Expression and Purification of Glutamine Synthetase Cloned from *Bacteroides fragilis*

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A glutamine synthetase (GS) gene, *glnA*, from *Bacteroides fragilis* was cloned on a recombinant plasmid pJS139 which enabled *Escherichia coli glnA* deletion mutants to utilize $(\text{NH}_4)_2\text{SO}_4$ as a sole source of nitrogen. DNA homology was not detected between the *B. fragilis glnA* gene and the *E. coli glnA* gene. The cloned *B. fragilis glnA* gene was expressed from its own promoter and was subject to nitrogen repression in *E. coli*, but it was not able to activate histidase activity in an *E. coli glnA ntrB ntrC* deletion mutant containing the *Klebsiella aerogenes hut* operon. The GS produced by pJS139 in *E. coli* was purified: it had an apparent subunit M_r of approximately 75000, which is larger than that of any other known bacterial GS. There was very slight antigenic cross-reactivity between antibodies to the purified cloned *B. fragilis* GS and the GS subunit of wild-type *E. coli*.

INTRODUCTION

The medically important obligate anaerobe *Bacteroides fragilis* is under investigation with regard to its genetic constitution, particularly the potential for the transfer of genetic material to the other microbial inhabitants of the mammalian gut. There has been some doubt regarding the possibility of expression of *Bacteroides* genes in other bacteria following a report by Guiney *et al.* (1984) which indicated that the ampicillin resistance gene from *Escherichia coli* was not expressed in *B. fragilis* and that the clindamycin resistance gene from the *B. fragilis* pBFTM10 plasmid was not expressed in *E. coli*. However, the cloning and expression in *E. coli* of the fimbrial sub-unit protein, pilin protein, cellulase and chondroitin lyase genes from *Bacteroides nodosus*, *Bacteroides succinogenes* and *Bacteroides thetaiotaomicron* have been reported (Anderson *et al.*, 1984; Crosby & Collier, 1984; Elleman & Hoyne, 1984; Guthrie *et al.*, 1985).

Since *B. fragilis* is the anaerobic species most frequently isolated from human infections (Kasper *et al.*, 1979) we investigated the expression of its genes in *E. coli*. Glutamine synthetase (GS) is a central enzyme in the pathways of nitrogen metabolism for many bacteria and as its presence in *B. fragilis* was expected a gene library prepared from *B. fragilis* strain BF-1 was screened for expression of GS.

In the *Enterobacteriaceae* the structural gene for GS, *glnA*, is regulated by the products of the *ntrB* and *ntrC* genes which are linked to the *glnA* gene, and by the product of the unlinked *ntrA* gene (Garcia *et al.*, 1977; Kustu *et al.*, 1979; Pahel & Tyler, 1979; McFarland *et al.*, 1981; Magasanik, 1982). The *ntr* genes also regulate other operons involved in nitrogen metabolism.

GS from several bacterial genera has been purified utilizing the large size and heat resistance of the holoenzyme to separate it from other intracellular macromolecules (Streicher & Tyler, 1980; Bodasing *et al.*, 1985). We report the use of polyethylene glycol (PEG) and column chromatography for the purification of the cloned GS and describe the production of specific antibodies in rabbits.

Abbreviation: GS, glutamine synthetase.

Table 1. *Bacterial strains and plasmids*

Strain	Relevant genotype	Reference/source
<i>B. fragilis</i> BF-1	Wild-type bacteriocin ⁺	Mossie <i>et al.</i> (1979)
<i>E. coli</i> ET8051	<i>glnA ntrB ntrC</i>	Tuli <i>et al.</i> (1982)
<i>E. coli</i> YMC-10	<i>glnA⁺ ntrB⁺ ntrC⁺</i>	Backman <i>et al.</i> (1981)
<i>E. coli</i> YMC-11	<i>glnA ntrB ntrC</i>	Backman <i>et al.</i> (1981)
<i>E. coli</i> HB-101	Str ^r <i>glnA⁺ ntrB⁺ ntrC⁺</i>	Maniatis <i>et al.</i> (1982)
Plasmids		
pEcoR251	Ap ^r <i>EcoRI</i>	M. M. Zabeau, Plant Genetic Systems, Ghent, Belgium
pCI ₈₅₇	Kan ^r λ -P _R repressor(ts)	
pJS139	Ap ^r <i>glnA⁺</i>	This study
pJS139.1	Ap ^r <i>glnA</i> (<i>XhoI</i> deletion)	
pJS139.2	Ap ^r <i>glnA⁺</i> (<i>StuI</i> deletion)	

