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Aggregation in *Azospirillum brasilense* Cd: Conditions and factors involved in cell-to-cell adhesion

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Abstract

Aggregation of the root-inhabiting, asymbiotic N-fixing *Azospirillum brasilense* Cd (ATCC-29729), was studied. Aggregation occurred towards the end of the exponential phase and during the stationary phase. More aggregates were formed in media supplemented with organic acids than in those containing sugars as a sole carbon source. Maximum growth with no aggregation was obtained in a medium containing both fructose and malate as carbon sources. Aggregation was increased by poly-L-lysine and carbodiimide as well as by increasing the C/N ratio and decreasing combined nitrogen in the growth medium. Aggregates were stable at pH levels of >8 and <6, but dispersed at pH 7.1. Treatment of Azospirillum with NaEDTA resulted in loss of both aggregative capacity and the ability of adsorb to wheat roots without losing cell viability. When extracted bacteria were suspended in their dialysed NaEDTA extract, both their aggregative and adsorptive capacities were restored.

The dialysed NaEDTA extract agglutinated bacterial cells and red blood cells, especially of type O. When the extract was run through a sepharose gel, it separated into three main fractions, of which only one showed agglutinating capacity. Gel electrophoresis of this fraction revealed a single band (MW 97,000) which reacted positively to Schiff's reagent and Coomassie brilliant blue R-250, typical to a glycoprotein. Bacterial agglutination by this fraction was strongly inhibited by D-glucose, melibiose and α-metyl glucoside. No evidence as to the involvement of cellulose fibrils in aggregation was found. It is suggested that glycoprotein(s) and glucose residues located on the outer surface of the cells are involved in aggregation of Azospirillum.

Introduction

Aggregation, or autoagglutination of cells, is a widespread phenomenon (Calleja, 1984). The term flocculation has been used to describe a specific case of aggregation which occurs during growth and involves polysaccharides formation (Sadasivan and Neyra, 1985). Aggregation of asymbiotic N-fixing bacteria such as Azospirillum, Klebsiella and Azotobacter may affect their dispersal, survival in soil, N-fixing capacity and adsorption to plant roots (Madi *et al.*, 1988). Deinema and Zevenhuizen (1971) related flocculation in some gram negative bacteria, including Rhizobium and Azotobacter, to the formation of cellulose fibrils. Sadasivan and Neyra (1985) showed that flocs of Azospirillum were resistant to desiccation; they related flocculation and floc formation in *Azospirillum brasilense* and *A. lipoferum* to β-linked exopolysaccharides, possibly cellulose. Recently, studies of aggregation and pellicle formation in Azospirillum by electron microscopy (Madi *et al.*, 1988) revealed the presence of an extracellular layer in cells from the stationary phase. The purpose of this work was to study the conditions and the nature of factor(s) involved in aggregation of *A. brasilense* Cd and their possible involvement in its adsorption to wheat roots.

Methods

Bacterial strain and growth conditions

Azospirillum brasilense Cd (ATCC-29729) was used. Bacteria were grown in shaking liquid cultures in 100-mL Erlenmeyer flasks, each of which contained 20 mL malate synthetic medium supplemented with 9.3 m*M* (NH_4Cl (Okon *et al.*, 1977). The flasks were incubated in a rotary shaker (150 rpm) for 48 h at 30°C.

Quantitative estimation of aggregates formation

Aliquots of liquid culture containing aggregates were transferred to a conical tube and allowed to stand for 15 min. Aggregates settled to the bottom of the tube while most of the free cells remained in suspension. The supernatant was sampled and its turbidity measured with a Coleman spectrophotometer (model 6/20) at 420 nm. The culture was then mechanically dispersed by treatment in a tissue homogenizer (Heidolph RzR 50) for 1 min, and the total OD was measured. Percent aggregation was calculated as follows:

$$\% \text{ Aggregation} = \frac{OD_t - OD_s \times 100}{OD_t}$$

where OD_t = total optical density after mechanical dispersion and OD_s = OD of supernatant after aggregated had settled.

NaEDTA extraction

Extraction of Azospirillum cells with NaEDTA (ethylenediamine tetraacetic acid sodium salt) was done according to Eggest *et al.* (1983). Washed aggregates were suspended in a solution of 1 m*M* NaEDTA in 60 m*M* phosphate buffer (pH 6.8) adjusted to a final concentration of 1.6 gm cells (dry wt) L^{-1}. The mixture was then incubated with continuous stirring for 1 h at 20°C. The cells were then sedimented by centrifugation at 300 rpm for 10 min at 4°C, washed twice and resuspended in 30 m*M* phosphate buffer (pH 6.8) before testing for reaggregation and adsorption. For reaggregation tests, bacteria that had been extracted with 1 m*M* NaEDTA and washed were mixed with 1 m*M* NaEDTA extract previously dialysed for 24 h at 4°C against 1 m*M* $CaCl_2$ in phosphate buffer.

