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Carbohydrate binding activities of *Bradyrhizobium japonicum*: IV. Effect of lactose and flavones on the expression of the lectin, BJ38

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BJ38 is a galactose/lactose-specific lectin ($M_r \sim 38\,000$) found at one pole of *Bradyrhizobium japonicum*. It has been implicated in mediating the adhesion of the bacteria to soybean roots, leading to the establishment of a nitrogen-fixing symbiosis. When the ligand lactose is added to cultures of the bacteria for at least 1 h prior to harvesting the cells for BJ38 isolation, the yield of the protein was found to be elevated in a dose-dependent fashion. Half maximal stimulation was observed at ~50 μM; the effect was saturated at ~1 mM, where a 10-fold higher yield of BJ38 was obtained. Saccharides with a lower affinity for BJ38 than lactose yielded a correspondingly smaller induction effect when compared at a concentration of 1 mM. The higher level of BJ38 induced by lactose is also manifested by an elevated amount of BJ38 detectable at the cell surface and by a higher number of *B. japonicum* cells adsorbed onto soybean cells. Surprisingly, the induction of BJ38 expression seen with lactose was also observed with certain, but not all, flavonoids that induce the *nod* genes of the bacteria; genistein mimicked the induction observed with lactose, whereas luteolin failed to stimulate BJ38 production.

Keywords: lectin; gene expression; cell-cell adhesion

Introduction

In previous studies [1], we documented that *Bradyrhizobium japonicum* exhibits four saccharide-specific binding activities: (a) adsorption to Sepharose beads derivatized with lactose (Lac); (b) autoagglutination (star formation); (c) binding to cultured soybean (SB-1) cells; and (d) adhesion to soybean roots. In all four of these assays, galactose (Gal) inhibited the binding, but C-2 derivatives of the monosaccharide (e.g., *N*-acetyl-D-galactosamine, GalNAc) failed to yield the same effect. Mutants of *B. japonicum*, isolated on the basis of a defect in one binding activity (SB-1 cell binding), showed a concomitant loss in the other three binding capacities. These observations suggested that all of these carbohydrate-specific processes may be mediated by the same component and mechanism.

On the basis of these observations, we began to search for Gal/Lac-specific carbohydrate-binding proteins from *B. japonicum*. We reported the purification of one such lectin, designated BJ38 ($M_r \sim 38\,000$), that bound Lac and Gal but showed much lower affinity toward GalNAc [2]. A polyclonal antiserum generated against BJ38 was used in transmission electron microscopy and in confocal fluorescence microscopy to localize the lectin at the cell surface of the bacterium [3]. More importantly, the lectin was found at only one pole of the cell, coincident with the attachment site for: (a) autoagglutination of other *B. japonicum* cells; (b) adhesion to soybean cells; and (c) absorption to Lac-Sepharose beads. These results indicate that the localization of BJ38 is consistent with a suggested role for this bacterial lectin in the polar binding of *B. japonicum* to other cells and surfaces.

In the course of these studies, we observed that the yield of BJ38 purified from *B. japonicum* was consistently higher when isolated from cultures containing Lac than from corresponding cultures without the disaccharide. Therefore, a systematic study was undertaken to document the requirements' specificity, and the consequences of this Lac effect. We report in the present communication the data derived from such a study, as well as the surprising observation that the Lac induction effect can also be mimicked by flavonoid compounds that are a part of the signalling components between the rhizobium bacteria and their leguminous hosts.

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Materials and methods

Bacterial cultures and isolation of BJ38

B. japonicum cells (R110d) were obtained from the laboratory of the late Dr Barry Chelm (Michigan State University). The bacterial strain was maintained on agar plates containing yeast extract gluconate (YEG) as described before [3]. Liquid cultures of *B. japonicum* were initiated by inoculating *B. japonicum* (3-day-old cells) from YEG agar plates into 50 ml YEG and culturing the bacteria on a gyratory shaker (100 rpm, 30 °C) for two days. This culture was then inoculated into 2 l of YEG. After 2 days, aliquots of *B. japonicum* (300 ml) were inoculated into 1.5 l of YEG and the bacteria cultured for different lengths of time (see below).

Routinely, saccharides and flavonoids were added to this final liquid culture step to determine their effect on BJ38 expression. Lactose was obtained from Kodak (Rochester, New York); mannose, GalNAc, genistein, apigenin, naringenin from Sigma (St. Louis, MO); and glucose from MCB (Cincinnati, OH). Dr Franz De Bruijn (Michigan State University) kindly provided luteolin.

For the isolation of BJ38, *B. japonicum* cells were harvested by centrifugation (11 000 × **g**, 15 min) in a GS3 rotor and the bacteria were lysed by passage through a French Press (20 000 psi). The lysate was centrifuged at 27 000 × **g** for 30 min and the supernatant fraction was precipitated by ammonium sulphate (65% saturation). The precipitate was centrifuged and resuspended in 3 ml phosphate-buffered saline (PBS) and dialised against PBS overnight at 4 °C. The bacterial lectin was then isolated by affinity chromatography on a Lac-Sepharose column [2]. Lac-eluted fractions containing BJ38 were identified by SDS-PAGE [4] and silver staining [5]. Samples were first concentrated by precipitation with 0.015% deoxycholate–7.2% trichloroacetic acid [6], washed with acetone, resuspended in sample buffer of the SDS-PAGE system, and subjected to electrophoresis. The gels were silver-stained and the intensities of the bands corresponding to BJ38 were quantitated on the basis of comparison with the staining intensities of known amounts of an arbitrary standard, carbonic anhydrase. Protein determination of cell extracts was performed by the method of Bradford *et al.* [7].

