Diverse roles of the GlcP glucose permease in free-living and symbiotic cyanobacteria

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Certain cyanobacteria can form symbiotic associations with plants, where the symbiont supplies the plant partner with nitrogen and in return obtains fixed sugars. We recently showed that in the symbiotic cyanobacterium *Nostoc punctiforme*, a glucose specific permease, GlcP, is necessary for the symbiosis to be formed. Results presented here from growth yield measurements of mutant strains with inactivated or overexpressing sugar transporters suggest that GlcP could be induced by a symbiosis specific substance. We also discuss that the transporter may have a role other than nutritional once the symbiosis is established, i.e., during infection, and more specifically in the chemotaxis of the symbiont. Phylogenetic analysis shows that the distribution of GlcP among cyanobacteria is likely influenced by horizontal gene transfer, but also that it is not correlated with symbiotic competence. Instead, regulatory patterns of the transporter in *Nostoc punctiforme* likely constitute symbiosis specific adaptations.

While most cyanobacteria are photoautotrophs and use ammonium or nitrate as a source of nitrogen, other metabolic capacities are not uncommon within this group of organisms. The symbiotic cyanobacterium *Nostoc punctiforme* is able to use N_2 as the nitrogen source and to grow heterotrophically using sugars as carbon source.[1] In plant-cyanobacteria symbiosis, the cyanobacterium, which has low photosynthetic activity, provides the plant with fixed nitrogen, while the plant supplies the cyanobacterium with sugars.[2]

We recently characterized a glucose-specific permease (GlcP) as being necessary for the cyanobacterium *N. punctiforme* to form symbiosis with the plant *Anthoceros punctatus*.[3] The key observation of our work was that mutants of *N. punctiforme* in which glucose uptake was abolished did not infect *A. punctatus*. The gene encoding GlcP is part of a cluster, *frtA1-frtA2-frtB-frtC-glcP—oprB*, in which at least the *frt* genes and *glcP* may be co-transcribed. The *frt* genes encode an ABC-type transporter for fructose, and *oprB* encodes an outer membrane sugar porin. We did not determine, however, the exact role of GlcP in symbiosis or its regulation. Possibly the transporter has a role in the mature symbiosis, by taking up sugars supplied by the plant for nutrition, but a second role could be in chemotaxis.

Glucose-Supported Growth

When grown in light in the presence of either fructose or glucose, the growth yield of the *N. punctiforme* wild type increased (**Fig. 1**). In contrast, mutant strain CSME1A, which exhibits minimal transport activity of both glucose and fructose,[3] showed the lowest growth yields, and strain CSME11, which has a low glucose transport activity but retains a partial activity of fructose transport,[3] showed a limited increase in growth yield specifically with fructose. On the other hand, glucose increased the yield of the strain overexpressing *glcP* (CSME1B). These results

indicate that in free-living cultures of *N. punctiforme* GlcP is not expressed at levels permitting the maximal growth that would be possible with glucose, and likely that GlcP is not induced by this sugar.

The Frt transporter is neither significantly induced by fructose in *N. punctiforme* (J. C. Meeks, personal communication). In contrast, the Frt transporters of the non-symbiotic cyanobacteria *Anabaena variabilis* and *Nostoc* sp. strain PCC 7107.are induced by fructose.[4,5] The regulation of the sugar transporters GlcP and Frt in *N. punctiforme* is probably adapted to symbiosis. The transporters could be regulated by a symbiosis-specific mechanism (via a plant derived molecule different from glucose), which may include the nearby located *hrm* genes that are involved in hormogonia differentiation.[6]

Does GlcP have a Role in Chemotaxis?

Previous studies have shown that inactivation of various genes involved in N_2 fixation results in *N. punctiforme* still forming colonies in the plant partner that are however unable to provide the plant with fixed nitrogen.[6] This is in contrast to the *glcP* mutants, which formed no visible symbiotic colonies, implying that GlcP has a role during the establishment of the symbiosis. The fact that inactivation of *glcP* does not impair hormogonia differentiation or motility may imply that the early role of GlcP is related to chemotaxis. Indeed, *Nostoc* is known to be chemotactic toward glucose but not fructose,[7] and N-stressed glands of *Gunnera* plants are enriched in soluble sugars prior to symbiosis.[8] Genes encoding homologs of *E. coli* methyl-accepting chemotaxis proteins (MCPs) are present in *N. punctiforme*, but gene inactivation studies have failed to identify any of these MCPs as involved in the symbiotic chemotaxis response (J. C. Meeks, personal communication). GlcP belongs to the Major Facilitator Superfamily (MFS) type of transporters. Chemotaxis

dependent on MFS transporters was previously shown for *Pseudomonas putida* and *Ralstonia eutropha*.[9,10] In both organisms, the transporter-encoding genes cluster together with genes encoding metabolic enzymes of the transported compound, similar to the *hrm* genes that are located immediately upstream of the *frt-glcP* genes in *N. punctiforme*.[6] This could suggest the involvement of a metabolic intermediate as an intracellular signal, i.e., metabolic-dependent chemotaxis.