Blocking of charged groups on cell surface

Bacteria were grown on synthetic medium in a shaker for 24 h to an OD of 0.7 at 450 nm, and were then harvested and resuspended in 60 m*M* phosphate buffer (pH 6.8) to an OD of 0.4. The following agents were then added to a final concentration of 1 mg mL^{-1}: 1-ethyl 3-(3-dimethylaminopropyl) carbodiimide, which blocks carboxyl groups (Feldner *et al.*, 1983); poly-L-lysine, a polycation which binds to negatively-charged groups (Ohyama *et al.*, 1979). The treated bacteria were incubated for 4 h on a rotary shaker (150 rpm) at 30°C while their aggregation was followed.

Detection of cellulose fibrils

Cellulose fibrils were detected by a modification of a procedure used by Matthysse *et al.* (1981). Azospirillum aggregates (1.6 gm dry wt.L^{-1}) were pre-treated with 1 *N* HCl at 100°C for 2 h, collected by centrifugation, washed twice with distilled water and incubated with 100 μg mL^{-1} of cellulase from *Trichoderma viride* (Sigma) in 50 m*M* citrate buffer pH 4.9 at 45°C for 72 h, or subjected to digestion with 6 *N* HCl at 100Ç for 24 h. The released sugars were identified by thin layer chromatography (TLC), using glucose and cellobiose as markers, on silica gel plates (Merck, Darmstadt, W Germany). The running solvent was a mixture of ethyl acetate. acetic acid, methanol and water (2:3:3:12). The developing solvent consisted of 2 gm diphenylamine, 2 mL aniline, 100 mL acetone and 15 mL H_2SO_4. After spraying, the plates were incubated at 100°C for 5 min. Sugars appeared as brown spots on the plates.

Gel filtration

Dialysed NaEDTA extract containing 0.2 mg mL^{-1} proteins and 14 μg mL^{-1} polysaccharides (expressed as glucose equivalent with Anthrone reagent) was fractionated on a column of Sepharose 6B (1.8 × 100 cm), and eluted with

0.1 *M* NaCl at a flow rate of 1.0 ml min^{-1}. Fractions were collected using LKB fraction collector. Individual fractions of 1.2 mL were examined for agglutination capacity and for light absorption at 280 nm, using a Kontron spectrophotometer (Uvikon 820).

Agglutination capacity of NaEDTA extract

The capacity of the dialysed NaEDTA extract and its fractions (eluted by gel filtration) to agglutinate erythrocytes was followed by using serial two-fold dilution of the extract and its fractions in V-shaped microtiter plates. Each well contained 100 μL of the NaEDTA extract or its fraction, diluted in 0.1 *M* phosphate buffered saline (PBS) pH 7, and 100μL of 25-fold PBS-diluted blood cells type O, A, or B. The agglutination titer was defined as the reciprocal of the highest dilution capable of agglutinating erythrocytes.

The capacity of NaEDTA extract and its fractions to agglutinate Azospirillum cells, was determined as follows: Cells were harvested by centrifugation at 20,000 *g* for 15 min at 4°C, washed twice with PBS, and fixed with 1% glutaraldehyde in PBS for 30 min at 4°C. Fixed cells were washed once in PBS, suspended in 0.1 *M* glycine in PBS, and brought to a final concentration of 5×10^9 mL^{-1} PBS. Five μg aliquots of this suspension were mixed with 5μL of the NaEDTA extract or its fractions on a microscope glass slide. Agglutination was instantly determined microscopically, at a 400-fold magnification, using a Zeiss phase-contrast microscope (Barak *et al.*, 1985).

Effect of sugars on bacterial agglutination

Five μL aliquots of the tested sugar (0.5 *M*) were mixed with 5 μL of Azospirillum suspension (5×10^9 mL^{-1}) and with 5 μL of the fraction showing agglutinating activity (peak I) in a total volume of 15μL.

Electrophoresis

The crude extract from NaEDTA-treated cells, concentrated to 1 mg protein mL^{-1} and peak I (P – I) containing 0.16 mg protein μL^{-1}, were boiled separately, in a solution containing 1% SDS and 1% β-mercaptoethanol, for 10 min. Electrophoresis was performed on 7.5% polyacrylamide gels (PAGE) in 0.1% SDS. The gels were stained with Coomassie brilliant blue R (Sigma). High molecular protein standards (Sigma) were used to estimate molecular weight.

