Interaction of Azospirillum lipoferum with Wheat Germ Agglutinin Stimulates Nitrogen Fixation

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In vitro, the nitrogen fixation capability of A. lipoferum is efficiently increased in the presence of wheat germ agglutinin (WGA). A putative WGA-binding receptor, a 32-kDa protein, was detected in the cell capsule. The stimulatory effect required N-acetyl-d-glucosamine dimer (GlcNAc di) terminated sugar side chains of the receptor and was dependent on the number of GlcNAc di links involved in receptor-WGA interface. Binding to the primary sugar binding sites on WGA had a larger stimulatory effect than binding to the secondary sites. The WGA-receptor complex generated stimulus led to elevated transcription of the nifH and nifA genes and of the glnA gene cluster but not of the glnB gene from its own promotor. There may well be a signalling cascade contributing to the regulation of nitrogen fixation.

Biological nitrogen fixation is one of the most important processes in the natural environment: it is the major pathway for the reduction of dinitrogen molecules from air to give ammonia and subsequently glutamine and other nitrogen-containing molecules. Diazotrophic Azospirillum lipoferum enters into associative symbiosis with the roots of several cereals (rice, maize, wheat, and sorghum), tomato, legumes, etc. (16). Under conditions of low oxygen tension and ammonia limitation, bacteria fix nitrogen both in association with roots and in the free-living state (35). A. lipoferum is considered as a potential phytostimulator, since it significantly increases plant growth. Incompatible with nitrogen fixation (30). NifA activity is modulated by the PII protein (encoded by glnB), the intracellular signal transmitter, in response to the nitrogen status of the cell. glnB is clustered with glnA, the structural gene of glutamine synthetase (GS), required for ammonia assimilation. glnB is expressed under control of three nitrogen-regulated promoters, glnBr, glnBp2, and glnApr, active in nitrogen access, nitrogen fixation, and ammonia assimilation, respectively (reviewed in reference 11). In A. lipoferum, the nifHK operon under NifA-like control has been identified (17), and the glnB gene, contiguous with glnA, has been described. However, the PII protein has not yet been characterized (reviewed in reference 15).

In this report, we describe the specificity of the recognition event between WGA and the WGA-binding receptor on the cell surface of A. lipoferum. We also report evidence of an enhanced nitrogen fixation capacity of the bacterium, as a consequence of this interaction. Several target genes of the

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WGA-induced stimulus (nifH, nifA, and glnB) were identified by enhanced expression of the corresponding promoter-lacZ fusions. A preliminary characterization of the putative capsular WGA-binding receptor is reported.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. A. lipoferum SpBr17R is rifampicin-resistant derivative of SpBr17, the wild-type strain (46). SpBr17R: Tn2706 (Rif’ Cm’) is a WGA nonbinding (WGA−) mutant strain. A. brasilense Sp7 (46), Azotobacter vinaldelii UW136 (14), and Rhizobium phymatum UNF122 (24) are wild-type strains. pAW1142: Tn2706 (Cm’ Te’) (40) was used as a transposon donor for mutagenesis. pRK2013 (Kmr) is a WGA nonbinding (WGA−) mutant strain. A. brasilense Sp7 (46), Azotobacter vinaldelii UW136 (14), and Rhizobium phymatum UNF122 (24) are wild-type strains. pAW1142: Tn2706 (Cm’ Te’) (40) was used as a transposon donor for mutagenesis. pRK2013 (Kmr) is a WGA nonbinding (WGA−) mutant strain. A. brasilense Sp7 (46), Azotobacter vinaldelii UW136 (14), and Rhizobium phymatum UNF122 (24) are wild-type strains. A. lipoferum SpBr17R by triparental plate mating using the pRK2013 helper plasmid.

RESULTS

Isolation of a WGA− strain of A. lipoferum. A. lipoferum was defective for FITC-WGA binding was isolated from a Tn2706-mutagenized population of A. lipoferum SpBr17R. FITC-WGA-stained preparations of the wild-type and mutant strains are shown in Fig. 1A and B. In the wild-type cells, binding of FITC-WGA produced fluorescence mostly coupled, without polarity, to the cell capsules. Up to 96% of the cell population was positively stained. In contrast, the WGA− strain showed an FITC-WGA dark phenotype, with less than 1% of the cell population showing dim fluorescence. The mutant strain was Luria-Bertani broth (42). Minimal medium for A. lipoferum cultures were selected by determining the number of CFU per milliliter in plating assays. Chloramphenicol, tetracycline, rifampicin, and kanamycin were used at concentrations of 10, 20, 30, and 50 μg/ml, respectively.

