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PII: S0005-2736(17)30412-1
DOI: https://doi.org/10.1016/j.bbamem.2017.12.020
Reference: BBAMEM 82669

To appear in:

Received date: 28 July 2017
Revised date: 30 November 2017
Accepted date: 25 December 2017

Please cite this article as: Muriel C.F. van Teeseling, Roland Benz, Naomi M. de Almeida, Mike S.M. Jetten, Rob J. Mesman, Laura van Niftrik , Characterization of the first planctomycetal outer membrane protein identifies a channel in the outer membrane of the anammox bacterium Kuenenia stuttgartiensis. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Bbamem(2017), https://doi.org/10.1016/j.bbamem.2017.12.020

Characterization of the first planctomycetal outer membrane protein identifies a channel in the outer membrane of the anammox bacterium *Kuenenia stuttgartiensis*

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Highlights

- *Kuenenia stuttgartiensis* membranes harbour two major pore-forming proteins
- Kustd1878 is a cation-specific pore with a conductance of 170-190 pS in 1 M KCl
- Immunogold localization shows the presence of Kustd1878 in the outermost membrane
- This OMP further evidences that *Planctomycetes* have a Gram-negative cell envelope

Abstract

Planctomycetes are a bacterial phylum known for their complex intracellular compartmentalization. While most *Planctomycetes* have two compartments, the anaerobic ammonium oxidizing (anammox) bacteria contain three membrane-enclosed compartments. In contrast to a long-standing consensus, recent insights suggested the outermost *Planctomycete* membrane to be similar to a Gram-negative outer membrane (OM). One characteristic component that differentiates OMs from cytoplasmic membranes (CMs) is the presence of outer membrane proteins (OMPs) featuring a ß-barrel structure that facilitates passage of molecules through the OM. Although proteomic and genomic evidence suggested the presence of OMPs in several *Planctomycetes*, no experimental verification existed of the pore-forming function and localization of these proteins in the outermost membrane of these exceptional microorganisms. Here, we show via lipid bilayer assays that at least two typical OMP-like channel-forming proteins are present in membrane preparations of the anammox bacterium *Kuenenia stuttgartiensis*. One of these channel-forming proteins, the highly abundant putative OMP Kustd1878, was purified to homogeneity. Analysis of the channel characteristics via lipid bilayer assays showed that Kustd1878 forms a moderately cation