Use of [^{125}I] anti-BJ38 to quantitate cell surface expression of BJ38

The generation and characterization of the anti-BJ38 antiserum used in this study have been reported previously [3]. The immunoglobulin fraction of this antiserum was isolated by absorption onto protein A-Sepharose (Sigma, St Louis, MO) and was then labelled with ^{125}I by the chloramine T method [8]. $Na^{125}I$ (1 mCi) was added to 100 µl of antibody and chloramine T (1 mg ml^{-1}; 25 µl) was added to start the reaction. The reaction was stopped with the addition of 25 µl of sodium metabisulphite (1 mg ml^{-1}) in PBS. Free ^{125}I was removed by passage through an AG1X8 ion exchange column (Bio-Rad Laboratories, Richmond, CA). Radioactivity was determined in a γ-spectrometer counter (LKB Instruments, Inc., Rockville, MD).

For quantitation of cell surface BJ38, aliquots of *B. japonicum* cells (5×10^6) were first incubated in PBS containing 5% bovine serum albumin (BSA) for 1 h at 4 °C. ^{125}I-labelled anti-BJ38 (specific activity = 11.7 Ci g^{-1}) was added to the *B. japonicum* suspension. After 4 h, individual samples were filtered through a membrane filter (pore size 0.22 µm, Millipore, Naperville, IL) that had been presoaked in 5% BSA/PBS. The membrane was washed three times in 0.1% BSA/PBS and the bound radioactivity determined.

B. japonicum *binding to SB-1 cells*

SB-1 cells, originally derived from soybean roots (*Glycine max* (L.) Merr. cv. Mandarin) were kindly provided by G. Lark (Department of Biology, University of Utah, Salt Lake City, UT). Continuous cultures were maintained in 1B5C media by transferring 15 ml of culture (4 day old) to 50 ml fresh 1B5C and culturing the suspension cultures at 27 °C on a gyratory shaker in the dark.

For quantitation of bacterial binding to SB-1 cells, *B. japonicum* cells were washed three times in YEG to remove the saccharides that had been added to the YEG media. A binding assay described by Ho *et al.* [9] was then used. SB-1 cells (2 day old, 0.5 ml of cell suspension (20% v/v)) were incubated with 10^9 *B. japonicum* in a 35 mm culture dish. After incubation for 0.5, 2 and 4 h, the cells were transferred to 12 × 75 mm culture tubes and washed three times with PBS by centrifugation (460 × **g**, 2 min) and resuspension.

Bacterial cells bound to the soybean host were quantitated by radioimmunoassay, using anti-Brj antibody (antibody generated against whole *B. japonicum* cell) [9]. The cells were incubated with anti-Brj antibody (75 µg ml^{-1} containing 10^6 cpm [^{125}I] anti-Brj (specific activity 2.32 Ci g^{-1})) at 4 °C. The samples were washed three times by centrifugation (460 × **g**, 2 min) in 0.1% BSA/PBS and the bound radioactivity was quantitated to compare the relative amount of *B. japonicum* binding to SB-1 cells. In parallel, the conclusions derived from the quantitative assay were confirmed qualitatively by microscopic examination.

Fluorescence microscopy

B. japonicum cells were attached to coverslips as described [3]. The cells were blocked in 5% BSA/PBS for 2 h, followed by incubation in either preimmune or anti-BJ38 antiserum (1:20 in 5% BSA/PBS). Following washing in PBS three times, fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibody (1:50 dilution; Boehringer Mannheim, Indianapolis, IN) in 5% BSA/PBS was added. After 1 h incubation, the samples were washed three times with PBS. Stained cells were visualized with a fluorescence microscope

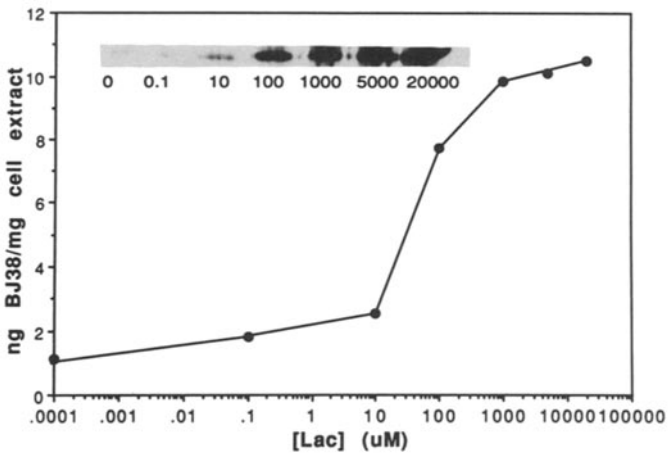

Figure 1. Dose-response curve of the effect of Lac on the production of BJ38. *B. japonicum* cells were cultured for 27 h in YEG media that had been supplemented with different concentrations of Lac. The bacterial cells were lysed by French pressing and the cell extracts were fractionated over a Lac-Sepharose column. BJ38 was eluted with 0.1 M Lac. The amount of lectin was quantitated by comparing the intensities of the silver-stained polypeptide bands (inset) with the staining intensities of known amounts of carbonic anhydrase, and is presented in the Figure as ng of BJ38 isolated per mg of cell extract.

