Glucose and trehalose PTS permeases of *Spiroplasma citri* probably share a single IIA domain, enabling the spiroplasma to adapt quickly to carbohydrate changes in its environment

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*INTRODUCTION*

*Spiroplasma citri* is a plant-pathogenic mollicute phylogenetically related to Gram-positive bacteria. Spiroplasma cells are restricted to the phloem sieve tubes and are transmitted from plant to plant by the leafhopper vector *Circulifer haematoceps*. In the plant sieve tubes, *S. citri* grows on glucose and fructose, whereas in the leafhopper haemolymph the spiroplasma must grow on trehalose, the major sugar in insects. Previous studies in this laboratory have shown that fructose utilization was a key factor of spiroplasmal pathogenicity. To further study the implication of sugar metabolism in the interactions of *S. citri* with its plant host and its leafhopper vector, genes encoding permease enzymes II (EIIGlc and EIITre) of the *S. citri* phosphoenolpyruvate : glucose and phosphoenolpyruvate : trehalose phosphotransferase systems (PTS) were characterized. Mapping studies revealed that the EIIGlc complex was split into two distinct polypeptides, IIA Glc and IICB Glc, encoded by two separate genes, *crr* and *ptsG*, respectively. As expected, *S. citri* polypeptides IIA Glc and IICB Glc were more phylogenetically related to their counterparts from Gram-positive than to those from Gram-negative bacteria. The trehalose operon consisted of three genes *treR*, *treP* and *treA*, encoding a transcriptional regulator, the PTS permease (EIITre) and the amylase, respectively. However, in contrast to the fructose-PTS permease, which is encoded as a single polypeptide (IIABCFru) containing the three domains A, B and C, the trehalose-PTS permease (IIITre) lacks its own IIA domain. No trehalose-specific IIA could be identified in the spiroplasmal genome, suggesting that the IIBTtre permease probably functions with the IIA Glc domain. In agreement with this statement, yeast two-hybrid system experiments revealed that the IIA Glc domain interacted not only with IIB Glc but also with the IIBTtre domain. The results are discussed with respect to the ability of the spiroplasma to adapt from the phloem sap of the host plant to the haemolymph and salivary gland cells of the insect vector.

*Abbreviation:* PTS, phosphoenolpyruvate : sugar phosphotransferase system.

The GenBank accession numbers for the IIITre, IIA Glc and IICB Glc regions of *S. citri* sequences reported in this paper are AY230005, AY230006 and AY230007, respectively.

Sequence alignments are included as supplementary data with the online version of this paper at http://mic.sgmjournals.org.

*PPTS, phosphoenolpyruvate : sugar phosphotransferase system.*
tissues of their phloem sap-feeding insect vectors (Fletcher et al., 1998). They also multiply in the herbaceous plant *Catharanthus roseus* (periwinkle) in which they induce symptoms similar to those observed in their original host. However, despite numerous attempts, phytoplasmas have not yet been cultured in *vitro* whereas spiroplasmas, and in particular *S. citri*, have been cultured since 1970 (Saglio et al., 1971, 1973). Since then, *S. citri* has been extensively characterized at the molecular level and methods for efficient transmission to periwinkle plants by its leafhopper vector *Circulifer haematoceps* have been developed (Bové et al., 1989; Bové, 1997; Foissac et al., 1996). Eventually, as molecular genetic tools such as transposon and homologous recombination mutagenesis have been made available, *S. citri* has become a model organism to investigate how plant-pathogenic mollicutes cause diseases in plants and to study the molecular mechanisms of insect transmission (Renaudin, 2002; Bové et al., 2003). In *S. citri*, several components, including toxins and lactic acid, have been suggested to play a role in disease development (Daniels, 1983). *S. citri* infection was also shown to cause a shortage of auxins, and it was proposed that utilization of plant sterols could result in a deficit of growth regulators (Chang, 1998). However, no conclusive experiments could confirm these hypotheses. More recently, it was shown that phytoplasma infection of *Catharanthus roseus* severely impaired carbohydrate partitioning, suggesting that sugar metabolism was involved in the interactions of mollicutes with their host plants (Lepka et al., 1999).