METHODS

Bacterial strains and plasmids. *B. fragilis* strain BF-1 was described by Mossie *et al.* (1979). The plasmid pEcoR251 was a gift from M. M. Zabeau, Plant Genetic Systems, Ghent, Belgium. This positive selection vector contains the *E. coli* *EcoRI* gene under the control of the λ rightward promoter, the ampicillin (Ap) resistance gene and the pBR322 origin of replication. It was derived from the pCL plasmids described by Zabeau & Stanley (1982). The *EcoRI* gene product, expressed at high levels by the λ promoter on pEcoR251, is lethal unless insertionally inactivated or regulated by plasmid pCI₈₅₇ which contains a temperature-sensitive λ repressor gene (Remaut *et al.*, 1983). The *EcoRI* gene has a single *Bgl*II restriction enzyme site which can be used for the insertion of DNA digested with *Bgl*II, *Bam*HI or *Sau*3AI. The *E. coli* strains used are described in Table 1.

B. fragilis was grown under anaerobic conditions in a complex medium which contained (g l⁻¹) Difco tryptic soy broth (24), Difco yeast extract (10), glucose (1) and L-cysteine hydrochloride (0.5) (Southern *et al.*, 1984). The *E. coli* strains were grown in LB (Luria-Bertani) broth containing (g l⁻¹) Difco Bacto-tryptone (10), Difco yeast extract (5) and NaCl (5) (Davis *et al.*, 1980), or in CSH glucose minimal medium (Miller, 1972) supplemented as described in the text.

Preparation of DNA. Plasmid DNA was prepared by the alkali-hydrolysis method of Ish-Horowitz & Burke (1981); *B. fragilis* chromosomal DNA was prepared as follows. Bacterial cells collected from 100 ml of a late-exponential-phase culture (about 10⁹ cells ml⁻¹) were suspended in 5 ml 0.01 M-Tris/HCl buffer (pH 7.8) containing 10 mM-EDTA and 8.0 mM-glucose. *Streptomyces griseus* protease (Merck) (10 mg) and 100 mg SDS were added and gently mixed to dissolve. The resulting cell lysate was extracted with a phenol/chloroform/amylic alcohol (50:49:1, by vol.) mixture (Marmur, 1961), to remove proteins and lipids, until the supernatant was clear. After precipitation with an equal volume of 2-propanol, the pellet was redissolved in 5 ml 10 mM-Tris/HCl, 1 mM-EDTA buffer at pH 7.9 (TE buffer).

Construction of the *B. fragilis* genomic library and selection of the GS gene. *B. fragilis* DNA was digested with sufficient *Sau*3AI restriction endonuclease (Boehringer-Mannheim) to produce a spectrum of DNA fragment sizes, and was then fractionated on a sucrose density gradient. DNA was precipitated from those fractions which were shown by agarose gel electrophoresis to contain fragments of 3–10 kb. Plasmid pEcoR251 was digested to completion with *Bgl*II, precipitated with ethanol and redissolved in TE buffer.

After ligation (Davis *et al.*, 1980), DNA was used to transform competent *E. coli* HB101 cells; transformants containing recombinant pEcoR251 were selected on Luria agar containing Ap (125 µg ml⁻¹). *E. coli* HB101 Ap^r colonies were pooled and recombinant pEcoR251 plasmid DNA was extracted and used to transform the *E. coli* YMC-11 *glnA* deletion mutant. GS transformants were selected on minimal medium containing (NH₄)₂SO₄ as the sole nitrogen source. The pJS139 recombinant plasmid was isolated and characterized by restriction mapping using standard procedures (Maniatis *et al.*, 1982).