(Zeiss D-7802 Obserkochen; excitation filter, 546 nm; chromatic beam splitter, 580 nm; barrier filter, 590 nm).

Glutamine synthetase assay

Glutamine synthetase activity was determined using the γ-glutamyl transferase (γ-GT) assay described by Bender *et al.* [10]. Cell extract (μl amouts) was added to the γ-GT assay mixture consisting of 135 mM imidazole, 18 mM hydroxylamine, 0.27 mM $MnCl_2$, 25 mM potassium arsenate, 0.36 mM sodium ADP, pH 7.55. The samples were incubated at 37 °C for 5 min. The reaction was initiated by addition of L-glutamine (20 mM final concentration). Addition of 'stop-mix' (0.2 M $FeCl_3 . 6H_2O$, 0.12 M trichloroacetic acid and 0.25 M HCl) terminated the reaction. Precipitate was removed from the samples by centrifugation and the absorbance at 540 nm was determined. Using the value that 1 μmol of glutamyl hydroxamate gives 0.532 U of absorbance at 540 nm as determined by Bender *et al.* [10], the specific activity of glutamine synthetase was calculated and expressed as μmol γ-glutamylhydroxamate produced per minute per mg cell extract.

Results

Lactose induction of BJ38 expression in B. japonicum

In the protocol for the isolation of BJ38, liquid cultures of *B. japonicum* were initiated by inoculating the bacteria maintained on agar plates in YEG medium. This initial liquid culture (50 ml) was expanded twice (for details, see Materials and Methods). The final liquid culture (~2 l) reached late logarithmic phase in ~27 h, at which time the cells were harvested for lectin purification. Addition of Lac to the final liquid culture resulted in a 10-fold increase in the yield of BJ38, when compared to the corresponding yield from cultures without Lac (Fig. 1). The effect of Lac was dose-dependent, with saturation at ~ 1 mM. Half maximal effect was observed at ~50 μM.

Three sets of experiments were performed to determine the length of time required to observe the Lac effect. First, Lac was added to a final concentration of 1 mM at various times during the final liquid culture and all the cultures were harvested at the same time (i.e. at the end of the 30 h period of the final liquid culture). In this scheme, the cell density and growth phase of the bacteria at the time of harvest were kept constant, while the initial time (cell density and growth phase of bacteria) of exposure to Lac, as well as the length of exposure to Lac, were allowed to vary. The results showed that Lac added at early time points during the final liquid culture yielded the greatest effect (Fig. 2A). Thus, addition of Lac at 2 h and 8 h of the final liquid culture resulted in approximately eight times higher yield of BJ38 than if Lac was added immediately before the harvest. In such an experimental scheme, we could not distinguish whether the observed differences were due to a difference in the length of exposure of the *B japonicum* cells to Lac or to the time (cell density and/or growth phase) of the initial exposure.

Therefore, the second experimental scheme tested the effect of Lac addition and harvest at various times throughout the final liquid culture period of 30 h, keeping the length of exposure constant at 4 h. The results showed that exposure to Lac early during the final liquid culture period yielded the highest amount of BJ38, with a monotonic decrease in the yield with later times of exposure (Fig. 2B). It should be noted that the yield of BJ38 is expressed as ng of BJ38 isolated per mg of cell extract so that the value is normalized to the total cell number used in the sample. Thus, the results suggest that the cell density and/or growth phase of the bacteria was critical at the time of Lac addition. In this experiment, however, the density (and the growth phase) of the *B. japonicum* cells at the time of harvest for BJ38 was different for each sample. Therefore, a third experimental scheme was devised to eliminate this variable. Lac was added at the initiation of the final liquid culture period and the cells were harvested after different lengths of exposure to the saccharide. The results showed that the control samples (*B. japonicum* cells without any Lac) yielded 'baseline' levels of BJ38, while Lac-treated cells yielded increasing amounts of BJ38 with longer exposures to the saccharide (Fig. 3). Exposures as short as 45 min were sufficient for appreciable enhancement of the yield of BJ38. The high yield of BJ38 in the sample that received 21 h of exposure to Lac was important because this showed that