A significant breakthrough came from genetic studies in *S. citri*, showing that mutants unable to use fructose produced very mild symptoms in spite of multiplication (Postma et al., 2003). In *S. citri*, several components, including toxins and lactic acid, have been suggested to play a role in disease development (Daniels, 1983). *S. citri* infection was also shown to cause a shortage of auxins, and it was proposed that utilization of plant sterols could result in a deficit of growth regulators (Chang, 1998). However, no conclusive experiments could confirm these hypotheses. More recently, it was shown that phytoplasma infection of *Catharanthus roseus* severely impaired carbohydrate partitioning, suggesting that sugar metabolism was involved in the interactions of mollicutes with their host plants (Lepka et al., 1999).

### METHODS

#### Strains, growth media and transformation.

*Escherichia coli* TOP10 [*F* recA1 araD139 (araD-139) galK1GalR4 endA1 supE44 thi-1 leuB6 tryR] was used as the host strain for cloning experiments and plasmid propagation. *E. coli* competent cells (Invitrogen) were transformed by heat shock at 42°C. *S. citri* GII-3 wild-type (wt) strain was originally isolated from its leafhopper vector *C. haematoceps* captured in Morocco (Vignault et al., 1980). Phytoplasmas were grown at 32°C in HSI or SP4 medium (Whitcomb, 1983). *Saccharomyces cerevisiae* (MATa/MAA, ura3-52, his3-A200/His3Δ200, trpl-901/trpl-901, leu2-3,112/Leu2-3,112, gal4-542/gal4-542, gal80-538/gal80-538, URA3::GAL1-LacZ/URA3::(GAL4-17-mers)-3-CYC1LacZ, LYS2/lys2::GAL1-HIS3) used in two-hybrid analyses were obtained through mating of strains HF7c and Y187 (Louvet et al., 1997). Yeast cultures were grown either in YPD complete medium or in SD selective medium (Sherman et al., 1986). Yeast transformations were performed by the lithium acetate method (Gietz et al., 1995).

#### DNA and RNA analyses.

Total DNA from *S. citri* cells was extracted with the Wizard Genomic DNA Purification Kit (Promega). Restricted DNA was fractionated by agarose gel electrophoresis, blotted onto positively charged nylon membranes by the alkali transfer procedure and hybridized with appropriate [digoxigenin]dUTP-labelled probes using standard stringency conditions (Sambrook et al., 1989). Hybridization signals were detected with anti-digoxigenin-alkaline phosphatase-conjugate and HNPP (2-hydroxy-3-naphthoic acid-2'-phenylalanilide phosphate) as the substrate, following the supplier’s instructions. Fluorescent signals were detected using a Fluor-S Multimager phosphoimager (Bio-Rad). Total RNA was extracted from phytoplasma cells by using the Tri-reagent (Sigma). The RNA samples were treated with RQ1 RNase-free DNase (Promega), separated by agarose gel electrophoresis and transferred onto charged nylon membranes according to standard procedures (Sambrook et al., 1989). Hybridization with the appropriate probes was performed as described above for Southern blots except that washing was carried out at 50°C. Probes specific for the *S. citri* genes treP, crr, ptsG and spiralin were obtained by PCR amplification of plasmid DNA with primer pairs PER2/PER3, DHAn1/DHAb2, GP6/GP7 and SR14/SR16, respectively (Table 1).

#### PCR and RT-PCR.

First-strand cDNA was synthesized from 1 μg DNase-treated RNA mixed with 2-4 μl 200 mM dNTP, 2-4 μl 0-1 mM DTT, 3 μl 100 mM 3’ primer (DHAb2 or DHHBg2) and 30 units RNase-OUT (Invitrogen) to a total volume of 20 μl. RNA was heat-denatured at 65°C for 5 min before adding 200 units Superscript II reverse transcriptase (Invitrogen). The reverse transcription was performed over 70 min at 40°C and the enzyme was heat-denatured for 5 min at 70°C. PCR was performed with 2 μl of the product of the reverse transcription or 40 ng genomic DNA. Primers used for amplification are listed in Table 1. PCR conditions were 94°C for 1 min, 55°C for 1 min, 72°C for 1 min. The PCR products were separated by agarose gel electrophoresis.
Table 1. Primers used in this study

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*Bold letters indicate restriction enzyme sites. Lower-case letters indicate mismatched nucleotides.