GS assay. GS activity was assayed in crude cell extracts by the γ -glutamyltransferase assay which determines the total GS activity (Bender *et al.*, 1977). Specific enzyme activity was expressed as µmol γ -glutamyl hydroxamate formed min⁻¹ (mg protein)⁻¹.

Protein was determined using the dye-binding method of Bradford (1976).

Regulation of nitrogen metabolism operons. Utilization of arginine and low concentrations of glutamine as sole nitrogen sources (Ntr⁺ phenotype) was determined by growth on minimal medium containing glutamine (0.5 mM) or arginine (15 mM) as described by Tuli *et al.* (1982). Histidase activity was assayed using a modification of the method of Smith *et al.* (1971). Exponential-phase cells (2 ml) were well mixed with 0.5 ml toluene to permeabilize them. The cells were collected by centrifugation, resuspended in 0.2 ml 0.15 M-NaCl and stored at -60 °C until

assayed. For the assay a sample (20 μ l) was mixed with 100 μ l 1 M-diethanolamine/HCl buffer (pH 9.4), 10 μ l freshly made 0.5 M reduced glutathione in 0.1 M-potassium phosphate buffer (pH 7.4) and 550 μ l distilled water. After 5 min incubation at 37 °C, 100 μ l 0.1 M-histidine was added and mixed; incubation at 37 °C was then continued for 15 min. The reaction was stopped by addition of 1 ml saturated sodium tetraborate solution. The A_{277} was determined and the specific activity was expressed as A_{277} (mg protein)⁻¹ (arbitrary units).

β -Lactamase assay. The method of Sykes & Nordström (1972) was used to assay cultures of *E. coli* YMC-11 (pJS139) growing in minimal and Luria medium. The results are expressed as enzyme units (U) (mg protein)⁻¹.

DNA hybridization. Chromosomal DNA extracted from *E. coli* and *B. fragilis* was digested to completion using the restriction endonuclease *Xho*I; and fragments were then separated by agarose gel electrophoresis in Tris/acetic acid buffer (pH 7.8). The DNA fragments were transferred to a nitrocellulose membrane (Hybond-N; Amersham) according to Smith & Summers (1980). A ³²P-labelled probe was prepared from purified pJS139 DNA using a nick-translation kit (Amersham) according to the manufacturer's directions. The membrane was treated according to the methods of Meinkoth & Wahl (1984) for prehybridization and hybridization. Conditions of hybridization were such that a 50% homology between the probe and chromosomal DNA should have been detected.

Extraction and purification of the pJS139 GS gene product. The enzyme was extracted from *E. coli* YMC-11 (pJS139) cells which had been grown overnight in Luria broth, and which were then diluted into nitrogen-free CSH minimal medium and shaken at 37 °C for 2 h. The stabilizer of GS, *N*-cetyl-*N,N,N*-trimethylammonium bromide (Merck), was added to the culture to a final concentration of 0.1 mg ml⁻¹; incubation was then continued for 10 min. The cells were collected by centrifugation and resuspended in 1/100th of the culture volume of extraction buffer (50 mM-imidazole/HCl, 20 mM-MgCl₂, 0.1 mg phenylmethylsulphonyl fluoride ml⁻¹ and 20 mM-2-mercaptoethanol, pH 7.15); the cells were then disrupted by sonication. After centrifugation the supernatant was kept on ice; NaCl was added to 0.1 M and PEG (*M*_w 6000) to 4% (w/v). After 4 h the mixture was centrifuged and PEG added to the supernatant to bring it to 6% (w/v); the mixture was then kept at 4 °C overnight.

The pellet was collected, dissolved and loaded onto a Sephacryl S1000 (Pharmacia) 250 × 10 mm column, equilibrated and eluted with column buffer (1%, w/v, KCl, 10 mM-imidazole/HCl, pH 7.15) at 0.2 ml min⁻¹. Fractions (1 ml) were collected and assayed for GS activity and analysed by SDS-PAGE (Laemmli, 1970; O'Farrell, 1975). Estimates of the cloned GS subunit *M*_r were obtained from 8% polyacrylamide gels.

