

Mind the PVCs

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With this letter, we would like to draw attention to bacteria belonging to the PVC (Planctomycetes-VerrucomicrobiaChlamydiae) superphylum. Members of Planctomycetes (Ward, 2010) are widely distributed environmental bacteria occurring in soil, freshwater, marine and wastewater environments. Verrucomicrobia (Hedlund, 2010) also exhibit a wide distribution, found in soil, marine habitats, hot springs, anoxic rice paddy soils and marine sediments, and even in the intestine of metazoa [including humans (Derrien et al., 2008; 2011)]. Most Chlamydiae are pathogens commonly found in mammals and multicellular eukaryotes, although many new lineages are associated with marine water and free-living amoebae (Horn, 2008; Corsaro et al., 2009). The PVC superphylum, as currently defined (Wagner and Horn, 2006), also includes Lentisphaerae [marine species and inhabitants of the animal gastrointestinal tract, anaerobic sludge and landfill (Cho et al., 2004)], and the two candidate phyla Poribacteria [bacterial associates of marine sponges (Fieseler et al., 2004)], and OP3 [environmentally detected but uncharacterized (Rohini Kumar and Saravanan, 2010)].

PVC bacteria were initially classified as independent phyla because of their very different habitats and phenotypes (Fuerst, 2013). Phyla are practically defined as bacteria sharing greater than or equal to 85% 16S ribosomal RNA gene sequence identity (Hugenholtz et al., 1998). However, more recent analyses have proposed an assemblage of these phenotypically diverse organisms into a broad superphylum structure. A superphylum is a grouping of related phyla that form looser higher-level clades in phylogenetic trees. PVC bacteria form such a superphylum, i.e. they share less than 85% sequence identity but are still recognized as more related to each other than to members of other phyla. The original PVC superphylum proposal (Wagner and Horn, 2006) is now well supported and accepted, including in recent reconstructions of the tree of life using different methods and/or tools (e.g. Ciccarelli et al., 2006; Hou et al., 2008; Letunic and Bork, 2011; Yutin et al., 2012; Rinke et al., 2013). This grouping is additionally supported by characteristics shared by most but not all members of the group (Devos and Reynaud, 2010; Reynaud and Devos, 2011; Devos, 2013; Fuerst, 2013).

Although the Chlamydiae have long been a focus of microbiological research because of their role as mammalian pathogens, the Planctomycetes, Verrucomicrobia and related phyla have more recently emerged as fascinating subjects for research in evolutionary cell biology, ecology and biotechnology, and human health. This research has been stimulated by the growing availability of PVC genome sequences, recently summarized by (Kamneva et al., 2013) to include nine planctomycete species, nine verrucomicrobial species and two members of the Lentisphaerae. Chlamydial genomes are much more numerous because of their early selection as genome sequencing targets, for their importance to human health and small genome size. Highlights in PVC research over the past few years include:

-Evolutionary cell biology: Some members of PVC (particularly the planctomycetes) appear to deviate from our 'classical' definition of bacteria in many aspects. Subsets of PVC organisms lack some 'typical' bacterial properties (Ward and Klotz, 2010), such as binary fission [with most Planctomycetes dividing by budding (Ward, 2010)], the cell division protein FtsZ [present in Verrucomicrobia, but lost in Planctomycetes Chlamydiae (Pilhofer et al., 2008)] and the 'universal' bacterial cell wall polymer, peptidoglycan, absent in Planctomycetes and undetected in Chlamydiae (König et al., 1984; Pavelka, 2007). Some of them also possess other characters not usually seen in bacteria and typically associated with other domains of life, such as a complex and dynamic endomembrane system (Lee et al., 2009a,b; Santarella-Mellwig et al., 2010), proteins that structurally resemble eukaryotic membrane coat-like proteins (Santarella-Mellwig et al., 2010), tubulin (Jenkins et al., 2002), sterols (Pearson et al., 2003) and endocytosis-like behaviour (Lonhienne et al., 2010). Some PVC members also display unique properties not observed anywhere else in the tree of life, such as ladderane lipids in the anammox (Sinninghe Damsté et al., 2002), unique C1 transfer enzymes in planctomycetes (Chistoserdova et al., 2004) and a new subtype of ribulose-1,5- biphosphate carboxylase/oxygenase in verrucomicrobiae (Khadem et al., 2011). The evolutionary origins of these unique constellations of cellular and biochemical features are difficult to determine, especially given the 'ORFan black hole' of PVC genomes; more than 50% of predicted proteins in PVC genomes are of unknown function. The evolution of eukaryote-like features in PVC cells is the subject of vigorous debate in the PVC research field (Devos and Reynaud, 2010; McInerney et al., 2011; Reynaud and Devos, 2011; Budd and Devos, 2012; Devos, 2012; Fuerst and Sagulenko, 2012). Possibilities of origin include a homologous relationship, lateral gene transfer, alternative trees of life that feature a deep branching point for PVC organisms (Jun et al., 2010) or convergence. Homology could be illuminating for the origin of our own (eukaryotic) cells. Conversely, a relationship based on analogy could provide fascinating examples of convergent evolution. In either case, a better understanding of evolutionary processes contributing to PVC cell biology could have immense significance for understanding the evolution of cell organization and complexity. Clarifying the 'PVC exception to the bacterial definition' problem appears as an attractive field of research for years to come (Fuerst, 2013).