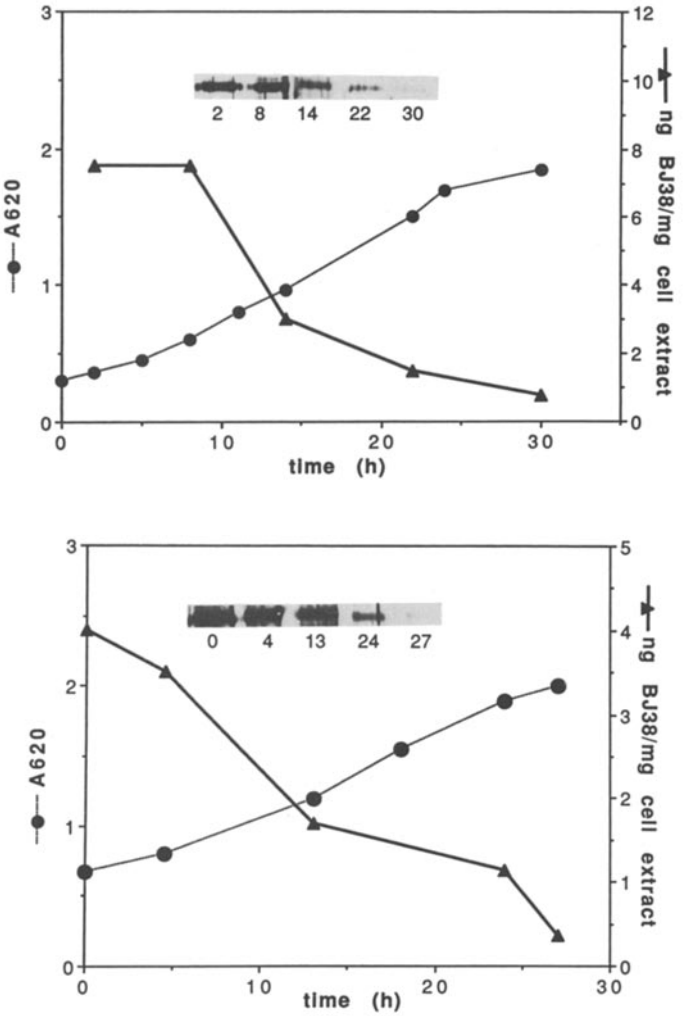

Figure 2. The effect of the growth phase of *B. japonicum* cells on the Lac induction of BJ38 expression. Lac was added to a concentration of 1 mM at various times during the final liquid culture (300 ml of a 2 l *B. japonicum* culture passaged into 1.5 l YEG). In A, all cultures were harvested at the same time (~30 h after initiation of the final liquid culture). In B, the cultures were harvested at different times (in each case, 4 h after the addition of Lac). The cell density of the sample at the time of harvest is presented as absorbance at 620 nm (A620 ●). The amount of BJ38 isolated is expressed as ng BJ38 per mg cell extract (▲). The inset shows the intensities of the silver-stained bands of the isolated BJ38. These experiments were repeated three times, with essentially the same results.

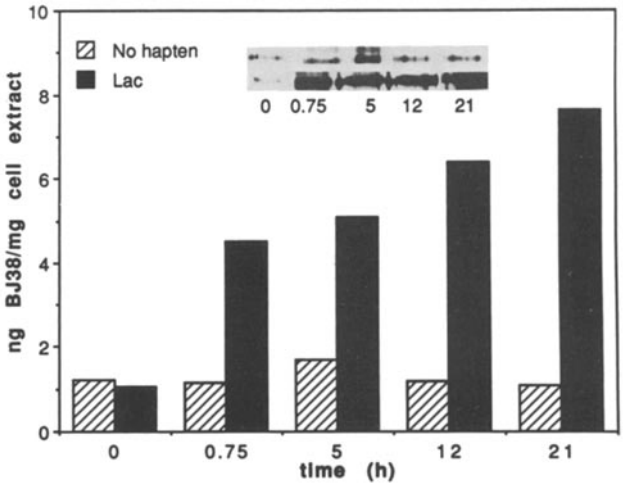

Figure 3. The effect of time exposure of *B. japonicum* cells to Lac on the induction of BJ38 expression. Lac was added to a concentration of 1 mM at the initiation of the final liquid culture (300 ml of a 2 l *B. japonicum* culture passaged into 1.5 l YEG) and the cultures were grown for the indicated lengths of time before harvesting the cells for BJ38 isolation. In parallel, final liquid cultures containing no saccharide were grown for the same lengths of time prior to harvesting for BJ38 isolation. The amount of BJ38 isolated is expressed as ng BJ38 per mg cell extract. The inset shows the intensities of the silver-stained bands of isolated BJ38. This experiment was repeated three times.

the high cell density and the stationary phase of the bacteria at the time of harvest did not impair the Lac effect.

Specificity of the lactose induction effect

The specificity of the Lac effect was tested in terms of two key questions: (a) can other saccharides mimic the effect of Lac? (b) does Lac affect the expression of other *B. japonicum* proteins, besides BJ38? Glutamine synthetase was chosen as an irrelevant protein control, to be compared to BJ38. Thus, aliquots of the same *B. japonicum* culture were inoculated into the final liquid YEG step that contained either no hapten (control) or various saccharides (1 mM). For each of these final liquid cultures, the amount of BJ38 isolated and the specific activity of glutamine synthetase were quantitated (Fig. 4). Neither Lac, nor any of the other saccharides, altered the level of activity of glutamine synthetase. On the other hand, the stimulatory effect of Lac on the yield of BJ38 was much more pronounced than the other saccharides. The eight-fold induction due to Lac should be compared with the four-fold induction seen with Gal and the 2.5-fold effect due to GalNAc. This relative order of the ability to induce BJ38 expression is similar to the relative binding affinity of BJ38 for the three saccharides [2]. Finally, Man, which does not bind to BJ38 (2), had no effect on BJ38 expression.

