

First detection of a 'Candidatus Phytoplasma solani'-related strain infecting sugar beet in Switzerland

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Sugar beet (Beta vulgaris) is the sole sugar crop in Switzerland, currently cultivated on approximately 16,000-18,000 ha, mainly distributed along the Central Plateau (Mahillon et al., 2022). The Swiss sugar industry is threatened by the disease "Syndrome Basses Richesses" (SBR) which significantly reduces sugar yield (Mahillon et al., 2022). Disease surveillance is done annually and has revealed that SBR has become widespread in the country in recent years.

Two phloem-restricted pathogens are associated with SBR, the Stolbur (16SrXII group) phytoplasma 'Candidatus Phytoplasma solani' ('Ca. P. solani') and the γ -proteobacterium 'Candidatus Arsenophonus phytopathogenicus' ('Ca. A. phytopathogenicus') (Gatineau et al., 2002; Sémétey et al., 2007). Both are transmitted by planthoppers, Pentastiridius leporinus (Hemiptera: Cixiidae) being the main vector in sugar beet (Gatineau et al., 2002). Until 2023, only 'Ca. A. phytopathogenicus' had been detected in sugar beet with SBR symptoms in Switzerland. In 2024, DNA extracts (obtained using a CTAB-based method) from seven samples showing SBR symptoms collected in the north-eastern regions (Figure. 1) tested positive for phytoplasma using a quantitative PCR method based on detection of the phytoplasma 23S rRNA gene (Hodgetts et al., 2009). Phytoplasma infection was confirmed by nested PCR amplification of the 16S rRNA gene using the P1/P7 and R16F2n/R16R2 primer pairs used for universal phytoplasma identification, according to EPPO Standard PM7/133 (European and Mediterranean Plant Protection Organization, 2018). Further characterisation of the 'Ca. P. solani' isolate was done by nested PCR amplification of the tuf gene using fTuf1/rTuf1 and fTufAY/rTufAY primers pairs (Schneider & Gibb, 1997). Amplicons were purified (NucleoFast, Macherey-Nagel,

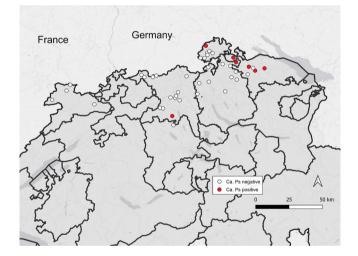


FIGURE 1 Detection of 'Candidatus Phytoplasma solani' in Switzerland in 2024. Red and white dots indicate the locations of sampling as well as presence (red)/absence (white) of the pathogen

Germany) and directly sequenced (Fasteris, Plan-les-Ouates, Switzerland). Phylogenetic trees were generated with the MEGA software version 11.0.13 using the Maximum Likelihood method in a bootstrap test (500 replicates) and 'Candidatus Phytoplasma mali' as the outgroup. BLAST sequence comparison of the partial 16S rRNA gene sequence (1177 bp) showed a 100% shared identity with 'Ca. P. solani' (group 16SrXII) isolates from sugar beet in Germany (OQ717667) and Poland (PP716579) (Figure 2a), belonging to subgroup 16SrXII-P.

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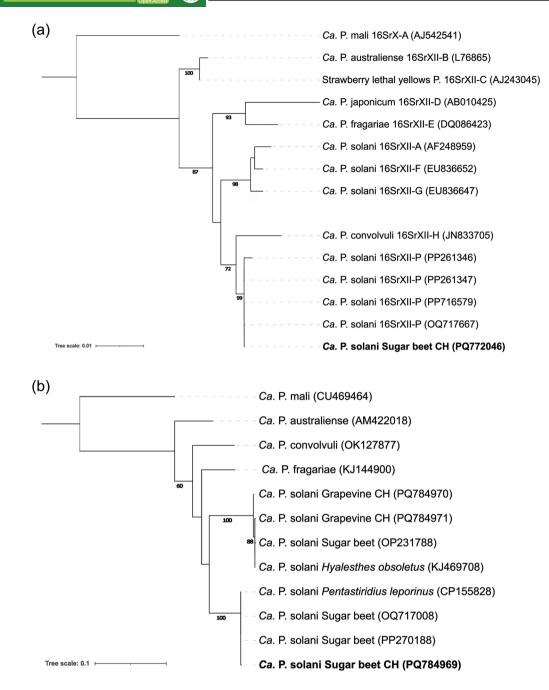


FIGURE 2 Phylogenetic analysis based on (a) 16S rRNA gene sequence and (b) *tuf* gene sequence of the Swiss '*Candidatus* Phytoplasma solani' ('*Ca.* P. solani') isolate from sugar beet (CH, in bold) compared with other phytoplasma isolates. Bootstrap support values >60% are labeled. Scale bar shows the number of substitutions per site

Sequences of the *tuf* gene (943 bp) showed only one single nucleotide polymorphism difference, an A to G mutation at position 16 of the Swiss isolate, when compared with those of three German phytoplasma isolates from either sugar beet or *P. leporinus*. This supports the hypothesis that the Swiss '*Ca*. P. solani' isolate is enclosed within the phylogenetic cluster of these three German phytoplasmas (Figure 2b), and it is closely related to phytoplasma isolates from grapevine and *Hyalesthes obsoletus*. Sequences have been submitted to the GenBank database under Accession Nos. PQ772046 (16S rRNA) and PQ784969, PQ784970 and PQ784971 (*tuf*), respectively.

This is the first record of both detection and genetic characterisation of 'Ca. P. solani' infecting *B. vulgaris* in Switzerland. The phytoplasma is associated with SBR disease in the northeast of the country, close to Germany where 'Ca. P. solani' is prevalent in sugar beet fields affected by SBR. This finding coincides with the first detection of 'Ca. A. phytopathogenicus' in this area in 2024. Interestingly, sugar beet production in western Switzerland, known as the region most heavily affected by SBR, and where 'Ca. A. phytopathogenicus' has been present for several years, has so far remained free of 'Ca. P. solani'. The results also suggest a complex epidemiology for closely related 'Ca. P. solani' isolates, which can affect different crop plants like sugar beet and grapevine, and are very likely to be transmitted by two distinct Hemiptera: Cixiidae populations.

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