Identification of glycoproteins

Gels following SDS–PAGE or gels stained with Coomassie blue R were fixed overnight in 50% methanol containing 7% acetic acid. The gels were washed twice with distilled water for 1 min and oxidized by submergence for 1 h in a solution of 1% periodic acid and 5% acetic acid. The gels were then washed with distilled water three times for 1 min each and reduced by submergence for 20 min in a solution of 5% acetic acid containing 5% sodium metabisulfite and submerged overnight in Schiff's reagent (Sigma) for detection of glycoproteins.

Protein assays

Proteins were determined according to Sedmak and Grossberg (1977).

Plant material

Wheat cultivar *Triticum aestivum* cv. Miriam was used for adsorption experiments. Seeds were surface disinfected with 1% NaOCl for 10 min, washed 3 times with sterile distilled water, and then allowed to germinate for 72 h on moist sterile filter paper.

Statistical analysis

Experiments were performed using 3–5 replicates. Significance at the 5% level was considered as indicating true differences by Duncan's multiple range test.

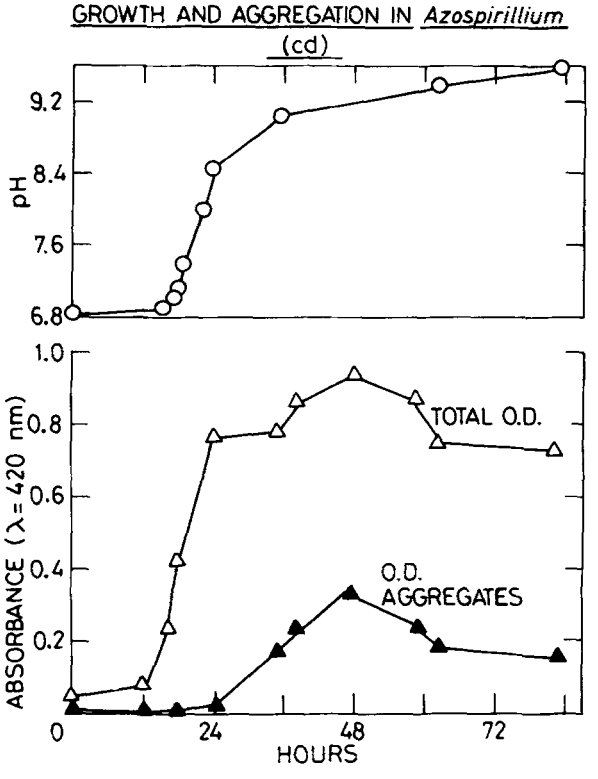

Fig. 1. Growth, pH change and aggregation of *A. brasilense* Cd in shaking liquid culture.

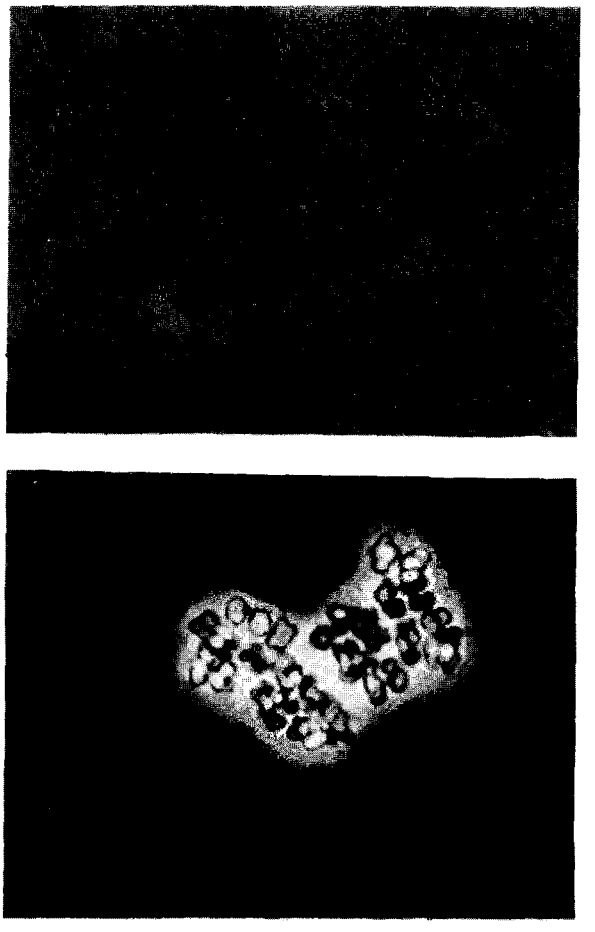

Fig. 2. Microscopic observation of *A. brasilense* Cd aggregates under a phase contrast microscope. **a** 12 h-old cells showing an elongate form. **b** 24 h-old small aggregates. The cells appear more rounded and refractile than the elongate 12 h-old cells.