RESULTS

Gene organization of the trehalose and glucose PTS enzymes II

From data of the ongoing S. citri genome sequencing project, short sequences encoding putative peptides with high similarities to the trehalose- and glucose-specific PTS enzyme II (permeases) were identified. Then, the DNA inserts from the relevant plasmids were sequenced to determine the complete sequences of the PTS permease genes. The restriction maps as well as the gene organization of the regions encompassing the trehalose operon and the glucose PTS permease genes are presented in Fig. 1. Sequences of the 6-2 kbp region of the trehalose operon were found to contain six ORFs in the same orientation, each one starting with an ATG initiation codon preceded by a RBS sequence and finishing with a TAA or TAG stop codon (Fig. 1a). The encoded polypeptides were 282, 199, 87, 329, 522 and 549 aa long, respectively. The first two polypeptides (ORFs 1 and 2) were found to share significant homology with rRNA methylases and GTPases, respectively. The highest scores were 41% identity and 60% similarity between ORF1 and a Mycoplasma pneumoniae transcriptional regulator, the trehalose PTS permease (EIITre) and the amylase, respectively. In contrast, ORF3 showed no significant homology with known proteins. Also, based on similarities of their predicted translation products with proteins in the databases, the last three ORFs (ORFs 4, 5 and 6) were identified as the treR, treP and treA genes, encoding a transcriptional regulator, the trehalose PTS permease (EIITre) and the amylose, respectively. Inverted repeat sequences characteristic of p-independent transcription terminators were found downstream of the GTP-binding

45 s, 53 °C for 45 s and 72 °C for 45 s for RT-PCR and for 90 s for PCR. The number of cycles was 40 for PCR and 20, 30 and 40 for RT-PCR experiments.

Yeast two-hybrid analysis. The use of plasmids pACT2 (Clontech) and pOD80 (accession no. 008932) to study protein–protein interactions in a Saccharomyces cerevisiae two-hybrid system has been described previously (Louve et al., 1997). DNA sequences corresponding to domains IIA Glc, IIB Glc and IIB Tre of the S. citri enzyme II (permeases) were amplified with primer pairs DHA1n1/DHA2b2, DHBGn1/DHBGb2 and DHTn1/DHTb2, respectively (Table 1). PCR amplification was performed with the proofreading Platinum Pfx DNA polymerase (Invitrogen) as recommended by the supplier. Restriction sites Ncol or BamHI were included in the primers to fuse the amplified DNA fragments either to the GAL4 DNA-binding domain of pOD80 or to the GAL4-activating domain of pACT2. The resulting constructions pAA (pACT2 + IIA Glc), pAG (pACT2 + IIA Tre), pAT (pACT2 + IIB Tre), pOA (pOD80 + IIA Tre), pOG (pOD80 + IIB Glc) and pOT (pOD80 + IIB Tre) carrying the translational fusions were verified by sequencing. The insert-free pACT2 and pOD80 plasmids were also tested as negative controls. Transformed cells carrying the various plasmid combinations were selected by plating on SD medium lacking tryptophan and leucine (SD-TL). Then, protein interactions were tested by streaking transformants on SD medium lacking tryptophan, leucine and histidine (SD-TLH). To confirm protein interactions β-galactosidase assays were carried out as described previously (Barthe et al., 1998).