Evolutionary Considerations

To further investigate the biological context of GlcP, we searched for homologs in other bacteria, including four symbiotic cyanobacteria whose genomes were recently sequenced, the symbiont of *Azolla*, *Nostoc azollae* 0708, two symbionts of diatoms, *Richelia intracellularis* and *Calothrix rhizosoleniae* SC01, and the symbiont of a prymnesiophyte, UCYN-A.[11-13] A Blast search against the nr database showed that the top 12 scoring sequences, with 64–84% sequence identity, were all cyanobacterial (**Table 1**), while four other cyanobacterial sequences showed 53–55% sequence identity. Remaining cyanobacterial hits had 20–30% sequence identity. Hits for the four symbiotic cyanobacteria were, if at all present, found in this low scoring group (**Table 1**). It thus appears that the presence or absence of GlcP is not by itself related to symbiotic competence, and that sugar uptake mechanisms are diverse in cyanobacterial symbionts. Indeed, a glucose transporter different from GlcP was recently characterized in marine picocyanobacteria.[14]

Except for the 12 cyanobacterial sequences, most homologous sequences were retrieved from Deltaproteobacteria, Actinobacteria, and Gammaproteobacteria. These sequences also show considerable sequence similarity (50–68% identity), and thus GlcP appears to be a widespread and conserved type of transporter. We constructed a phylogenetic tree over the 100 sequences with highest sequence similarity to *N.*

punctiforme GlcP. The top scoring cyanobacterial sequences all cluster together and may have been acquired from Deltaproteobacteria (**Fig. 2**). The strains containing these GlcP sequences do however not cluster together in the cyanobacterial 16S phylogenetic tree (compare for example with Shih et al.),[15] but are instead found in several distantly related branches. The presence and distribution of GlcP in cyanobacteria thus likely reflect events of intra-phylum horizontal gene transfer and possibly gene loss, with an unknown contribution of each of these mechanisms. Two additional independent acquisitions may have happened, one taking a gammaproteobacterial protein into *Moorea producens* and another taking an actinobacterial protein into *Synechococcus/Prochlorococcus*.

The GlcP permease can have a nutritional role, facilitating heterotrophic or mixotrophic growth, in most cyanobacteria in which it is present, and additionally appears to have been recruited to accomplish symbiotic functions in *N. punctiforme.* These adaptations seem to have included changes in regulatory patterns, since the regulation of GlcP appears to differ between *N. punctiforme* and the non-symbiotic cyanobacteria.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

<jrn>1. Meeks JC, Campbell EL, Summers ML, Wong FC. Cellular differentiation in the cyanobacterium *Nostoc punctiforme*. Arch Microbiol 2002; 178:395-403; PMID:12420158; http://dx.doi.org/10.1007/s00203-002-0476-5</jrn>

<edb>2. Bergman B, Ran L, Adams DG. Cyanobacterial-plant symbiosis: signal and development. *In* A Herrero, E Flores, eds, The Cyanobacteria: Molecular Biology, Genomics and Evolution, Caiser Academic Press, Norfolk, UK; 2008: pp 447-473.</edb>

<jrn>3. Ekman M, Picossi S, Campbell EL, Meeks JC, Flores E. A *Nostoc punctiforme* sugar transporter necessary to establish a Cyanobacterium-plant symbiosis. Plant Physiol 2013; 161:1984-92; PMID:23463784; http://dx.doi.org/10.1104/pp.112.213116</jrn>

<jrn>4. Ungerer JL, Pratte BS, Thiel T. Regulation of fructose transport and its effect on fructose toxicity in *Anabaena* spp. J Bacteriol 2008; 190:8115-25; PMID:18931119; http://dx.doi.org/10.1128/JB.00886-08</jrn>

<jrn>5. Schmetterer G, Flores E. Uptake of fructose by the cyanobacterium *Nostoc* sp. ATCC 29150. Biochim Biophys Acta 1988; 942:33-7; http://dx.doi.org/10.1016/0005-2736(88)90271-4</jrn>