Results

Relation between growth and aggregation

Aggregation of Azospirillum in a shaking liquid culture occurred towards the end of the stationary phase. The pH of the medium increased during growth and aggregation (Fig. 1). Examination under a phase contrast microscope revealed that the usually elongated cells had become refractile and rounded, and that the cells were entrapped within an extracellular layer (Fig. 2a and b).

Effect of carbon source

Flasks containing a synthetic medium without malate but supplemented with different sugars and organic acids (46 m*M*) as sole carbon sources were inoculated with Azospirillum. After 48 h of growth, optical density, aggregation level and pH of the medium were recorded. More aggregates were formed on media containing organic acids than on those containing sugars as a sole carbon source (Fig. 3). Maximum aggregation was observed where malate was the sole carbon source, while some was evident where D-fructose or D-galactose served as the sole source of carbon. No aggregation was observed in a medium containing L-arabinose. The growth of Cd on fructose, succinate and L-arabinose was maximal, while the growth on D-galactose was poor. No growth was observed on media containing D-mannose, D-glucose or D-fucose. In organic acid-supplemented media, pH levels increased to 8.9–9.5, while in sugar amended media it remained at 6.8–7.0. Bacteria were also grown on a malate synthetic medium supplemented with various sugars. Growth and aggregation level

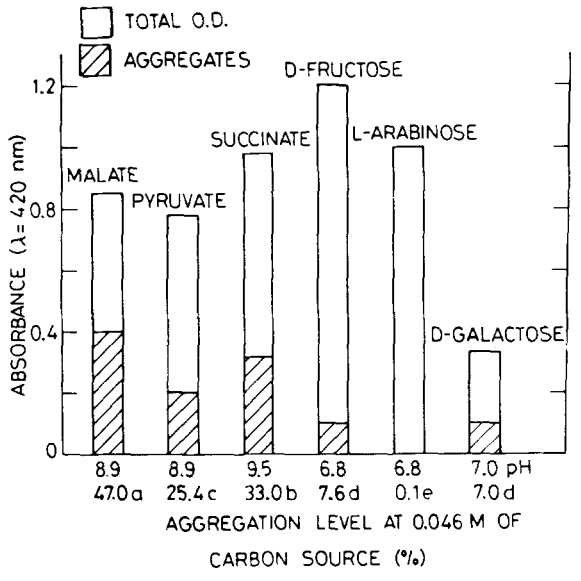

Fig. 3. Effect of various carbon sources on aggregation level of *A. brasilense* Cd. Aggregation values accompanied by *the same letter* in each column do not differ significantly at $p = 0.05$.

were measured after 48 h. Aggregation of *A. brasilense* Cd was affected by sugars with no apparent relationship to their use as a carbon source (Table 1). Thus, in the presence of fructose and malate growth reached OD $\geqslant$ 2 but aggregation dropped from 46% to 0.1%, (as compared to OD = 1.2 and 7.6% aggregation on fructose alone). D-glucose, D-mannose and D-fucose did not affect growth but aggregation was significantly inhibited.

Table 1. Effect of some sugars[a] on growth and aggregation of *A. brasilense* Cd in malate synthetic medium

Carbon source[c]	Growth (OD[b])	Aggregation (%)
Control	0.88 ± 0.05b	46.0 ± 5.1a
D-fructose	2.00 ± 0.10a	0.1 ± 0.01d
L-arabinose	0.90 ± 0.06b	42.5 ± 2.5a
D-galactose	0.89 ± 0.02b	46.3 ± 4.6a
D-glucose	0.87 ± 0.06b	32.1 ± 1.0b
D-mannose	0.88 ± 0.05b	22.2 ± 1.1c
D-fucose	0.86 ± 0.04b	22.3 ± 3.4c

[a] Effect of sugars on growth and aggregation was examined in presence of 0.046 *M* malate in the growth medium. Final sugar concentration: 0.046 *M*.

[b] Total OD, λ + 420 nm.

[c] *Azospirillum brasilense* does not utilize glucose, mannose and fucose.

Values followed by the same letter do not significantly differ at $p = 0.05$.