Effect of lactose induction on cell surface expression on BJ38

Thus far, the expression of BJ38 has been assayed at the level of amount of the lectin that can be purified by Lac-Sepharose affinity chromatography. The question is, therefore, raised whether the induction of BJ38 expression by Lac is also manifested at the level of increased amounts of BJ38 at the cell surface. BJ38 at the cell surface was detected in two assays, using a polyclonal rabbit antiserum specific for the lectin [3]. First, rabbit anti-BJ38 was labelled with ^{125}I and the amount of antibody required to saturate the BJ38 found on the cell surface of *B. japonicum* was

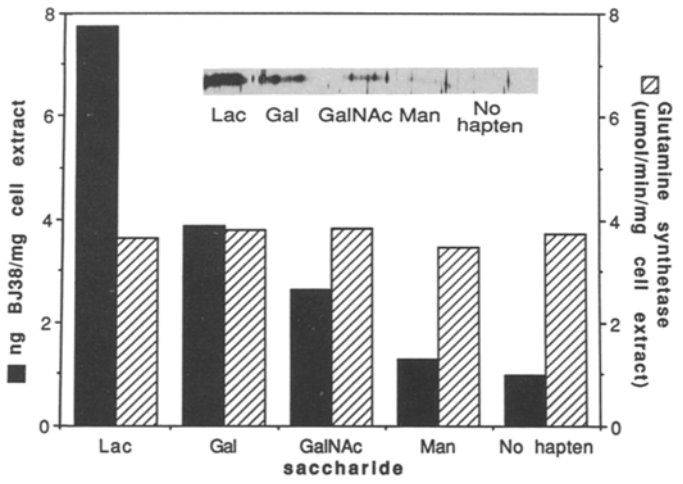

Figure 4. Saccharide specificity in the induction of BJ38. Final liquid cultures of *B. japonicum* were incubated for 10 h in the presence of 1 mM concentrations of Lac, Gal, GalNAc, and Man, or without any saccharide. The amount of BJ38 isolated (inset) was quantitated and is expressed as ng BJ38 per mg cell extract. In parallel, the specific activity of glutamine synthetase in the cell extracts was also determined (see Materials and methods). This experiment was reproduced three times.

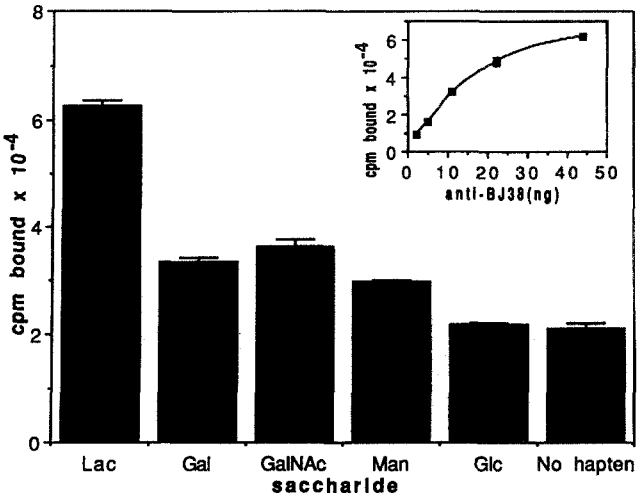

Figure 5. Quantitation of the effect of saccharides on the cell surface expression of BJ38. *B. japonicum* cells were cultured in the presence of Lac, Gal, GalNAc, Man, and Glc (all at 1 mM), or without any saccharide. Bacteria from each treatment (5×10^6 cells) were then blocked with 5% BSA/PBS for 1 h and then incubated for 4 h with 44 ng of [^{125}I]anti-BJ38. The bacteria were then filtered through a Millipore filter (pore size 0.22 μm) and the membrane washed three times in PBS. The amount of radioactivity retained on the membrane was then determined. The data represent means ± SD of triplicate determinations. Inset: Determination of the amount of anti-BJ38 needed to saturate the BJ38 found on the cell surface of *B. japonicum*. *B. japonicum* (5×10^6) were incubated with various amounts of [^{125}I]anti-BJ38 for 4 h. *B. japonicum* was then filtered through a Millipore filter and the amount of bound radioactivity determined.

determined by quantitating the radioactivity bound as a function of antibody added (Fig. 5, inset). Using a saturating dose of ^{125}I-labelled anti-BJ38 (~44 ng), the level of BJ38 expressed on the cell surface was compared for *B. japonicum* cells derived from liquid cultures containing various saccharides (1 mM) or containing no hapten (control). The presence of Lac in the final liquid culture greatly increased the amount of BJ38 at the cell surface, accessible to binding by ^{125}I-labelled anti-BJ38 (Fig. 5). The other saccharides had little or minimal effects. This includes GalNAc and Gal, which yielded 2.5-to four-fold induction effects when the expression of BJ38 was assayed at the level of isolation of bulk amounts of the lectin.

Second, immunofluorescence staining with rabbit anti-BJ38 was used to determine the fraction of *B. japonicum* expression BJ38 at the surface. Approximately 40% of the bacteria derived from final liquid culture containing Lac (1 mM) stained with anti-BJ38 (Fig. 6a). In contrast, bacteria derived from final liquid cultures that contained no hapten or a control saccharide (1 mM glucose) yielded 10% immunofluorescent cells (Fig. 6b and 6c). Thus, assaying at the level of antibody-accessible BJ38, the induction effect of Lac is manifested mainly as an increase in the percentage of cells exhibiting cell surface exposed lectin.