Random Tn2706 mutagenesis. pAW1142: Tn2706 was introduced into A. lipoferum SpBr17R by triparental plate mating using the pRK2013 helper plasmid. A WGA− strain was selected from among the fluorescein isothiocyanate (FITC-) WGA-stained cells. The stimulatory effect of WGA on the cell surface (48). The effect of WGA on the cell surface (48).

Detection of capsular WGA-binding components. Capsules of 3-day-old cells, grown on solid minimal medium, were suspended in phosphate-buffered saline (PBS), incubated with lectins (40 μg of lectin/10^7 cells/ml) for 30 min at 37°C, and then washed in PBS. Lectin binding was quantified (Perkin-Elmer MFP 44B fluorimeter) by measuring mean epifluorescence at 525 nm (E_{525}) (10^7 cells/ml). WGA-Neu and WGA-Glc were obtained by incubation of WGA with a molar excess of NeuNAc or GlcNAc at 30°C for 1 h, and unbound ligand was removed by molecular sieving on Sephadex G-25 gels.

Stimulation of nitrogenase activity by WGA. The effect of purified WGA in vitro on nitrogen fixation of the wild-type and WGA− strains was investigated (Fig. 1C and D). In the absence of WGA, a similar optimum for nitrogen fixation was determined at 0.5% oxygen, both in the wild type and in the WGA− strain. Under lower oxygen tensions, the nitrogen-fixing capacity decreased. The optimum oxygen requirement for nitrogen fixation was not modified by addition of WGA (data not shown).

In the presence of WGA, wild-type bacteria produced a maximal nitrogenase activity, 4.7-fold higher than that in the absence of WGA. The greatest stimulation was during the exponential phase of growth. The stimulatory effect of WGA was dose dependent, and maximal stimulation was obtained

TABLE 1. Plant lectins

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Plant source</th>
<th>Recognized terminal sugar residue of the cognate receptor</th>
<th>Reference or description</th>
</tr>
</thead>
<tbody>
<tr>
<td>WGA</td>
<td>Triticum vulgare</td>
<td>α(2→3)- or α(2→6)-linked NeuNAc</td>
<td>1, 38</td>
</tr>
<tr>
<td>WGA-Neu</td>
<td>None</td>
<td>GlcNAc&lt;sub&gt;β&lt;/sub&gt;</td>
<td>WGA primary sugar-binding sites saturated with NeuNAc&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WGA-Glc</td>
<td>None</td>
<td>GlcNAc&lt;sub&gt;β&lt;/sub&gt;</td>
<td>WGA primary and secondary sugar-binding sites saturated with GlcNAc&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DSA</td>
<td>Datura stramonium</td>
<td>GlcNAc&lt;sub&gt;β&lt;/sub&gt;</td>
<td>6</td>
</tr>
<tr>
<td>SNA</td>
<td>Sambucus nigra</td>
<td>NeuNAc&lt;sub&gt;α&lt;/sub&gt;-Gal&lt;sub&gt;β&lt;/sub&gt;</td>
<td>44</td>
</tr>
<tr>
<td>MAA</td>
<td>Maackia amurensis</td>
<td>GlcNAc&lt;sub&gt;α&lt;/sub&gt;-Gal&lt;sub&gt;β&lt;/sub&gt;</td>
<td>47</td>
</tr>
<tr>
<td>GMA</td>
<td>Glycine max</td>
<td>GalNAc, α-lactosyl-</td>
<td>31</td>
</tr>
<tr>
<td>LCA</td>
<td>Lens culinaris</td>
<td>α-Mannosyl-α(1→2)Gal</td>
<td>25</td>
</tr>
<tr>
<td>UEAI</td>
<td>Ulex europaeus</td>
<td>α-Fucosyl-α(1→2)Gal</td>
<td>22</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Materials and Methods. DSA, Datura stramonium agglutinin; SNA, Sambucus nigra agglutinin; MAA, Maackia amurensis agglutinin; GMA, Glycine max agglutinin; LCA, Lens culinaris agglutinin; UEAI, Ulex europaeus agglutinin I; GalNAc, N-acetyl-β-D-galactosamine; Gal, D-galactose.
with 1 μg of WGA/10^7 cells/ml (data not shown). Stimulation with WGA did not increase the nitrogen-fixing capacity of the WGA^− strain. Under conditions of nitrogen fixation, the wild-type and WGA^− strains grew similarly but slowly, and their growth rate was not affected by addition of WGA (Fig. 1C and D). Presumably, the poor growth was due to the oxygen-depleted conditions of the cultures.