In silico analyses. DNA and protein sequence analyses were performed using the programs proposed by Infobiogen (http://www.infobiogen.fr/index.html). The BLAST program was used to search homologies in general databases (http://www.ncbi.nlm.nih.gov/BLAST/) or in the Spiroplasma kunkelii partially sequenced genome (http://www.genome.ou.edu/spiro_blast.html). Multiple alignments were done with MULTALIN (Corpet, 1988; http://www.toulouse.inra.fr/lgc/multalin/multalin.html). Phylogenetic trees were constructed by using the MEGA2 program (Kumar et al., 2001; http:// evolution.genetics.washington.edu/phylip.html).
protein gene and downstream of the treA gene, suggesting that ORF3 and the treR, treP and treA genes are transcribed to a single polycistronic mRNA. Indeed, Northern blot analyses revealed that the IIA\(^{\text{Glc}}\) and IICB\(^{\text{Glc}}\) domains of the permease were encoded by two distinct genes, each one occurring as a single copy. These genes were annotated crr and ptsG as they certainly represent orthologues of the crr and ptsG genes of E. coli (sequence alignments are included as supplementary data with the online version of this paper at http://mic.sgmjournals.org). As shown in Fig. 2, none of the restriction fragments were found to hybridize with both the crr- and ptsG-specific probes. For example, probe crr hybridized with the 7-3 kbp NsiI and 11-8 kbp HpaI fragments (Fig. 2a, lanes 4 and 5) whereas the ptsG probe hybridized with NsiI and HpaI fragments of 4-2 and 3-6 kbp, respectively (Fig. 2b, lanes 4 and 5). Sequence analyses of the 4-2 kbp NsiI fragment hybridizing with the ptsG probe (Fig. 2b, lane 4) showed this fragment to contain four ORFs, including the ptsG gene (ORF2), which encodes the glucose PTS permease polypeptide IICBGlc (Fig. 1b).

Upstream of ptsG, ORF1 encodes an 82 aa polypeptide for which no significant homology was found. In contrast, the 350 aa ORF3 and the truncated ORF4 located downstream of ptsG encode hypothetical, conserved proteins with no known function in the case of ORF3 and with striking similarities to serine-threonine protein phosphatases in the case of ORF4. These two ORFs are orthologues of the yloN and yloO genes of Bacillus subtilis (Foulger & Errington, 1998).

The gene organization of the trehalose operon is similar to that of the fructose operon described previously (Gaurivaud et al., 2000b). However, alignment of the amino acid sequences of the two permeases indicated that, in contrast to the fructose permease, which contains all three domains (IIBC\(^{\text{Fru}}\)), the treP-encoded trehalose permease (IIBC\(^{\text{Tre}}\)) lacked domain IIA (see Fig. 5). In addition, no trehalose-specific IIA\(^{\text{Tre}}\) domain could be identified elsewhere in the spiroplasmal genome. In the case of the glucose-specific PTS, PCR amplification of genomic DNA with primer pair GP1/GP2 (see positions of the primers in Fig. 1b and c) yielded negative results, suggesting that, in contrast to the situation in Mycoplasma genitalium and Mycoplasma pneumoniae, the three domains IIA, IIB and IIC are not fused into a single polypeptide IIBC\(^{\text{Glc}}\) encoded by a single gene. Indeed, Southern blot analyses revealed that the IIA\(^{\text{Glc}}\) and IICB\(^{\text{Glc}}\) domains of the permease were encoded by two distinct genes, each one occurring as a single copy. These genes were annotated crr and ptsG as they certainly represent orthologues of the crr and ptsG genes of E. coli (sequence alignments are included as supplementary data with the online version of this paper at http://mic.sgmjournals.org). As shown in Fig. 2, none of the restriction fragments were found to hybridize with both the crr- and ptsG-specific probes. For example, probe crr hybridized with the 7-3 kbp NsiI and 11-8 kbp HpaI fragments (Fig. 2a, lanes 4 and 5) whereas the ptsG probe hybridized with NsiI and HpaI fragments of 4-2 and 3-6 kbp, respectively (Fig. 2b, lanes 4 and 5). Sequence analyses of the 4-2 kbp NsiI fragment hybridizing with the ptsG probe (Fig. 2b, lane 4) showed this fragment to contain four ORFs, including the ptsG gene (ORF2), which encodes the glucose PTS permease polypeptide IICBGlc (Fig. 1b).