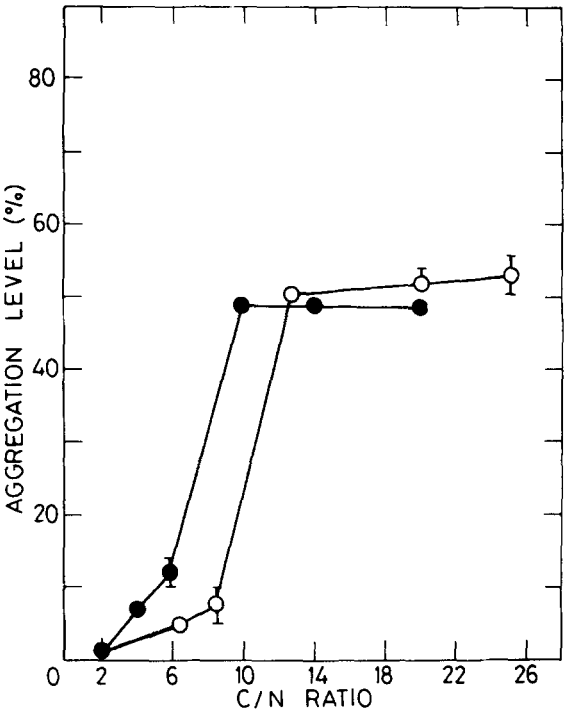

Fig. 4. Effect of carbon to nitrogen (C/N) ratio on aggregation of *A. brasilense* Cd. ● C/N ratio obtained by changing the C-concentration. ○ C/N ratio obtained by changing the N-concentration.

Effect of C/N ratio

Bacteria were grown for 48 h on a liquid synthetic medium at a constant malate concentration (37 m*M*) with increasing NH_4Cl concentrations (3.7–14.9 m*M*). At the same time, bacteria were grown at a constant NH_4Cl concentration (9.3 m*M*) with increasing malate concentration (7.4–74.5 m*M*). An increase in the C/N ratio resulted in an increase in aggregation level. No further change in aggregation was observed in Cd at C/N ratio levels higher than 10 (Fig. 4).

Influence of surface charge blockers

Blocking of carboxyl groups by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Feldner *et al.*, 1983) did not affect aggregation, while poly-L-lysine, a polycation which binds to negative charges (Ohyama, 1979), increased it (Fig. 5). These results indicate toward the possible involvement of negative charges in aggregation of Azospirillum cells.

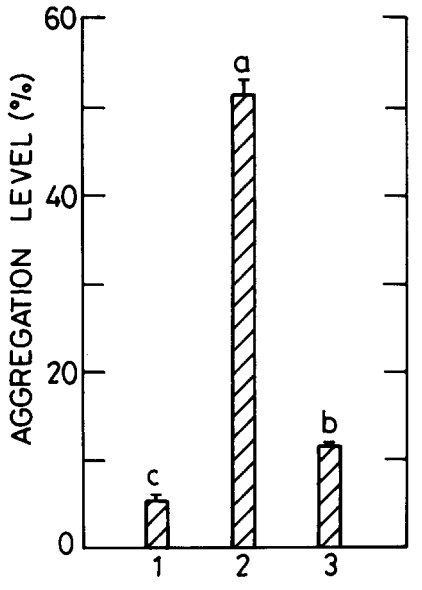

Fig. 5. Effect of surface-charge blocking compounds on aggregation of *A. brasilense* Cd. 1. Phosphate buffer (pH 6.8, 60 m*M*). 2. Poly-L-lysine. 3. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. Values accompanied by *the same letter* in each column do not differ significantly at $p = 0.05$.

Effect of pH on aggregation stability

Azospirillum brasilense Cd was grown on a synthetic medium for 48 h, and 5 mL aliquots were taken from the culture containing aggregates. The aggregates were allowed to settle and the supernatant was decanted. Aggregates were then incubated with 5 μL of phosphate buffer 60 m*M* at different pH levels. The OD of the supernatant was measured after 2 h of incubation, and the residual aggregates were then dispersed mechanically by means of a tissue homogenizer.

Percent aggregate dispersion was calculated as: $(OD_s/OD_t) \times 100$ where OD_2 = OD of the supernatant after 2 h of incubation with phosphate buffer at various pH levels. Aggregate stability was affected by pH (Fig. 6). Maximum at pH 5.5. An increase in pH led to increased dispersion in Cd as well, and maximum dispersion was obtained at pH 7.2. At pH values higher than 7.2 the dispersion level of Cd decreased.

Possible involvement of cellulose in aggregation

The involvement of cellulose fibrils in aggregation of *Azospirillum brasilense* Sp. 7 has been suggested by Sadasivan and Neyra (1985). This possibility was investigated in *A. brasilense* Cd by removing non-cellulose polysaccharides with 1 *N* HCl followed by digestion of possibly present cellulose fibrils with cellulase or with 6 *N* HCl and detecting the released glucose and cellobiose by TLC. No glucose or cellobiose could be detected in the digestion products of the aggregates.