**Involvement of terminal sugar residues of the cell surface in the stimulation of nitrogen fixation by lectin.** Binding of lectins to the bacterium is essentially mediated by terminal sugar residues of the corresponding receptors on the cell surface. Terminal sugar residues on the surface of the wild-type and WGA^− cells were surveyed by a differential lectin binding assay, using FITC-labeled lectins with different sugar-binding specificities (Table 1). All lectins tested except UEAI, were able to attach to the wild-type cells, as assessed by epifluorescence microscopy, and no agglutination of cells was observed (at 1 μg of lectin/10^7 cells/ml) (data not shown). Consequently, terminal GlcNAcα, terminal α(2→3)- and/or α(2→6)-linked NeuNAc, GalNAc, and/or α-lactosyl-, α-mannosyl-, and/or α-glucosyl- but not 1-fucosyl-α(1→2)-linked D-galactose (Gal) residues are present on the cell surface. The specific fluorescence intensities (E525/10^7 cells/ml) of the cells treated with WGA, SNA, and MAA were comparable (Table 2). The fluorescence levels obtained with DSA and WGA-Neu were 23 and 66% lower, respectively, than that with WGA. No fluorescence was observed with WGA-Glc, as expected. The cell surface of the WGA^− strain was similarly probed with FITC-lectins. WGA, WGA-Neu, WGA-Glc, and DSA were not able to bind to the cells, whereas SNA and MAA showed a level of

![FIG. 1. Binding of WGA to A. lipoferum cells (A and B) and effect of WGA stimulus on nitrogen fixation (C and D). (A and B) FITC-WGA-stained wild-type (A) and WGA^− (B) cells visualized by epifluorescence microscopy (approximately ×3 × 10^3). (C and D) Time course of nitrogenase activity in the presence of WGA. After 2 h of nitrogenase derepression in nitrogen-free medium at 0.5% of oxygen, WGA (1 μg/ml) and acetylene (10% [vol/vol]) were added (time zero). Nitrogenase activity (nanomoles of C2H4/10^7 cells/milliliter) in the presence (●) and absence (▲) of WGA. ×, growth curve (growth is expressed in 10^7 cells/milliliter). The standard deviation was less than 12% of the value in each case. The reported values are means of three independent assays.](image)

**TABLE 2. Lectin-binding capacities of wild-type and WGA − strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Specific fluorescence intensity (E_{525}/10^7 cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WGA</td>
</tr>
<tr>
<td>SpBr17R (wild type)</td>
<td>924 ± 74</td>
</tr>
<tr>
<td>WGA</td>
<td>35 ± 4</td>
</tr>
</tbody>
</table>

*Three-day-old cells grown on solid minimal medium were stained with FITC-labeled lectins (at 40 μg/10^6 cells/ml). In the absence of lectin, the background fluorescence intensity was 15. The values are means of three independent assays.*
binding comparable to that obtained with the wild-type strain (Table 2).

In view of its specificity for sugar moieties (Table 1), WGA is linked at both sugar-binding sites to GlcNAc on the wild-type strain. Note that at the primary sugar-binding sites, linkage to terminal NeuNAc can also be established. WGA-Neu and DSA attach exclusively to GlcNAc, and SNA and MAA attach to terminal NeuNAc residues. As the WGA \textsuperscript{2} strain interacts with SNA and MAA but not with WGA, WGA-Neu, and DSA (Table 2), it probably possesses structurally modified or no GlcNAc, but has intact terminal NeuNAc residues on the cell surface.