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Northern blot hybridizations with the \textit{ptsG} probe revealed a unique mRNA of 2.2 kb, the size of which was in agreement with a monocistronic \textit{ptsG} transcript (Fig. 3c). Accordingly, a typical transcription terminator structure was found immediately downstream of \textit{ptsG} and sequences resembling the \textit{ptsH} and \textit{crr} genes were identified in the intergenic region between ORF1 and \textit{ptsG}. Sequencing a 5.2 kb region encompassing the \textit{ptsG} encoding \textit{crr} gene (ORF4) revealed four additional, putative ORFs, three of which (ORFs 1, 3 and 5 in Fig. 1c) did not show any significant homology with known proteins. Interestingly, the 578 aa ORF2 showed striking similarities with the \textit{ptsI} and \textit{crr} genes of the \textit{S. kunkelii} genome, encoding the HPr protein, \textit{ptsI} and \textit{crr} genes are organized in a single operon, in the \textit{S. kunkelii} genome, \textit{ptsI} and \textit{crr} genes are strongly suggests that, in contrast to \textit{M. capricolum}, the \textit{S. citri} \textit{ptsG} and \textit{crr} genes are transcribed to two distinct mRNAs. A similar organization of the \textit{ptsG} and \textit{crr} genes was also found to occur in \textit{S. kunkelii}. However, unlike the situation in \textit{E. coli} where \textit{ptsH}, encoding the HPr protein, \textit{ptsI} and \textit{crr} are organized in a single operon, in the \textit{S. citri} genome, \textit{ptsI} and \textit{crr} on the one hand and \textit{ptsH} on the other hand are located at two distinct loci on the chromosome.

\textbf{Transcription of the trehalose operon, \textit{ptsG} and \textit{crr} genes}

In the experiment shown in Fig. 3, total RNA was extracted from \textit{S. citri} cultures grown in HSI medium containing glucose (lanes 1), fructose (lanes 2) or trehalose (lanes 3) and hybridized with three different probes, \textit{treP} (Fig. 3a), spiralin (Fig. 3b and c) and \textit{ptsG} (Fig. 3c). Fig. 3a shows that the \textit{treP}-specific probe hybridized with a 4.5 kb signal corresponding to the transcription product of the whole operon. Interestingly enough, the hybridization signal was significantly stronger in the presence of trehalose, indicating that transcription of the trehalose operon is stimulated by trehalose (lane 3), but not by glucose (lane 1) and fructose (lane 2). The amount of spiralin mRNA (used as the control) was not significantly affected (Fig. 3b). Similarly, hybridization with the \textit{ptsG} probe revealed that glucose, but not fructose and trehalose, stimulated transcription of the glucose permease gene (Fig. 3c, lanes 1–3). Surprisingly, the \textit{crr} transcription product could not be detected by Northern blot hybridization (data not shown). However, its presence in spiroplasma cells was revealed by RT-PCR experiments (Fig. 4). As shown in the figure, the 428 bp amplified DNA fragment corresponding to the \textit{crr} mRNA could only be detected through at least 30 cycles of amplification (lanes 6 and 9) whereas the 278 bp fragment corresponding to the \textit{ptsG} transcript was detected through 20 cycles only (lanes 4, 7 and 10).

\textbf{Sequence analyses of the \textit{S. citri} glucose-PTS enzyme II}

The amino acid sequences of the \textit{S. citri} enzyme II polypeptides were aligned with those of various Gram-positive and Gram-negative bacteria (multiple alignments are included as supplementary data with the online version of this paper at http://mic.sgmjournals.org). To maximize the alignment agreement of the IICBG\textsuperscript{Clh} sequences, insertion loops were introduced into the \textit{S. citri} and \textit{S. kunkelii} spiroplasma sequences between regions IIC1 and IIC2 as well as between regions IIC2 and IIC3. Additional insertions

