Phytopathogenic Mollicutes
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Omics

The timetree of phytoplasmas reveals new insights into the relationships with their insect and plant hosts

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Abstract

Phytoplasmas are a diverse monophyletic group of phytopathogenic bacteria. No attempts have yet been made to the estimate the divergence times of phytoplasma lineages. Reltime molecular divergence time analyses using 16S rRNA gene sequence data of Acholeplasmataceae was performed. The timetree of phytoplasmas, provided here for the first time, and based on prior divergence time estimates for two nodes within the *Mollicutes*, estimates the split between phytoplasma and *Acholeplasma* at about 663 million years ago (Ma), and initial diversification of the crown phytoplasma clade at about 330 Ma. Overall, these results suggest that phytoplasmas have been associated with insect and plant hosts at least since the carboniferous period.

Keywords: bacteria, divergence time, diversification, evolution

Introduction

Phytoplasmas are a diversified monophyletic group of phytopathogenic bacteria (phylum *Tenericutes*, class *Mollicutes*, family Acheloplasmataceae) (Gundersen *et al*., 1994), strictly associated with phloem sieve elements of the plants and internal organs of the sap-sucking hemipteran insects (mainly Auchenorrhyncha and Psyllidae) that are their only known vectors (Weintraub and Beanland, 2006). Due to difficulties in *in-vitro* cultivation, phytoplasmas are either classified as taxa based on phylogenetic analysis of molecular data (Lee *et al*., 2000) or as provisional '*Candidatus*' species according to bacterial nomenclature rules (Murray and Stackebrandt, 1995; IRPCM, 2004). Thirty-five ribosomal groups and 169 subgroups have been recognized based on 16Sr genetic data (Trivellone, 2019). To facilitate finer-scale characterization of some subgroups, other markers, *e.g. secY*, *tuf*, and *secA*, have been used (Hodgetts and Dickinson, 2010). Despite the availability of molecular phylogenies of phytoplasmas and a general timetree for bacteria (Marin *et al*., 2016), no attempts have yet been made to estimate divergence times of the detected phytoplasma lineages.

Materials and Methods

The 16S rRNA gene sequence data were employed to reconstruct the phylogenetic relationships of Acholeplasmataceae, including all designated subgroups/ '*Candidatus* species' of phytoplasmas and acholeplasmas, as well as 25 other *Mollicutes* and 2 *Firmicutes* species as outgroups. The maximum likelihood analysis was performed on RAxML v8.2.11 using GTRGAMMAI model with 1,000 bootstrap replicates. To estimate phytoplasma divergence times, RelTime molecular divergence time analyses in MEGA X (Mello, 2018) was performed and applied to two calibrated nodes: the split of *Acholeplasmatales* from *Mycoplasmatales* + *Entomoplasmatales* (1,704.5-2,070 million years ago, Ma) (Sheridan *et al*., 2003; Marin *et al*., 2016), and the split of *Mycoplasmatales* from *Entomoplasmatales* (1,581-1,679.4 Ma) (Sjöstrand *et al*., 2014; Marin *et al*., 2016).

Results and Discussion

A timetree focused on phytoplasmas is provided here for the first time. The 16S rDNA Maximum Likehold tree supported phytoplasmas as a monophyletic group derived from *Acholeplasma*, saprophytic or parasitic bacteria associated with various plant and animal hosts (Hanajima *et al*., 2015). Phytoplasmas are sister to a clade containing the plant pathogen *Acholeplasma palmae* and the horse parasite *A. parvum* (Tully *et al*., 1994; Atobe *et al*., 1983). The split between phytoplasmas and *Acholeplasma* was estimated at about 663 Ma, much earlier than the emergence of land plants (481-584 Ma) (Kumar *et al*., 2017), suggesting that the phytoplasma ancestor was originally associated only with animals. Plant parasitism apparently evolved independently in several lineages of Acholeplasmataceae, including phytoplasmas after their divergence from *Acholeplasma*.

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The crown clade of phytoplasmas began to diversify about 330 Ma, which corresponds to the rise of *Spermatophyta* (289-337 Ma) (Kumar *et al*., 2017) and early radiations of phytophagous Hemiptera (about 300-386 Ma) (Johnson *et al*., 2018), suggesting that the rise of seed plants and insect vectors played a crucial role in the phytoplasma diversification. Two major phytoplasma clades of phytoplasmas were recovered: one comprised the ribosomal groups 16SrI, XII, XIII, XVI-XVIII, XXIII, XXVIII and XXXI, which began diversifying about 130 Ma ago; the other contained the remaining groups and began to diversify about 257 Ma ago. The timetree suggests that a rapid radiation of phytoplasmas began after the emergence of angiosperms (168-194 Ma) (Kumar *et al*., 2017) and that many of the numbered 16S ribosomal groups appeared between 20 and 50 Ma ago. Overall, these results suggest that the carboniferous was an important period for the initial spread and diversification of phytoplasmas and, therefore, that the diversity of phytoplasmas may have been driven by co-evolution, and co-radiations, with plants and insect vectors. Besides providing crucial information for understanding the evolutionary history of lineages, this first timetree of phytoplasmas offers new insights into the understanding of the biological relationships with their insect, plant and ancestor hosts. To overcome the lack of a fossil record in bacteria, which could provide calibration points closer to the tips of the phylogeny, more detailed analyses of the 16SrV group using faster-evolving genes are underway. These will incorporate a date estimates for more recent divergence events that can be used to refine the overall divergence time estimates for phytoplasmas.

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