<jrn>6. Meeks JC. Molecular mechanisms in the nitrogen-fixing *Nostoc*-bryophyte symbiosis. Prog Mol Subcell Biol 2006; 41:165-96; PMID:16623394; http://dx.doi.org/10.1007/3-540-28221-1_9</jrn>

<jrn>7. Nilsson M, Rasmussen U, Bergman B. Cyanobacterial chemotaxis to extracts of host and nonhost plants. FEMS Microbiol Ecol 2006; 55:382-90; PMID:16466377; http://dx.doi.org/10.1111/j.1574-6941.2005.00043.x</jrn>

<jrn>8. Khamar HJ, Breathwaite EK, Prasse CE, Fraley ER, Secor CR, Chibane FL, Elhai J, Chiu WL. Multiple roles of soluble sugars in the establishment of *Gunnera-Nostoc* endosymbiosis. Plant Physiol 2010; 154:1381-9; PMID:20833727; http://dx.doi.org/10.1104/pp.110.162529</jrn>

<jrn>9. Ditty JL, Harwood CS. Conserved cytoplasmic loops are important for both the transport and chemotaxis functions of PcaK, a protein from *Pseudomonas putida* with 12 membrane-spanning regions. J Bacteriol 1999; 181:5068-74; PMID:10438780</jrn>

<jrn>10. Hawkins AC, Harwood CS. Chemotaxis of *Ralstonia eutropha* JMP134(pJP4) to the herbicide 2,4-dichlorophenoxyacetate. Appl Environ Microbiol 2002; 68:968-72; PMID:11823246; http://dx.doi.org/10.1128/AEM.68.2.968-972.2002</jrn>

<jrn>11. Ran L, Larsson J, Vigil-Stenman T, Nylander JA, Ininbergs K, Zheng WW, Lapidus A, Lowry S, Haselkorn R, Bergman B. Genome erosion in a nitrogen-fixing vertically transmitted endosymbiotic multicellular cyanobacterium. PLoS One 2010; 5:e11486; http://dx.doi.org/10.1371/journal.pone.0011486; PMID:20628610</jrn>

<jrn>12. Hilton JA, Foster RA, Tripp HJ, Carter BJ, Zehr JP, Villareal TA. Genomic deletions disrupt nitrogen metabolism pathways of a cyanobacterial diatom symbiont. Nat Commun 2013; 4:1767; http://dx.doi.org/10.1038/ncomms2748; PMID:23612308</jrn>

<jrn>13. Thompson AW, Foster RA, Krupke A, Carter BJ, Musat N, Vaulot D, Kuypers MM, Zehr JP. Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. Science 2012; 337:1546-50; PMID:22997339; http://dx.doi.org/10.1126/science.1222700</jrn>

<jrn>14. Muñoz-Marín MdelC, Luque I, Zubkov MV, Hill PG, Diez J, García-Fernández JM. *Prochlorococcus* can use the Pro1404 transporter to take up glucose at nanomolar concentrations in the Atlantic Ocean. Proc Natl Acad Sci U S A 2013; 110:8597-602; PMID:23569224; http://dx.doi.org/10.1073/pnas.1221775110</jrn>

<jrn>15. Shih PM, Wu D, Latifi A, Axen SD, Fewer DP, Talla E, Calteau A, Cai F, Tandeau de Marsac N, Rippka R, et al. Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. Proc Natl Acad Sci U S A 2013; 110:1053-8; PMID:23277585; http://dx.doi.org/10.1073/pnas.1217107110</jrn>

Figure 1. Response of *Nostoc punctiforme* to sugars. Yields of cultures of *Nostoc punctiforme* (orange bars) and mutants CSME1A (Δ*frt*::C.K3; gene cassette in opposite orientation to the *frt-glcP* operon) (red bars), CSME11 (Δ*glcP*-Δ*oprB*::C.K3) (blue bars), and CSME1B (Δ*frt*::C.K3; gene cassette in the same orientation as the *frt-glcP* operon, promoting overexpression of *glcP*) (black bars) after growth in liquid BG11 (nitrate-containing) medium for 10 d at 30 °C in the light (about 25 μmol m^{-2} s^{-1}), in the absence or presence of 10 mM fructose or glucose as indicated. See Ekman et al. (2013) for a detailed description of the mutants.