![](http://mic.sgmjournals.org)
were introduced between regions IIC2 and IIC3 of the IICBGlc sequences of the human mycoplasmas M. genitalium and M. pneumoniae. The alignments indicate that the S. citri polypeptides do possess the PTS permease-specific domains IIA, IIB and IIC (Fig. 5). In particular, they show that the histidine residues at positions 66 and 81 of the S. citri domain IIA correspond to E. coli His-75 and His-90, respectively, which are known to be involved in phosphoryl group transfer from HPr to IICBGlc via IIAGlc (Postma et al., 1993; Presper et al., 1989). Similarly, the three regions (IIC1, IIC2 and IIC3) of the IIC domain as well as domain IIB are highly conserved. In particular, three regions (IIC1, IIC2 and IIC3) of the IIC domain as well as domain IIB were also found in the S. citri domain IIB. These data strongly suggested that the S. citri IIA Glc domain to bind the IIB Tre as well as the IIB Glc domains was tested through yeast two-hybrid experiments. Construction of pACT2 and pODB80 recombinant plasmids carrying either one of the IIAGlc, IIB Glc or IIB Tre domains is described in Methods.

**Domain IIAGlc-binding analyses**

The hypothesis that the IIAGlc domain could function either with IICBGlc or with IIBC Tre was suggested by the following observations: (i) S. citri metabolizes fructose, glucose and trehalose; (ii) no IIA Tre was identified in the genomes of S. citri and S. kunkelii; (iii) the glucose permease gene organization IIAGlc + IICBGlc in Gram-positive bacteria seemed to correlate with the ability to grow in trehalose-rich environments such as insect haemolymph; and (iv) IIB Glc and IIB Tre of S. citri show strong sequence similarities in agreement with the fact that they belong to the same EII family. Therefore the ability of the S. citri IIA Glc domain to bind the IIB Tre as well as the IIB Glc domains was tested through yeast two-hybrid experiments. Construction of pACT2 and pODB80 recombinant plasmids carrying either one of the IIAGlc, IIB Glc or IIB Tre domains is described in Methods. *Saccharomyces cerevisiae* HY was transformed with various plasmid pairs (1–11) as indicated in the legend to Fig. 7 and transformants were selected by plating on SD-TL medium. For each plasmid combination, four individual transformants were selected. Colonies were dispersed in water and the undiluted cell suspensions, as well as 10–1 and 10–2 dilutions, were plated on selective medium SD-TLH. For each plasmid combination, all four transformants yielded identical results, which are presented in Fig. 7. Whereas all transformants grew on the SD-TL medium (Fig. 7b), only those carrying plasmid pairs 8, 9, 10 or 11 grew on the SD-TLH selective medium (Fig. 7a), indicating interactions between the relevant protein domains. The results clearly indicated that, in addition to the expected interaction between IIAGlc and IIB Glc (plasmid combinations 8 and 10), the IIA Glc domain interacted also with domain IIB Tre (plasmid combinations 9 and 11). These results were further confirmed by β-galactosidase assays, showing that the GAL4 promoter-lacz fusion was also activated in the yeast transformants carrying plasmid pairs 8, 9, 10 and 11 (data not shown). These data strongly suggested that the S. citri trehalose permease IIBC Tre, which lacks its own IIA domain, might function with the glucose permease IIAGlc domain to import trehalose.