Next, we attempted to identify which lectin-terminal sugar contact is involved in the stimulation of nitrogen fixation. Nitrogenase activities of the wild-type strain induced by the different lectin stimuli were determined (Fig. 2). The highest (4.4-fold) increase of nitrogenase activity was obtained by WGA, and lower but significant effects were observed after WGA-Neu and DSA stimulation (1.7- and 2.7-fold, respectively). SNA and MAA had no stimulatory effect on the nitrogenase activity. These results suggest that the GlcNAcdi residue of the cell surface is most probably involved in the stimulation of nitrogen fixation. Moreover, binding of GlcNAcdi to the secondary sugar binding site of WGA alone (tested with WGA-Neu) stimulated the nitrogen fixation, but to only 38% of the level resulting from WGA attached by both sugar-binding sites to the cell surface (Fig. 2). In addition, no subsequent increase in nitrogen fixation was produced by GMA and LCA, i.e., through GalNAc-\alpha-lactosyl- and \alpha-mannosyl-\alpha-glucosyl-linkages, respectively (data not shown).

In the WGA$^{-}$ strain, stimulation with neither WGA (Fig. 1D) nor WGA-Neu and DSA (data not shown) increased the nitrogen fixation. Thus, the defect in lectin binding (Table 2) was consistent with the absence of nitrogenase stimulation.

In addition to \textit{A. lipoferum}, the effects of WGA, DSA, and SNA on the nitrogen fixation of \textit{A. brasilense}, \textit{Azotobacter vinelandii}, and \textit{K. pneumoniae} were assayed. The nitrogenase stimulation rate and lectin-binding capacity of \textit{A. brasilense} Sp7 for WGA, DSA, and SNA were similar to those of \textit{A. lipoferum} (Table 3). In contrast, no change in nitrogenase activity of \textit{Azotobacter vinelandii} UW136 and \textit{K. pneumoniae} UNF122 was detected with the same lectin stimuli (data not shown).

**Stimulation of nifH, nifA, glnB, and glnA expression by lectin binding.** Next, we tested whether the lectin stimuli exert a regulatory effect through modulation of the transcription of \textit{nifH}, \textit{nifA}, \textit{glnB}, and \textit{glnA} genes. Promoter fusions \textit{nifH-lacZ}, \textit{nifA-lacZ}, \textit{glnBA-lacZ}, and \textit{glnA-lacZ} (plasmids pAB358, pAB576, pAB904, and pAB912, respectively) (12, 30) were introduced into the wild-type and WGA$^{2}$ strains. The beta-galactosidase activities of the plasmid-borne fusions were monitored upon stimulation with WGA, WGA-Neu, DSA, SNA, and MAA, under conditions of nitrogen fixation. For the wild-type strain (Fig. 2), the lectins linked to GlcNAc residues of the

![FIG. 2. Nitrogenase activity and expression of N-regulated genes in the wild-type strain in the presence of lectins. Lectins (at 1 \mu g/10^7 cells/ml) were added after 2 h of nitrogenase derepression. Nitrogenase activity (nanomoles of C_2H_4/10^7 cells/milliliter) and beta-galactosidase activity (Miller units/milligram of protein) were measured 4 h after addition of WGA. Error bars indicate the standard deviation of the mean. The standard deviation of nitrogenase activity was less than 13% of the value in each case. The values are means of three independent experiments.](https://jb.asm.org/content/131/14/3952.full.png)

**TABLE 3. Nitrogen fixation in the presence of lectins and lectin binding capacity of \textit{A. brasilense} Sp7.**

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Nitrogenase activity$^a$ (nmol of C_2H_4/10^7 cells/ml)</th>
<th>Specific fluorescence intensity$^b$ (E_525/10^7 cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1,018 ± 100</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>WGA</td>
<td>3,767 ± 452</td>
<td>1,116 ± 129</td>
</tr>
<tr>
<td>DSA</td>
<td>2,239 ± 300</td>
<td>1,328 ± 185</td>
</tr>
<tr>
<td>SNA</td>
<td>928 ± 102</td>
<td>1,273 ± 162</td>
</tr>
</tbody>
</table>

$^a$ Assayed as described elsewhere (21). Lectins (at 1 \mu g/10^7 cells/ml) were added after 2 h incubation under conditions required for nitrogen fixation, and activity was measured 4 h after addition of lectins.