Figure 2. Phylogenetic tree of the 100 top scoring sequences in GlcP Blast search against the nr database (NCBI). The sequences were aligned with Muscle software (http://www.drive5.com/muscle/) using default settings. The resulting alignments were used for constructing an approximately-maximum-likelihood phylogenetic tree with the Fasttree software using default settings (http://www.microbesonline.org/fasttree/). The tree was rooted with the *E. coli* LacY transporter, an MFS transporter of a different type (family) than GlcP. Cyanobacterial strains are shown in black text while clusters of other bacterial phyla are collapsed and shown in color. *In addition to 49 actinobacterial sequences this cluster also contains six gammaproteobacterial and one firmicutes sequence. **In addition to 17 gammaproteobacterial sequences this cluster also contains one verrucomicrobial sequence.

Table 1. Cyanobacteria with GlcP homologs and the highest scoring GlcP Blast hits in symbiotic cyanobacteria. (S.I., sequence identity.)

Strain	Annotation	Query cover	E-value	S.I.	Acces. No.
Top cyanobacterial Blast hits					
Nostoc punctiforme PCC 73102	sugar transporter	100%	0	100 %	YP_001868585.1
Fischerella muscicola	major facilitator transporter	100%	0	88	WP_016862018.1
Crinalium epipsammum PCC 9333	sugar transporter	99%	0	84%	YP_007144676.1
Synechocystis sp. PCC 7509	MFS transporter, sugar porter family	99%	0	83%	WP_009632434.1
Nodosilinea nodulosa	major facilitator transporter	99	0	80%	WP_017297719.1
Cyanothece sp. PCC 7822	sugar transporter	99%	0	74%	YP_003899859.1
Chlorogloeopsis	major facilitator transporter	100	0	74%	WP_016876260.1
Oscillatoria acuminata PCC 6304	sugar family MFS transporter	98%	0	74%	YP_007088081.1
Microcoleus sp. PCC 7113	sugar family MFS transporter	94%	0	76%	YP_007119491.1
Lyngbya sp. PCC 8106	major facilitator transporter	98%	0	72%	WP_009784998.1
Synechocystis sp. PCC 6803	unnamed protein product	99%	0	71%	CAA34119.1
Gloeocapsa sp. PCC 73106	MFS transporter, sugar porter family	98%	0	69%	WP_006529968.1
Moorea producens	major facilitator transporter	98%	0	64%	WP_008188284.1
Prochlorococcus marinus str. MIT 9303	hypothetical protein	99%	3E-172	55%	YP_001017959.1
Synechococcus sp. RS9917	major facilitator transporter	100%	1E-160	55%	WP_007100383.1
Synechococcus sp. CC9311	major facilitator transporter	97%	1E-145	56%	WP_011618711.1
Synechococcus sp. WH 8016	sugar transporter	41%	2E-76	61%	WP_006854589.1
Synechococcus sp. WH 8016	general substrate transporter	52%	1E-64	53%	WP_006854590.1
Symbiotic cyanobacterial Blast hits					
'Nostoc azollae' 0708	EmrB/QacA transporter	25%	7e-07	30%	YP_003721762.1
'Nostoc azollae' 0708	major facilitator superfamily protein	32%	0.022	24%	YP_003721661.1
cyanobacterium UCYN-A	glycosyl transferase	6%	0.13	42%	YP_003421499.1
cyanobacterium UCYN-A	arabinose efflux permease family protein	21%	0.34	26%	YP_003421471.1