- Ecology and biotechnology: PVC bacteria have long been known for their ecological ubiquity (host associated and free living; terrestrial and aquatic; and marine and freshwater), mostly detected through cultivation-independent approaches to microbial diversity assessment. But it is only in the past decade that their contributions to major biogeochemical cycles have been appreciated. Highlights include the discovery of methane oxidation in the Verrucomicrobia (Dunfield et al., 2007; Pol et al., 2007; Islam et al., 2008; Sharp et al., 2012) and the involvement of anammox planctomycetes in the anaerobic oxidation of ammonia (van de Graaf et al., 1995; Strous et al., 1999), a major nitrogen cycling pathway. The anammox process has been harnessed globally for the biological treatment of wastewater (Strous et al., 1997; Helmer et al., 2001; Jetten et al., 2001). While PVC-based biotechnology efforts have so far focused mainly on anammox, other planctomycetes might represent a valuable source for small bioactive molecules (Donadio et al., 2007) or enantioselective enzymes (Wallner et al., 2005).

- Human health: No member of the Planctomycetes and Verrucomicrobia has so far been reported as a human pathogen. However, the verrucomicrobial species *Akkermansia muciniphila* has been recognized as an important contributor to human gastrointestinal homeostasis. *Akkermansia muciniphila* is intimately associated with the gastrointestinal tract through the physiological process of mucin degradation (Derrien et al., 2008) and has been shown to affect host immune and cell development functions through studies in germ-free mice (Derrien et al., 2011). Correlative studies have also shown that *A. muciniphila* is depleted in gut inflammatory conditions such as ulcerative colitis (Rajilic-Stojanovic et al., 2013) as well as obesity (Santacruz et al., 2010; Karlsson et al., 2012; Everard et al., 2013). The ability of another verrucomicrobial species, *Verrucomicrobium spinosum*, to participate in pathogenic interactions with invertebrates has also been described (Sait et al., 2011).

Recent technical progress includes the development of genetic tools in Planctomycetes and Verrucomicrobia (Domman et al., 2011; Jogler et al., 2011), and high-resolution microscopy techniques (Lieber et al., 2009; Jogler et al., 2011; Pilhofer et al., 2011; Santarella-Mellwig et al., 2013) that allow a better deciphering of PVC cell biology and permit a better description of the PVC cell plan. PVC researchers have achieved several milestones in building their community in the past couple of years. These milestones include:

- The first book dedicated to PVC organisms: *Planctomycetes: Cell Structure, Origins and Biology* (Ed: John A. Fuerst) New York: Humana Press
- The first PVC-focused journal issue in *Frontiers in Evolutionary and Genomic Microbiology*: (http://www.frontiersin.org/Evolutionary_and_Genomic_Microbiology/researchtopics/Recent_advances_in_the_biology/491)
- The first conference on PVC organisms: 'EMBO Workshop on Planctomycetes-Verrucomicrobia-Chlamydiae Superphylum: Exceptions to the Bacterial Definition' (28 February–2 March 2013; Heidelberg, Germany; <http://events.embo.org/13-pvc/>)
- A special issue of the *Antonie van Leeuwenhoek* journal dedicated to the proceedings of this conference (<http://link.springer.com/journal/10482/104/4/page/1>)
- The first website entirely dedicated to those bacteria to form a link for the PVC community and platform for all PVC-related information (<http://www.pvcbacteria.org>).

In conclusion, PVC organisms are relevant to multiple areas of scientific endeavour, including evolutionary cell biology, ecology and biotechnology, and human health. The tools required to make rapid advances in understanding the biology of these organisms (particularly genetics) are becoming established, and the PVC research community has attained a critical mass, as evidenced by recent scholarly activity.