$^b$ Three-day-old cells grown on solid medium were stained with FITC-labeled lectins (at 40 \mu g/10^6 cells/ml). The values are means of three independent assays.
cell surface (WGA, WGA-Neu, and DSA) increased the expression of nifH-lacZ, nifA-lacZ and glnBA-lacZ fusions. β-Galactosidase activities obtained with WGA, WGA-Neu, and DSA stimuli were 2.9-, 1.8-, and 1.8-fold (nifH-lacZ), 5.7-, 3.3-, and 5.2-fold (nifA-lacZ), and 2.6-, 1.5-, and 2.1-fold (glnBA-lacZ), respectively, higher than the corresponding unstimulated β-galactosidase activities. Expression of glnA-lacZ was not enhanced by any lectin stimuli. Neither of the NeuNAc-specific lectins (SNA and MAA) had an effect on the transcription of any fusion. No increased transcription of the promoter specific lectins (SNA and MAA) had an effect on the transcription of the promoter.

TABLE 4. Nitrogen fixation in the presence of WGA in wild-type and WGA− strains carrying plasmid-borne extra copies of nifA (pAB53) and glnB (pAB914) genes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nitrogenase activitya (nmol of C2H4/107 cells/ml)</th>
<th>−WGA</th>
<th>+WGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpBr17R (wild type)</td>
<td>716 ± 55</td>
<td>2,019 ± 170</td>
<td></td>
</tr>
<tr>
<td>SpBr17R(pAB53)</td>
<td>1,520 ± 142</td>
<td>1,600 ± 122</td>
<td></td>
</tr>
<tr>
<td>SpBr17R(pAB914)</td>
<td>1,704 ± 105</td>
<td>1,533 ± 86</td>
<td></td>
</tr>
<tr>
<td>WGA</td>
<td>663 ± 61</td>
<td>624 ± 43</td>
<td></td>
</tr>
<tr>
<td>WGA (pAB53)</td>
<td>1,459 ± 157</td>
<td>1,436 ± 162</td>
<td></td>
</tr>
<tr>
<td>WGA (pAB914)</td>
<td>1,597 ± 127</td>
<td>1,653 ± 113</td>
<td></td>
</tr>
</tbody>
</table>

* Measured in the absence of WGA or 4 h after addition of WGA (1 μg/107 cells/ml) after 2 h of nitrogen derepression in nitrogen-free medium at 0.5% oxygen. The values are means of three independent assays.

was mainly attached to the cell capsules. We attempted to identify the capsular proteins including the WGA-binding components. The solubilized capsular material was separated by SDS-PAGE (Fig. 3A). The protein profiles of the wild-type and WGA− strains were comparable. Six distinct polypeptides were revealed. On the basis of their intensity, the 32-kDa band might correspond to a doublet of two closely comigrating polypeptides. The 45- and >116-kDa polypeptides are comparable in size to the 45- and 110-kDa protein components of the lateral and polar flagella of A. brasilense, respectively (5). A Western blot of the protein patterns was probed with DIG-conjugated WGA (Fig. 3B). WGA-binding signal was detected only in the wild-type and not in the WGA− preparation. This single WGA-binding component was identified as a 32-kDa polypeptide.

The WGA-binding component from capsule preparation of the wild-type strain was isolated by WGA affinity column chromatography. Both flowthrough and eluted fractions of the capsule preparation, separated by WGA-agarose matrix, were analyzed by SDS-PAGE (Fig. 3C). In agreement with the results of the DIG immunodetection experiment, a capsule component of a 32-kDa polypeptide was specifically retained in eluted fractions 2 and 3 but was no longer detected in the flowthrough fraction. In contrast, the 32-kDa protein of the capsule preparation of WGA− strain was obtained in the flowthrough fraction, without any specific retention by the WGA matrix (Fig. 3C). Consequently, the 32-kDa polypeptide may be a component of the WGA-binding receptor on the cell surface of A. lipoforum.

**DISCUSSION**

In this report we demonstrate that two GlcNAc-specific lectins, WGA and DSA, are able to stimulate the nitrogen-fixing capacity of A. lipoforum, presumably after formation of a complex with the corresponding receptor on the bacterium cell surface. The stimulus, which led to the up-regulation of nitrogen fixation, is absent when the lectin-bacterium interaction is prevented, as in the WGA− strain.