Rabbits were immunized by intra-muscular injection of approximately 200 μ g of the purified GS protein on days 1, 3, 7, 14 and 21; serum was collected on day 24.

Ouchterlony immunodiffusion plates were prepared (Weir, 1973) using 1% agarose in 0.1 M-Tris/borate buffer (pH 7.8). The central well contained the antiserum (20 μ l) and the four surrounding wells contained purified cloned GS (1), cell lysate of *E. coli* YMC-11 (pJS139) (2), cell lysate of *E. coli* YMC-10 GlnA⁺ (3) and cell lysate of *E. coli* YMC-11 *glnA* (4), containing 0.28, 0.08, 0.05 and 0 units of GS activity, respectively. The cells had been incubated under nitrogen-limiting conditions before lysis. After precipitin bands were apparent, the agarose gel was washed for 4 h in running water, dried onto Gelbond film (FMC Corp.), stained with aceto-orcein (2 g orcein in 100 ml 50%, v/v, acetic acid) and destained in absolute alcohol.

Western blotting from SDS-PAGE gels onto nitrocellulose membranes was done by the method of Towbin *et al.* (1979): the membrane was blocked with 10 mM-Tris/HCl (pH 7.4), 0.15 M-NaCl, 2% (w/v) nonfat dried milk (Johnson *et al.*, 1984) and 0.05% (v/v) Tween-20 overnight at room temperature. The antibody binding and the development of bands using a goat anti-rabbit serum conjugated to horseradish peroxidase was done according to Rybicki & von Wechmar (1982), except that Tween-20 was used in place of NP-40.

RESULTS

Cloning of the *B. fragilis* GS gene in *E. coli*

A library of *B. fragilis* DNA was established in *E. coli* HB101 by insertional inactivation of the *Eco*RI gene of pEcoR251. Plasmid DNA prepared from pools of clones (about 10000) containing *B. fragilis* DNA was used to transform the *E. coli* YMC-11 GlnA⁻ NtrB⁻ NtrC⁻ deletion strain. An *E. coli* YMC-11 GlnA⁺ transformant was isolated on minimal medium. The transformant contained a recombinant pEcoR251 plasmid.

The presence of a GS structural gene on this plasmid was confirmed by retransformation of *E. coli* YMC-11 GlnA⁻ NtrB⁻ NtrC⁻ and *E. coli* ET8051 GlnA⁻ NtrB⁻ NtrC⁻ deletion strains. After transformation approximately equal numbers of colonies were obtained on minimal medium (GlnA⁺) and Luria medium containing Ap (Ap^r). This recombinant plasmid was designated pJS139. Digestion of pJS139 with *Pst*I indicated that an approximately 8 kb DNA fragment was inserted into pEcoR251 (Fig. 1).

Fig. 1. Partial restriction and deletion map of pJS139. 1, Scale (kb); 2, pJS139 Ap^rGlnA⁺; 3, pEcoR251 Ap^r; 4, *B. fragilis* fragment; 5, pJS139.1 Ap^rGlnA⁻ (*Xho*I deletion); 6, pJS139.2 Ap^rGlnA⁺ (*Stu*I deletion).

Fig. 2. Hybridization of ³²P-labelled pJS139 with *B. fragilis* and *E. coli* DNA. (a) Ethidium-bromide-stained agarose gel electrophoresis of restriction digests of plasmid and chromosomal DNA: lane 1, pJS139-*Xho*I; 2, λ -*Pst*I; 3, *E. coli* HB101-*Xho*I; 4, *B. fragilis* BF-1-*Xho*I; 5, λ -*Bst*EII. (b) Autoradiograph of a Southern blot of the gel in (a): lanes 1 and 2, 24 h exposure; lanes 3, 4 and 5, 48 h exposure. The hybridization probe was ³²P-labelled pJS139, to which ³²P-labelled λ DNA was added as an internal reference.