**DISCUSSION**

The carbohydrate PTS catalyses transport and phosphorylation of carbohydrates in various obligate and facultative bacteria (i.e. those which also multiply in an arthropod host) such as the plant-pathogenic spiroplasmas which multiply in their leafhopper vectors, and *M. capricolum* and *Borrelia burgdorferi* which multiply in ticks (Cottew & Yeats, 1982). These Gram-positive bacteria with a IIAGlc + IICBGlc organization are indicated by arrows in Fig. 6. In contrast, all other Gram-positive bacteria, including *Bacillus subtilis* and *Streptococcus pneumoniae*, were found to exhibit glucose permeases with a IICBA Glc single gene organization.
anaerobic bacteria. The sugar-specific component of the system (EII complex) generally consists of three functional domains, two cytoplasmic proteins IIA and IIB and one integral membrane protein IIC, which occur as either protein subunits or domains of a multi-domain polypeptide (Saier & Reizer, 1992). In *Bacillus subtilis*, the glucose-PTS permease consists of a single polypeptide chain in which the three domains are fused in the order IICBA Glc (Zagorec & Postma, 1992), whereas in *E. coli*, the glucose-specific permease EIIGlc is split into two distinct polypeptides IIAGlc and IICB Glc encoded by two separate genes, *crr* and *ptsG*, respectively (Lengeler et al., 1994). The *ptsG*-encoded IICB<sub>Glc</sub> protein confers sugar specificity and mediates transport of glucose. In *S. citri*, orthologues of *crr* and *ptsG* were identified at two distinct loci on the chromosome. Protein comparisons showed that the *crr* and *ptsG* predicted products shared striking homologies with the previously described glucose PTS permeases. In agreement with the phylogeny of mollicutes, which have arisen from ancestors of low G+C, Gram-positive bacteria, the *S. citri* proteins IIA<sub>Glc</sub> and IICB<sub>Glc</sub> are more similar to their counterparts from Gram-positive than to those from *E. coli*.

Fig. 6. Phylogenetic trees of *crr*- (a) and *ptsG*- (b) encoded polypeptides. The trees are based on the multiple alignments generated by the MULTALIN program and analysed by neighbour-joining with the MEGA 2 program. The bar indicates the scale of branch length and represents 0.1 mutations per amino acid residue. The asterisks refer to the organisms which display a IIA<sup>Glc</sup>+IICB<sup>Glc</sup> organization and the open arrows indicate the Gram-positive bacteria which are able to multiply in arthropod hosts. The amino acid sequences were retrieved from the NCBI according to the following accession numbers: *E. coli* (gi|1787343 for *ptsG* and gi|1788757 for *crr*), *Yersinia pestis* (gi|15979646 for *ptsG* and gi|15980951 for *crr*), *Vibrio cholerae* (gi|9656557 for *ptsG* and gi|9655424 for *crr*), *Haemophilus influenzae* (gi|1574566 for *crr*), *Salmonella typhimurium* (gi|16419720 for *ptsG* and gi|16420972 for *crr*), *Buchnera aphidicola* (gi|2688579 for *ptsG* and gi|2688474 for *crr*), *Bacillus subtilis* (gi|2633760), *Clostridium acetobutylicum* (gi|15023438), *Staphylococcus aureus* (gi|1072417), *Staphylococcus epidermidis* (gi|27316568), *Borrelia burgdorferi* (gi|2688579 for *ptsG* and gi|2688474 for *crr*), *Streptococcus pneumoniae* (gi|15458255), *M. capricolum* (gi|602679), *M. pulmonis* (gi|14089430), *M. genitalium* (gi|1045745), *M. pneumoniae* (gi|1674328) and *M. penticans* (gi|26453594).
Gram-negative organisms. Unexpectedly however, the gene organization IIA\textsuperscript{Glc} + IICB\textsuperscript{Glc} of the \textit{S. citri} glucose PTS permease resembles that of \textit{E. coli} rather than that (IICA\textsuperscript{Glc}) found in \textit{Bacillus subtilis} and the mollicutes \textit{M. pulmonis}, \textit{M. pneumoniae}, \textit{M. genitalium} and \textit{M. penetrans}. Our studies showed that, in Gram-positive bacteria, the IIA\textsuperscript{Glc} + IICB\textsuperscript{Glc} organization was found to occur only in multiple-host bacteria such as \textit{Borrelia burgdorferi}, \textit{M. capricolum} (Zhu et al., 1994) and the spiroplasmas \textit{S. citri} and \textit{S. kunkelii}, all of which have the ability to multiply in an arthropod host. Knowing that trehalose is the major sugar in arthropod haemolymph (Becker et al., 1996), it is consistent that genes encoding the trehalose-PTS enzyme II (EIIT\textsuperscript{Tre}) were identified in the genomes of the insect-transmitted spiroplasmas \textit{S. citri} and \textit{S. kunkelii}, but not in the human and animal mycoplasmas \textit{M. genitalium} (Fraser et al., 1995), \textit{M. pneumoniae} (Himmelreich et al., 1996) and \textit{M. pulmonis} (Chambaud et al., 2001).