Moreover, the stimulus is efficient with plant-associative bacteria, such as A. lipoforum and A. brasilense, but not with the free-living diazotrophs Azotobacter vinelandii and K. pneumoniae. Interestingly, not only WGA but also other lectins of a few additional plants, colonized by Azospirillum (16), bind also to NACGlc (41).

The WGA stimulus elicited an increased expression of nifH-lacZ, nifA-lacZ, and glnBA-lacZ fusions and consequently a higher nitrogenase activity (Fig. 2). Presumably, the stimulus...
from the WGA-receptor complex enhances the transcription of nifA. The elevated level of NifA could, in turn, increase nifH expression. Indeed, extra copies of nifA, introduced into wild-type cells, were also able to enhance the nitrogenase activity (Table 4). Similarly, the WGA-receptor-elicted signal would also trigger the transcription of glnB. An increased level of the P_I protein would improve the conversion of excess NifA protein to its active form. Accordingly, when glnB was overexpressed in the wild-type cells, nitrogenase activity was enhanced (Table 4). Note that the plasmid-borne glnB promoter did not respond to the WGA stimulus (Fig. 2). However, the surplus ammonia, produced by WGA-triggered nitrogen fixation, could be assimilated by an elevated level of GS (12), since the glnB mRNA is synthesized in larger amounts. Both an enhanced nitrogen fixation, induced by the WGA stimulus, and subsequently an increased level of glutamine synthetase (GS) have been reported in A. brasilense (2).

No information is available about lectin-induced bacterial signalling pathways in plant-bacterium associations. In mammalian cells, the binding of WGA triggers cellular recognition, glucose transport and lipolysis, cell growth inhibition, control of morphological states and activation of transcriptional factors, and several lectin-induced signal transducing pathways have been reported (7, 43, 51). The sugar residues of the lectin binding receptors are believed to transmit the signal (20, 27).

The binding specificity of the lectins is generally determined by analyzing terminal sugars of the carbohydrate side chains of the corresponding receptors (22). Our data contribute to understanding the specific chemical recognition between WGA and the putative receptor of A. lipoferum. The GlcNAc\_residues are the specific receptor components at the WGA-receptor binding interface and are responsible for the biologically active stimulus for the nitrogen fixation machinery. Although NeuNAc residues are also present on the cell surface (evidenced by the binding of SNA and MAA) (Table 2), presumably they are not required for the WGA-binding: the WGA-strain binds with SNA and MAA but not WGA, WGA-Neu and DSA. Furthermore, the NeuNAc termini are not sufficient for the stimulation of nitrogen fixation, as shown in the case of the SNA-receptor and MAA-receptor interactions, both in the wild type and WGA strains (Fig. 2). Moreover, binding to the primary and secondary sugar binding sites on WGA were differentively effective in elucidating the biologically active stimulus. The receptor, linked exclusively by the secondary sites of WGA (WGA-Neu–receptor interaction), produced only 38% of the total stimulatory effect (Fig. 2). This agrees with the reduced WGA-Neu-binding capacity of the bacterium (Table 2). The lower level of stimulation may be due to a poorer accessibility of the secondary binding sites in the WGA molecule for the cognate sugar residues of the receptor (49).

The number of molar GlcNAc links between the receptor and WGA modulates several WGA-triggered biological functions (32). From hemagglutination inhibition assays, we estimate that DSA possesses two GlcNAc sites (unpublished data) whereas the WGA molecule has four. Thus, the different stimulation strengths of WGA and DSA (Fig. 2) may be explained by the different number of GlcNAc moieties in the WGA-receptor and DSA-receptor interfaces.

A 32-kDa polypeptide was isolated in vitro, as a putative capsular glycoprotein of WGA-binding receptor. The defective receptor protein of the WGA\_strain has a molecular weight similar to that of the wild type. Thus, the structural gene encoding the receptor protein is most probably not inactivated by the mutation in the WGA\_strain. Possibly the glycosylation pattern of the protein is affected preventing the cognate linkage with the sugar-binding sites on the WGA molecule.

The interaction between Azospirillum and WGA may contribute to the adhesion of the bacteria to the root surface and to establishing a nitrogen-fixing association of improved efficiency with the wheat host. Presumably, the elevated nitrogen fixation capacity of the bacterium is supported by enhanced carbon and energy supply from the host plant.

ACKNOWLEDGMENTS

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