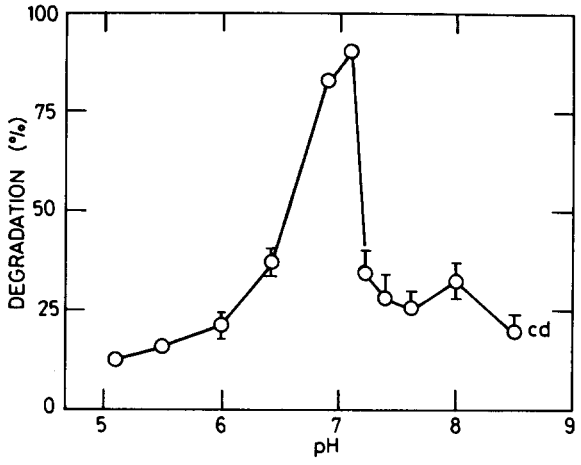

Fig. 6. Effect of pH on dispersion of aggregates of *Azospirillum* Cd.

Effect of chloramphenicol

Cells were grown on synthetic malate medium. When OD reached 0.4 (exponential phase), the medium was supplemented with 100 ppm (final concentration) of chloramphenicol which inhibit protein synthesis. Bacteria were harvested after 12 h and the aggregation level was measured. Aggregation was reduced from 37.3% in control to 6.4–1.4% in the chloramphenicol-treated cells, indicating the involvement of proteins in aggregation.

NaEDTA-extracted proteins and their possible involvement in aggregation

Cells were extracted with 1 m*M* NaEDTA, and washed with distilled water, suspended in: (a) 1 m*M* NaEDTA extract previously dialysed against 1 m*M* $CaCl_2$ in 60 m*M* phosphate buffer, (b) a solution of

Table 2. Effect of NaEDTA extract on adsorption of *Azospirillum brasilense* Cd to wheat roots

Treatment		Adsorption ($\times 10^6$ CFU/mL)
Extraction	Resuspension	
—	Phosphate buffer 60 m*M*, pH 6.8	17.0 ± 3.50
—	Dialysed NaEDTA extract	5.7 ± 0.20
NaEDTA, 1 m*M*	Phosphate buffer 60 m*M*, pH 6.8	3.58 ± 0.07
NaEDTA, 1 m*M*	Dialysed NaEDTA extract	16.0 ± 1.50

60 m*M* phosphate buffer (pH 6.8), containing 1 m*M* $CaCl_2$. The suspensions were adjusted to a final concentration of 1.6 gm cells (dry wt) L^{-1}, and incubated with gentle stirring for 20 h at 4°C. Treatment of Azospirillum cells with NaEDTA resulted in the reduction of the aggregative capacity from 40% in the control, to 1.5%. This treatment did not affect cells viability. When treated bacteria were suspended in their own, dialysed NaEDTA extract their aggregative capacity was partially restored to 28.6%, indicating the involvement of NaEDTA extracted proteins in aggregation.

Adsorption of NaEDTA extracted bacteria to wheat roots

Segments of 72-h-old etiolated wheat roots (1 g) were excised 0.5 to 2.5 cm from the root tip, and incubated for 30 min at 25°C in plastic tubes containing 5 mL of bacterial suspension adjusted to a final concentration of 5×10^8 CFU mL^{-1}. Following incubation the roots were blotted with filter paper to remove excess suspension and were then shaken (450 rpm) in 100-mL Erlenmeyer flasks containing 20 mL saline solution for 15 min to remove unattached bacteria. The segments were then homogenized in a waring blendor for 2 min. Cells were counted by plating serial dilutions of the homogenates according to Okon *et al.* (1977). The NaEDTA extracted bacteria lost their adsorptive capacity to wheat roots, and rgained it when supplemented with the NaEDTA extract (Table 2).

Agglutination of erythrocytes by crude NaADTA extract and its fractions

Unfractionated NaEDTA extract containing 2 mg mL^{-1} and 0.014 mg mL^{-1} of proteins and carbohydrates, respectively, agglutinated erythrocytes of type O, A and B at a titer of 1:1024, 1:128 and 1:256 respectively. Further agglutination tests were done using Sepharose-eluted fractions with type O erythrocytes only. The Sepharose-fractionated NaEDTA extract contained three peaks (Fig. 7). Of which, only peak I (P–I) fully agglutinated bacteria (Fig. 8) and type O erythrocytes at a titer of 1:256. Specific agglutinating activity of P–I increased 3.7-fold as compared with the unfractionated extract (Table 3). Gel electrophoresis of P–I (Fig. 9) revealed one band only, with a MW of 97,000, which reacted positively with both Schiff's reagent and with Coomassie-blue R, typical to a glycoprotein.