Effect of lactose induction on the adhesive properties of B. japonicum

Since Lac induction of BJ38 expression is manifested not only at the level of total BJ38 isolatable but also in terms of the fraction of cells exhibiting BJ38 detectable at the cell surface, the question is raised whether the capacity of *B. japonicum* to bind to soybean cells is also increased upon Lac induction. Bacteria derived from Lac-containing final liquid cultures indeed showed a high rate of adhesion to cultured soybean SB-1 cells (Fig. 7). Within 30 min following co-culture of SB-1 cells with *B. japonicum*, bacteria derived from Lac-induced cultures showed many cells attached to the soybean cells, compared with the corresponding co-culture using uninduced bacteria.

We had previously developed a radioimmunoassay, using an antiserum raised against whole *B. japonicum* cells (anti-Brj), to quantitate the number of bacteria bound on soybean cells [9]. The differences in the number of *B. japonicum* attached to SB-1 cells, qualitatively represented in the photomicrographs of Fig. 7, are corroborated by the quantitative data (Fig. 8). There was about a four-fold increase in the number of attached cells, comparing Lac-induced versus control samples, 30 min after co-culture. This difference decreased to about two-fold upon longer term culture.

Induction of BJ38 expression by flavones and derivatives

Using the same protocol for Lac induction, we have also tested the effect of several flavonoid compounds implicated

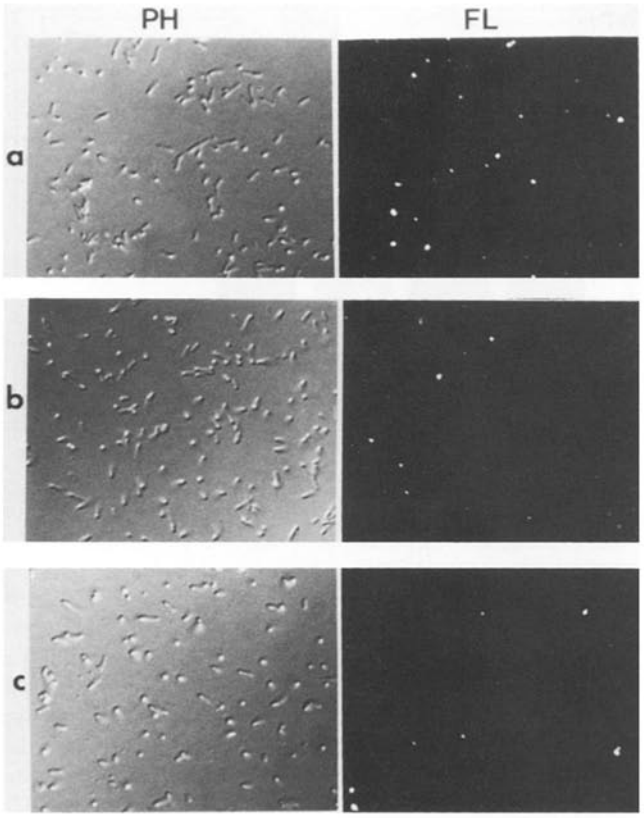

Figure 6. The effect of saccharides on the expression of BJ38 at the cell surface of *B. japonicum* as detected by immunofluorescence staining with anti-BJ38. Bacteria cells were cultured in the presence of 1 mM concentrations of (a) Lac; (b) Glc; and (c) no hapten for 24 h. The cells were then stained with anti-BJ38 as described in the Materials and methods. PH, phase contrast; FL, fluorescence.

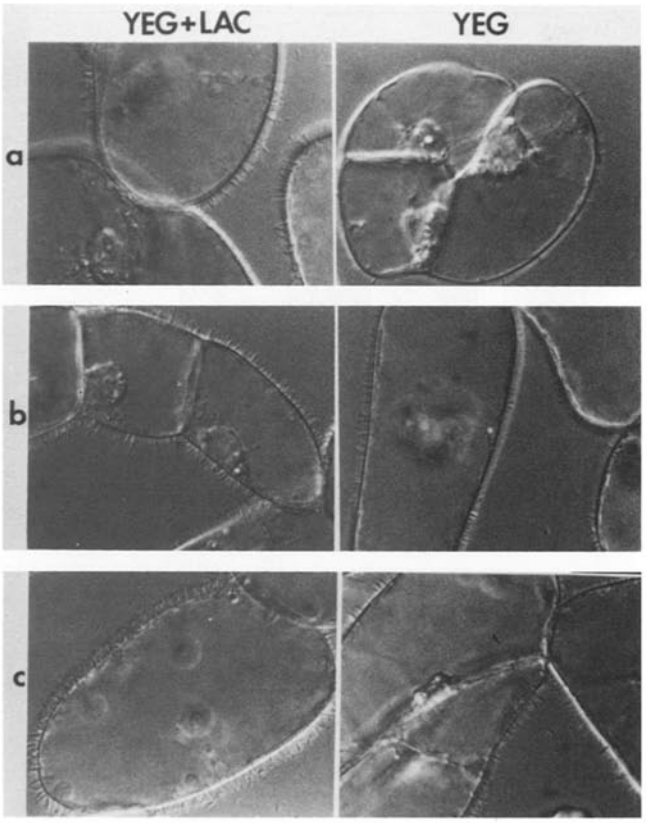

Figure 7. The effect of inclusion of Lac in the final liquid culture of *B. japonicum* on the subsequent binding of the bacteria to SB-1 soybean cells as observed by light microscopy. *B. japonicum* (10^9 cells) derived from final liquid cultures with (YEG + Lac) and without (YEG) saccharide were incubated with 0.5 ml cell suspension (20% v/v) of SB-1 cells as described in Materials and methods. Light micrographs were taken at (a) 0.5, (b) 2, and (c) 4 h after *B. japonicum* was added to the SB-1 cells.

