenetic analysis of the ribosomal proteins confirmed the ultrastructure- and genome-based assignment of UWE25 to the ‘Chlamydiaceae’ (Fig. 2a). The ribosomal protein trees were in agreement with 16S and 23S rRNA gene trees published previously and thus support the current rRNA gene-based classification of ‘Chlamydiaceae’ (Everett et al., 1999). The 16S rRNA and 23S rRNA genes of UWE25 showed sequence identities above 82.1 and 83.4\%, respectively, with all other members of the Chlamydiaceae. UWE25 hence meets the requirements of Everett et al. (1999) for inclusion in this order. Consistent with this finding and independent of the phylogenetic marker and treeing method applied, UWE25 formed a monophyletic group with all chlamydiae to the exclusion of all other bacteria (Fig. 2).

The rRNA genes of UWE25 were more than 90\% identical to those of members of the Parachlamydiaceae, indicating the affiliation of UWE25 with this family. Within the Parachlamydiaceae, the highest sequence identities were observed with *P. acanthamoebae* Bn\(^a\) (92.9\% for the 16S rRNA gene and 90.3\% for the 23S rRNA gene). According to Everett et al. (1999), 16S rRNA and/or 23S rRNA gene sequence identities to the closest relative of less than 95\% are indicative of a new genus. UWE25 can thus not be assigned to any previously described genus of the family Parachlamydiaceae. Consistently, all calculated phylogenetic trees clearly showed the monophyletic grouping of UWE25 with all other members of the family Parachlamydiaceae, but within this group UWE25 formed a deeply branching evolutionary lineage (Fig. 2b, c).

To confirm the rRNA gene-based phylogenetic inference, the RNase P RNA gene (*rnpB*) was analysed. *rnpB* has previously been shown to be an evolutionarily ancient and ubiquitous gene that is well-suited as a phylogenetic marker, and has been used as a tool for the discrimination of chlamydiae down to the species level (Hartmann & Hartmann, 2003; Herrmann et al., 2000). The *rnpB* sequence similarity of UWE25 to its closest relative *P. acanthamoebae* Bn\(^a\) (71.2\%) was lower than that between members of the genera *Chlamydia* and *Chlamydophila* (73.8–79.7\%), providing further support for the classification of UWE25 in a new genus. The distinct relationship of UWE25 to *P. acanthamoebae* Bn\(^a\) was also reflected in the *rnpB*-based phylogenetic tree (Fig. 2d). Therefore, based on Murray & Schleifer (1994), we propose the name ‘*Candidatus Protochlamydia amoebophila*’ for the designation of the *Acanthamoeba* sp. endosymbiont UWE25.

**‘Candidatus Protochlamydia amoebophila’**

‘*Candidatus Protochlamydia amoebophila*’ (Pro.to.chla. my’dia.a. Gr. adj. protos first, foremost; N.L. fem. n.
Chlamydia taxonomic name of a bacterial genus; N.L. fem. n. Protochlamydia referring to the similarity of these bacteria to the chlamydial ancestor; a.moe 'bo.phi.la. N.L. fem. adj. amoebophila loving amoebae, referring to the intracellular lifestyle within amoebae).

Phylogenetic position: phylum ‘Chlamydiae’, order Chlamydiales, family Parachlamydiaceae. ‘Candidatus Protochlamydia amoebophila’ represents a novel genus within the family Parachlamydiaceae. Other members of this tentative genus should have 16S rRNA genes with >95% identity to the 16S rRNA gene of ‘Candidatus Protochlamydia amoebophila’.
Coccoid Gram-negative reticulate bodies and elementary bodies, 0.5–1.0 μm in diameter. Not cultivable on cell-free media. Obligately intracellular symbiont of Acanthamoeba spp. surrounded by vacuolar membranes and dispersed in the host cell cytoplasm, occasionally in small clusters or morulae. Basis of assignation: 16S rRNA, 23S rRNA and RNase P RNA (GenBank accession no. AJ748539) genes, and complete genome sequence (genome size 2,414,465 bp; overall G+C content 35.8 mol%; GenBank accession number BX908798) (Horn et al., 2004). The original host Acanthamoeba sp. was isolated from a soil sample in western Washington State, USA; the current host is Acanthamoeba sp. UWC1 (Fritsche et al., 1993, 1998). Represented by isolate UWE25 (=ATCC PRA-7).

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References