Effect of sugars on agglutination capacity

The effect of 12 different sugars (0.5 μ) on the capacity of P–I to agglutinate bacteria was exam-

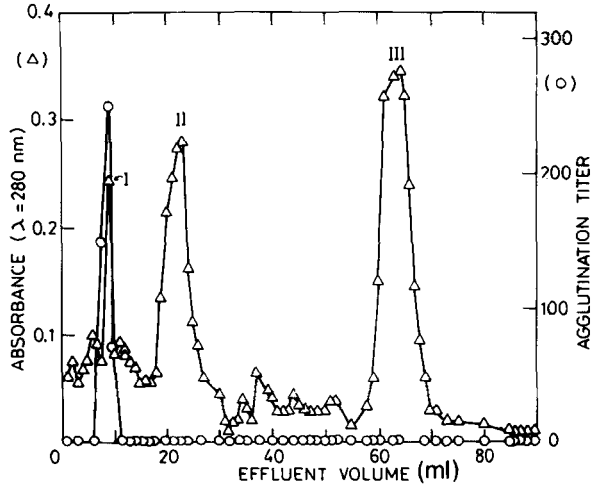

Fig. 7. Gel filtration of NaEDTA extract ot *A. brasilense* Cd. Fractionation was done on a column of Sepharose 6B. Each fraction contained 1.2 mL. △—△ UV adsorption; ○—○ agglutination titer of type O erytrocytes.

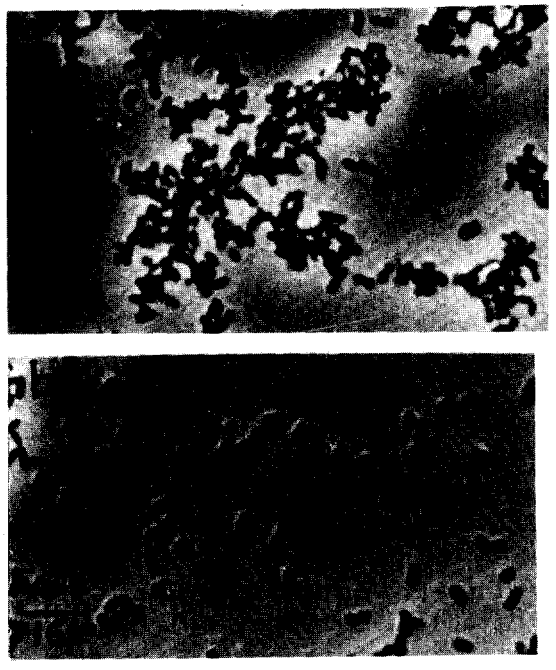

Fig. 8. Agglutination of *A. brasilense* Cd cells by fraction P–I. (**a**) Treatment, (**b**) control.

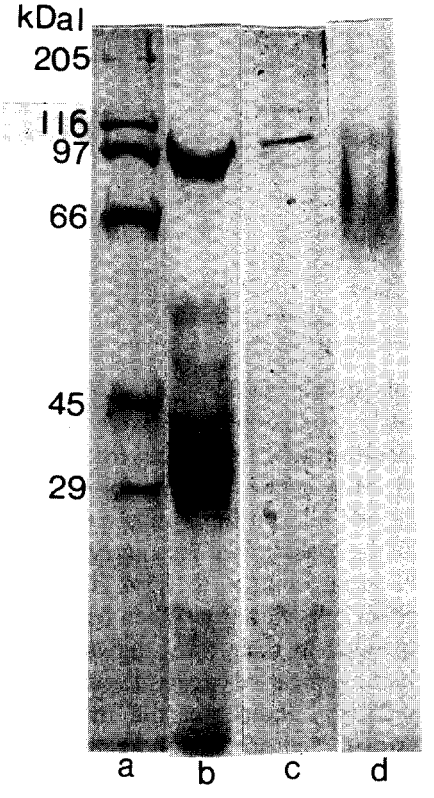

Fig. 9. SDS–PAGE of NaEDTA extract of *A. brasilense* Cd and fraction P–I of Cd extract. (**a**) Markers (see Material and Methods); (**b**) Cd; (**c**) fraction P–I stained with Coomassie blue, showing one positive band (97,000 M.W.); (**d**) P–I reacting positively to Schiff's reagent.

ined. Agglutination was strongly inhibited by D-glucose, melibiose and α-methyl glucoside. Some inhibition was also observed with D-mannose, D-fucose, D-galactose and D-fructose, whereas no inhibition could be observed with stachyose, L-mannose, N-acetyl-D-glucosamine, L-arabinose, and α-methyl mannoside.