in the induction of the *nod* genes that are required for early host responses in the *Rhizobium*-host plant signalling [11–15]. Thus, the flavonoids (2 μM) were included in the final liquid culture for 20 h prior to harvesting the bacterial cells for BJ38 isolation. The isoflavone genistein yielded a strong induction effect on BJ38 expression, comparable to that seen with the 'positive control', Lac (Fig. 9). In contrast, the flavone luteolin failed to yield any effect; the amount of BJ38 isolated corresponded to those of 'negative controls', Man and no hapten inducer. Finally, the flavone apigenin and the flavonone naringenin resulted in intermediate levels of induction of BJ38 expression.

Discussion

The key findings of this study include: (a) carbohydrate ligands of the lectin BJ38 can regulate the level of the protein; (b) this is manifested both in terms of the amount of protein that can be isolated on the basis of its carbohydrate-binding activity and in terms of cell surface exposed protein accessible to binding by a specific antibody; (c) among the saccharides tested, Lac was the best inducer, with a half maximal effect observed at ~50 μM and saturation at ~1 mM. When tested at 1 mM, the order of potency in stimulating the expression of BJ38 was Lac > Gal > GalNAc.

There does not appear to be a direct correlation between the amount of BJ38 isolated by affinity chromatography and the amount of BJ38 detected at the cell surface. Lac induced an eight- to 10-fold increase in the yield of BJ38, but the increase in the percentage of cells with BJ38 detectable at the cell surface is only about four-fold. Moreover, the increase in BJ38 assayed by these methods translates into a two-fold increase in the number of cells adhering to soybean cells. These results raise the question whether the increase in BJ38 is due to an elevated expression of the lectin in cells that already contain the protein or may represent a recruitment of a new subpopulation of *B. japonicum* cells that do not exhibit BJ38

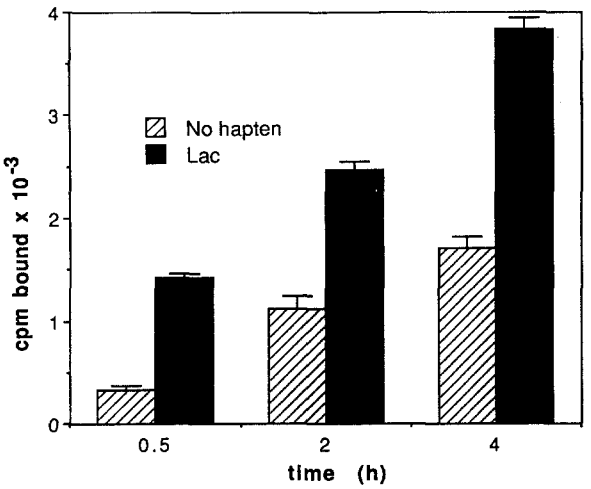

Figure 8. The effect of inclusion of Lac in the final liquid culture of *B. japonicum* on the subsequent binding of the bacteria to SB-1 soybean cells as quantitated by a radioimmunoassay. The experiment was carried out as in Fig. 7 and the number of bacteria binding to the SB-1 cells was quantitated using [^{125}I]anti-Brj as detailed in Materials and methods. The data represent means ± SD of triplicate determinations.

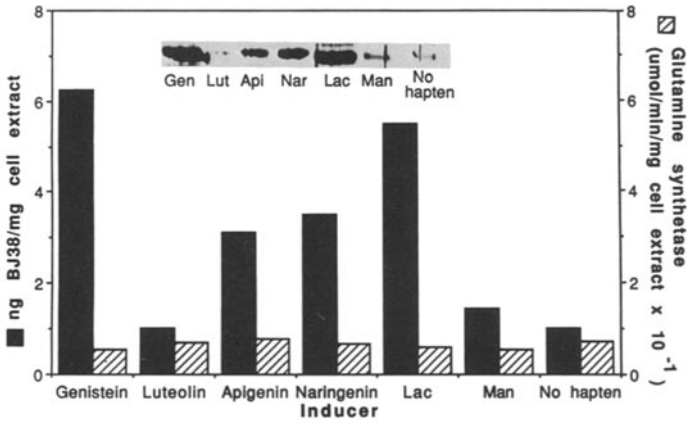

Figure 9. Comparison of the effect of flavones and saccharides on the induction of BJ38 expression in *B. japonicum* cells. Final liquid cultures of *B. japonicum* were incubated for 20 h in the presence of flavones (2 μM) or saccharides (1 mM) or with no addition. The amount of BJ38 isolated (inset) was quantitated and is expressed as ng BJ38 per mg cell extract. In parallel, the specific activity of glutamine synthetase in the cell extracts was also determined. This experiment was repeated three times.

in the absence of the carbohydrate ligand. It is not known how this subpopulation of bacteria might be different from those *B. japonicum* cells expressing the protein.

