



Phytopathogenic Mollicutes

An International Journal on Phytoplasma, Spiroplasma and other 'Phloem-limited Plant Pathogens'

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Malembic-Maher S, Salar P, Filippin L, Carle P, Angelini E and Foissac X 2010. Genetic diversity of European phytoplasmas of the 16SrV taxonomic group and proposal of '*Candidatus Phytoplasma rubi*'. *International Journal of Systematic and Evolutionary Microbiology*, DOI 10.1099/ijs.0.025411-0, in press

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New Research Highlights

Comments on “The culture of plant pathogenic phytoplasmas in axenic media” (Contaldo *et al.*, 2012)

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The findings reported prove that phytoplasmas, previously thought to be non-culturable, can now be grown on laboratory media. This is an important breakthrough in the study and management of phytoplasma-incited plant diseases.

Since phytoplasma discovery in 1967 (Doi *et al.*, 1967, Ishiie *et al.*, 1967; Maramorosch *et al.*, 2011), the proof of their pathogenicity is lacking due inability to isolate and grow them in culture. This roadblock has hindered studies of phytoplasma biology, biochemistry and physiology, although significant taxonomic progress has been achieved by the study of the 16S ribosomal gene and the full sequencing of genomes from four strains (Oshima *et al.*, 2006; Bai *et al.*, 2006; Kube *et al.*, 2008; Tran-Nguyen *et al.*, 2008).

The inability to fulfil Koch's postulates severely restricted the understanding of the roles of phytoplasmas in disease etiology and plant-insect-phytoplasma interactions.

In contrast to mycoplasmas, which cause an array of disorders in animals and humans, phytoplasmas resisted all attempts to culture them in cell-free media. However, following the application of techniques of molecular genetics, their enigmatic status was resolved and led to the designation of a new taxon named 'Candidatus phytoplasma' (IRPCM, 2004). Despite reduced genome size in comparison to their ancestors, retain an independent metabolism that allows them to survive in environments as diverse as plant phloem and insect haemolymph. This versatility is a unique property among microbes, shared only with some animal- or plant-infecting viruses and a few other microorganisms such as the causal agent of malaria.

The paper describes how, employing specific commercially available media, it was possible to

achieve the axenic growth of seven phytoplasmas belonging to diverse ribosomal groups.

Contrary to the prevailing dogma in plant pathology, therefore, phytoplasmas, like mycoplasmas, can indeed be grown independently from the host(s). The commercial medium used for phytoplasma isolation and cultivation utilizes media available for purchase at Phytoplasmas *in vitro* Ltd. (Reigate, UK), although their composition is proprietary; a patent was submitted (Bertaccini *et al.*, 2012) to cover the commercial exploitation of the methodology. However, research carried out for scientific purposes, is not restricted by the patent.

Another key point for *in vitro* cultivation is that micropropagated periwinkle shoots infected with phytoplasmas were used for initial isolation. These can be obtained from the official collection of phytoplasma strains in micropropagation (<http://www.ipwgn.net.org/index.php/collection.html>) (Bertaccini *et al.*, 1992).

In spite of the relatively long time required for isolation in liquid medium, colony growth of phytoplasmas in agar usually occurs within two to five days, which makes the technology more easy applicable once isolation is obtained. When observed with a binocular microscope, the colonies are comparable in size and appearance to those of mycoplasmas. PCR amplification using phytoplasma specific primers under previously described conditions, confirmed that the colonies were indeed identical to the phytoplasmas that were initially transferred to the agar plates. Identification using RFLP analysis and direct sequencing of selected amplicons confirmed phytoplasma identity.

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