Discussion

The conditions and factors involved in cell-to-cell adhesion of *Azospirillum brasilense* Cd and its absorptive capacity to wheat roots were studied in this work. Aggregation of Cd occurred towards the end of the exponential phase. It is possible that during their early-log phase stage, Azospirillum cells lack the receptor(s) which participate in their aggregation. Extraction of the cells with NaEDTA resulted in reduction of their adsorptive capacity to wheat roots, without affecting their viability. On the other hand, addition of this fraction to the extracted cells reconstituted their adsorptive capacity. In contrast to the report of Sadasivan and

Table 3. Agglutination titer of type O erythrocytes by NaEDTA extract and the active fraction (P–I)

Tested fraction	Volume (mL)	Protein ($mg \cdot mL^{-1}$)	Total polysaccharides ($mg \cdot mL^{-1}$)	Titer[a]	Specific[b] activity
Crude NaEDTA extract	1	2	0.014	1024	518
P–I	1.2	0.16	traces	256	1920

[a] Reciprocal of the highest NaEDTA extraction that agglutinated red blood type O.
[b] Titer × mg^{-1} protein × mL.

Neyra (1985), concerning *A. brasilense* Sp. 7, no cellulose fibrils could be detected in *A. brasilense* Cd aggregates. No glucose or cellobiose (possible products of cellulose digestion) were released from aggregates which had been pre-treated with 1 *N* HCl to remove polysaccharides other than cellulose, washed and further incubated with cellulase or hydrolyzed by 6 *N* HCl. Sadasivan and Neyra (1985) also reported on the increase in aggregation levels when fructose served as a sole carbon source in *A. brasilense* Sp. 7, in contrast to the prominent reduction in aggregates of fructose-grown cells reported in this work for *A. brasilense* Cd.

The observation reported by us that fructose supports growth of *A. brasilense* Cd at a high level, contrasted earlier reports by Okon *et al.* (1976) that *A. brasilense* Cd grew poorly on media containing fructose as the sole carbon source, but it is in agreement with the results reported by Das and Mishra (1983). We also obtained maximum yield of Cd (OD $\geqslant$ 2.0) and lowest aggregation level (0.1)% when the medium was supplemented with both fructose and malate. An increase in the C/N ratio and a decrease in combined nitrogen led to increased aggregation of Azospirillum. Deguid and Wilkinson (1961) reported that capsule size and the amount of polysaccharides per cell in *Klebsiella aerogenes* were 20 fold higher when bacteria were grown on limiting concentrations of N and P, than when grown in the presence of limiting concentrations of carbon source. In the case of Azospirillum, increased aggregation was probably caused by elevation of exopolysaccharides synthesis at high C/N ratios or at low nitrogen concentrations (Madi *et al.*, 1988). Negatively charged groups located on the cell surface may also be involved in aggregation of Azospirillum, as indicated by the enhanced aggregation in presence the polycation poly-L-lysine, which may act as a bridge between cells. Aggregation was also affected by pH. Foster and Bowen (1982) reported that bacteria have a net negative charge at pH levels near neutral, since electrostatic charge changes with variations in pH. In the case of Azospirillum it is possible that the net charge reaches a maximum at pH 7.2, resulting in repulsion and disaggregation.

Inhibition of aggregation by treatment of bacteria from the exponential phase with chloramphenicol, which specifically inhibits protein synthesis, indicates direct and/or indirect involvement of structural or functional proteins in aggregation. The presence of a glycoprotein (MW 97,000) in the active fraction (P–I) which participates in the agglutination of Azospirillum cells and erythrocytes have been demonstrated by gel electrophorsis. The involvement of glucose residues in the activity of P–I has been demonstrated by inhibiting agglutination with D-glucose, melibiose, and α-methyl glucoside. Aggregation was prevented in the cellular slime mole *Dictyostelium discoideum* by treatment with oligosaccharides which reacted with cell surface lectins (Rosen *et al.*, 1976). Similarly, aggregation level of growing Azospirillum culture decreased in the presence of glucose, mannose, fucose and fructose. Moreover, the Azospirillum cell surface is capable of binding concanavalin-A, a lectin with specific affinity to mannose and glucose (Madi *et al.*, 1988).

Our findings suggest that negative electrostatic charges and a glycoprotein capable of interacting with glucose and mannose receptors located on the outer cell surface (Madi *et al.*, 1988) are involved in aggregation of *A. brasilense* Cd and in its adsorption to wheat roots.

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