Interestingly, the \textit{S. citri} trehalose permease (IIICT\textsuperscript{Tre}) encoded by the \textit{treP} gene of the trehalose operon lacks its own IIA\textsuperscript{Tre} domain. In several \textit{E. coli} EIII complexes and in particular that for trehalose, the IIA domain has been lost and it has been functionally replaced by the glucose IIA\textsuperscript{Glc} protein. In this case, the IIA domain is not sugar-specific and phosphorylates a subset of several PTS IIB domains (Lengeler et al., 1994). In \textit{S. citri}, which uses both glucose and trehalose, the yeast two-hybrid experiments clearly indicated that the IIA\textsuperscript{Glc} domain could interact not only with the IIB\textsuperscript{Glc} domain but also with the trehalose-specific IIB\textsuperscript{Tre} domain. Crystallographic studies of the IIA\textsuperscript{Glc} of \textit{M. capricolum} have suggested that interactions between domains IIA\textsuperscript{Glc} and IIB\textsuperscript{Glc} are hydrophobic with no sequence specificity (Huang et al., 1998). Therefore, the ability of the \textit{S. citri} IIA\textsuperscript{Glc} to bind the two distinct domains IIB\textsuperscript{Glc} and IIB\textsuperscript{Tre} would be explained by the non-discriminating nature of the interactions. However, genetic studies will be required to definitively demonstrate that \textit{in vivo} the \textit{S. citri} trehalose permease functions with the IIA\textsuperscript{Glc} domain to import trehalose.

The occurrence of two distinct genes \textit{crr} and \textit{ptsG} encoding IIA\textsuperscript{Glc} and IICB\textsuperscript{Glc} polypeptides is consistent with the ability of IIA\textsuperscript{Glc} to function with both IICB\textsuperscript{Glc} and IIICT\textsuperscript{Tre}. However, its biological significance is not known. The constituents of the bacterial PTS have undergone extensive shuffling during their evolution (Reizer & Saier, 1997). From our studies, the gene organization of the glucose permeases in Gram-positive bacteria did not correlate with a specific phylogenetic branch, but instead seemed to be more related to the ecological niches of the bacteria. \textit{In vitro} analyses of the \textit{E. coli} glucose PTS showed that a fusion protein made of IIC\textsuperscript{Glc}, IIB\textsuperscript{Glc} and IIA\textsuperscript{Glc} domains linked together exhibited higher phosphotransferase activity than a mixture of the isolated subunits (Mao et al., 1995). These data suggested that the linking of functional domains could be an advantage regarding the activity of the permease. It is noteworthy that, among mollicutes, the mycoplasmas exhibit a glucose permease in which the three domains are linked, whereas the \textit{S. citri} enzyme is split into two distinct polypeptides. Selection of such an organization, which could be disadvantageous regarding enzyme activity, might be related to the spiroplasma life cycle. \textit{In vitro}, \textit{S. citri} metabolizes fructose, glucose and trehalose. The finding that the transcription of the PTS permeases genes was stimulated by the presence of the relevant carbohydrate in the medium (Gaurivaud et al., 2001; this study) reflects the adaptive capacity of \textit{S. citri} to grow in ecologically different niches. In the plant sieve tubes, \textit{S. citri} grows on fructose and glucose, whereas in the leafhopper, the spiroplasma must grow on trehalose, the major sugar in insect haemolymph. Therefore, when \textit{S. citri} is acquired by the insect from the plant, adaptation from glucose and fructose to trehalose could be achieved with a IIBC+IIA organization, provided that the IIA
component functions with both IICB_Glc and IICB_Tre. Similarly, when the spiroplasma moves from the insect haemolymph, where it relies on trehalose, to the cells of the salivary glands where glucose is the main sugar, a common IIA domain would help the spiroplasma to rapidly adapt from one host or host-compartment to another.

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