At the molecular level, it is not known whether the Lac induction effect occurs at the transcriptional levels. For example, it is possible that ligand binding merely protects or sequesters the lectin from proteolytic degradataion, thus resulting in high levels of the lectin. It seems more likely, however, that the induction effect is a result of transcriptional regulation of the BJ38 gene. Transcriptional regulation has been observed in the control of several saccharide binding proteins which mediate the transport of maltose, arabinose, xylose and lactose into bacteria [16–19]. In these systems, the genes encoding for the binding proteins are located in operons that are regulated by proteins which either activate or repress transcription of the operon. The transcription of these genes is regulated by the haptens of their respective gene products. In the case of BJ38, Lac is not the only saccharide inducer. Moreover, the potency of various saccharides in stimulating BJ38 expression follows the same order of affinities between the lectin and its carbohydrate ligands. For example, we had previously quantitated that the binding of the disaccharide Lac was 13-fold higher than that of Gal, which in turn was about 18-fold higher than that of the GalNAc [2]. Consistent with this relative order of affinities, Lac induced an eight- to 10-fold increase in BJ38, while the effects of Gal and GalNAc were four- and 2.5-fold, respectively. This raises the distinct possibility that it is BJ38 itself that is binding to the promoter region and regulating the transcription of its own gene. Binding of carbohydrate ligands precludes BJ38 from repressing the transcription of its own gene, possibly due to a ligand-induced conformational change.

To our surprise, we found that several flavonoid compounds known to regulate the *nod* genes of rhizobium [11–15] also induced BJ38 expression. In accordance with previous reports [11, 20], genistein, which was most potent in inducing *nodABC* expression, was also the most potent inducer of BJ38. Similarly, the flavone luteolin had negligible effects with respect to both *nodABC* and BJ38 induction and apigenin yielded induction levels intermediate between genistein and luteolin in both assays. Naringenin also induced BJ38, at a level below that of genistein but comparable to apigenin; this flavanone had no effect on *nodABC* induction in *B. japonicum* cells [11, 20].

These results raise the possibility that the BJ38 gene, demonstrated to be saccharide-sensitive, is also controlled by NodD, which mediates the activation of other *nod* genes [21, 22]. This scheme suggests that BJ38 may belong to the family of *nod* genes necessary for successful infection of the host plant. Such a possibility is intriguing in light of the fact that sequence analyses of known *nod* genes suggest that the *nodABC*, *nodH*, *nodL*, *nodM* and *nodZ* gene products are capable of binding carbohydrate [22–25]. These carbohydrate binding proteins function as enzymes proposed to be involved in the synthesis of low molecular weight lipooligosaccharide compounds such as the *nod* factors essential for successful nodulation. In our present case, however, BJ38 would be a carbohydrate binding product of a *nod* gene whose function is to mediate the attachment of *B. japonicum* to soybean roots [1]. This present scheme, however, does not preclude the possibility that flavonoids may stimulate BJ38 transcription through bacterial response

pathways other than *nodD*. For instance, studies by Parniske *et al.* [26] have also demonstrated isoflavonoid-induced resistance of *B. japonicum* to the phytoalexin glyceollin. This resistance has been demonstrated in $nodD_1D_2YABC$ deletion mutants, suggesting alternative sites of genistein recognition.

At the functional level, the reasons for such a dual control of BJ38 expression can only be speculated at this point. Lac and Gal could function to enhance *B. japonicum* attachment to soybean roots. Gal is one of the major constituents of the plant cell wall and may serve as a plant signal to enhance bacterial attachment to the soybean root through elevated levels of BJ38. Our present observation that an increase in *B. japonicum* binding to soybean roots is paralleled by an increase in the levels of BJ38 provide correlative evidence that this may indeed be so. A similar role for saccharide mediated induction has been proposed for *Agrobacterium tumefaciens*. Ankenbauer and Nester have reported that ChvE, a periplasmic sugar binding protein mediates the induction of the *vir* genes in *Agrobacterium tumefaciens* [27, 28], leading to the infection of the host plant. As most of the sugars able to bind to ChvE and induce the *vir* genes are constituents of plant cell wall polysaccharides, it was postulated that these compounds served to signal the bacterium of the presence of the host plant and, therefore, activate the bacterium for infection. In a similar fashion, soybean wall constituents, specifically Gal, may serve as signals to *B. japonicum* and activate that bacterial strain's attachment to soybean roots.

Flavonoids could also function to enhance *B. japonicum* attachment to soybean roots. In previous studies [29], we had documented that both BJ38 and *B. japonicum* bind preferentially to the emergent root hair zone of the soybean root. This preferential binding has been attributed to recognition of specific carbohydrate structures on the soybean root by BJ38. In studies on the spatial distribution of flavones capable of inducing *nod* gene expression along the plant root surface, the emergent root hair zone has also been shown to be the zone of maximum induction of bacterial *nod* genes by flavonoids [30–33]. Given these observations and the results documented in the present study, induction of BJ38 expression by flavonoids would lead to an enhanced binding of *B. japonicum* to soybean roots and, in particular, to the emergent root hair zone.

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