Figure 1

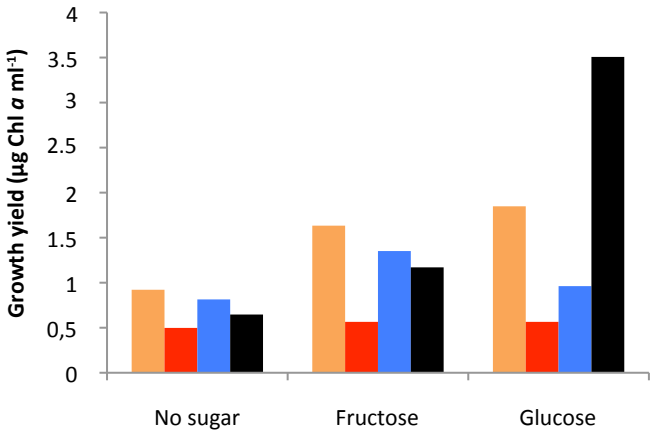
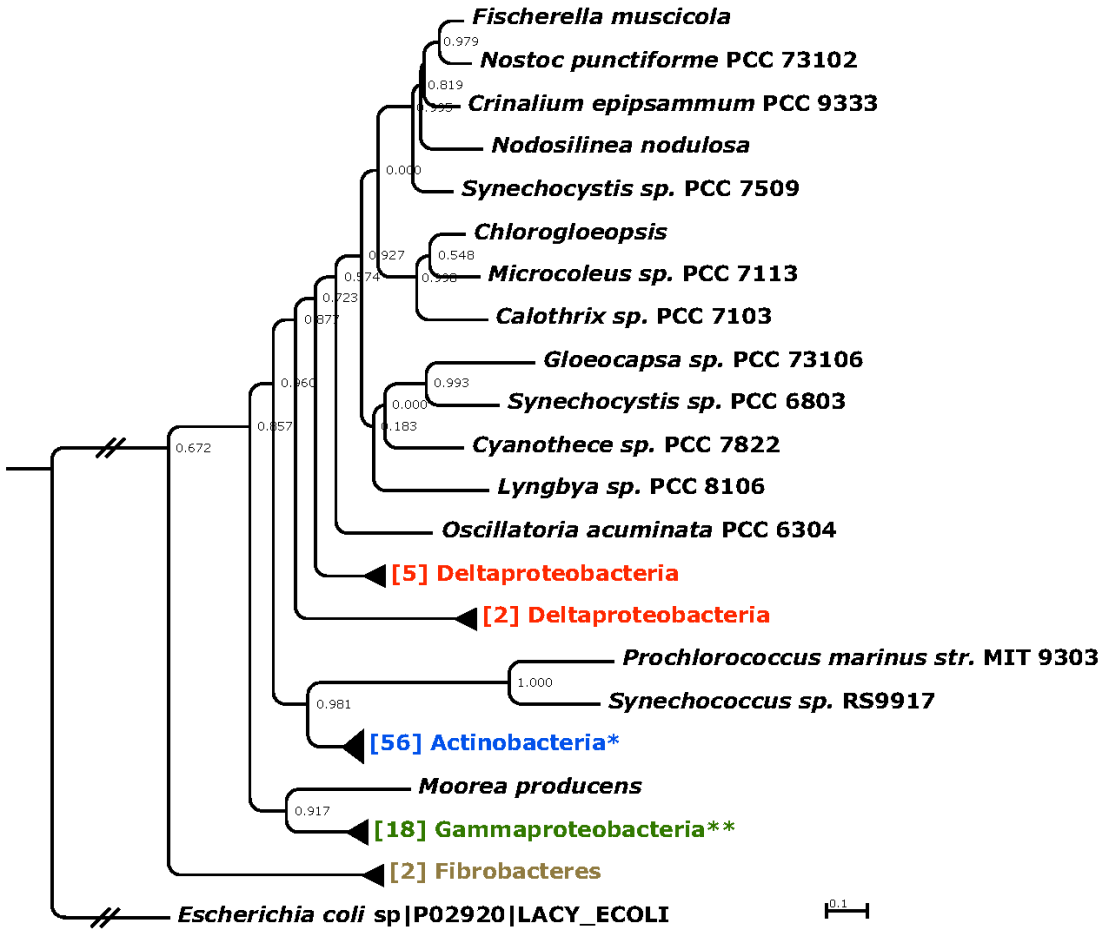

Growth yield (µg Chl *a* ml^{-1})
4
3.5
3
2.5
2
1.5
1
0,5
0
No sugar
Fructose
Glucose

Figure 2


Fischerella muscicola
0.979
Nostoc punctiforme PCC 73102
0.819
Crinalium epipsammum PCC 9333
Nodosilinea nodulosa
0.000
Synechocystis sp. PCC 7509
Chlorogloeopsis
0.927
0.548
Microcoleus sp. PCC 7113
0.574
0.723
0.877
Calothrix sp. PCC 7103
Gloeocapsa sp. PCC 73106
0.960
0.993
Synechocystis sp. PCC 6803
0.857
0.000
0.183
Cyanothece sp. PCC 7822
0.672
Lyngbya sp. PCC 8106
Oscillatoria acuminata PCC 6304
[5] Deltaproteobacteria
[2] Deltaproteobacteria
Prochlorococcus marinus str. MIT 9303
1.000
0.981
Synechococcus sp. RS9917
[56] Actinobacteria*
Moorea producens
0.917
[18] Gammaproteobacteria**
[2] Fibrobacteres
Escherichia coli sp|P02920|LACY_ECOLI
0.1