Characterization of the recombinant plasmid pJS139

The restriction map of pJS139 was obtained by complete single or double digestions with restriction endonucleases (Fig. 1). The location of the DNA region determining the GlnA⁺ phenotype was determined by the isolation of pJS139 deletion plasmids. The GS gene was located within a 4.2 kb fragment of the 8 kb insert, which was close to the junction of the *B. fragilis* DNA insert and the pEcoR251 DNA. Excision of a 2.7 kb fragment of DNA from this region using *Xho*I abolished the GlnA⁺ phenotype (pJS139.1), while excision of a 4.0 kb plasmid fragment bounded by *Stu*I restriction sites (pJS139.2) did not cause the loss of the GlnA⁺ phenotype.

The origin of the 8 kb insert in pJS139 was determined by Southern blotting and DNA hybridization between *B. fragilis* chromosomal DNA and ³²P-labelled pJS139 (Fig. 2). The

Table 2. *Relative levels of GS and histidase activity*

Strains were grown in CSH glucose minimal medium (Miller, 1972) with 15 mM-glutamate as nitrogen source and then diluted in minimal medium supplemented as follows: H, 15 mM-glutamate, 15 mM-glutamine; L, 15 mM-glutamate, 0.15 mM-glutamine; N, 1 g (NH₄)₂SO₄ l⁻¹ in CSH minimal medium; F, nitrogen-free CSH minimal medium. Samples were assayed after 3 h incubation at 37 °C in shake flasks. Histidase activity is defined in Methods. GS activity is expressed as $\mu\text{mol } \gamma\text{-glutamyl hydroxamate formed min}^{-1} (\text{mg protein})^{-1}$. SEM values were 5–10% of the values in the Table.

Strain	Medium	GS activity				Histidase activity		
		H	L	N	F	H	L	F
YMC-10 GlnA ⁺ Ntr ⁺		0.08	1.24	1.1	2.49	12.55	24.25	32.85
YMC-11 <i>glnA ntr</i>		0	0	0	0	6.35	6.65	15.55
YMC-11 pJS139 GlnA ⁺ <i>ntr</i>		0	0.05	1.25	4.15	7.65	6.6	14.3

plasmid pJS139 has two *Xho*I fragments of approximately 9.3 and 2.7 kb. The 2.7 kb fragment is internal to the putative *B. fragilis glnA* insert, but is adjacent to the junction between the vector and insert DNA. Therefore ³²P-labelled pJS139 was hybridized to both *B. fragilis* chromosomal DNA and pJS139 digested with *Xho*I: a positive 2.7 kb hybridization signal was detected in both digests (Fig. 2a, lanes 2 and 5). A second band of hybridization of approximately 10 kb was also observed. No DNA hybridization was detected between ³²P-labelled pJS139 and a *Xho*I digest of chromosomal DNA from the *E. coli* HB101 *glnA*⁺ strain.

Regulation of the cloned GS activity

GS activity was readily detectable in *E. coli* YMC-11 containing pJS139 and *E. coli* YMC-10 grown under nitrogen-limiting conditions, while no GS activity was detected in extracts of *E. coli* YMC-11 or ET8051 grown under any conditions (Table 2).

GS activity in *E. coli* YMC-11 containing pJS139 was partly repressed by ammonium salts but was fully repressed by glutamate and glutamine. GS activity of the *E. coli* YMC-10 wild-type strain was partly repressed by glutamate and ammonium salts but was fully repressed by glutamine (Table 2).

In *E. coli* and *Salmonella typhimurium* the *ntrB* and *ntrC* genes, which are closely linked to the GS gene, regulate not only the GS gene but also a high-affinity glutamine and a high-affinity arginine transport system (Kustu *et al.*, 1979). These *ntr* genes also activate the histidine utilization operon (Magasanik, 1982).

The ability of the various *E. coli* strains to grow on minimal medium containing low concentrations of arginine and glutamine was determined: while the ET8051 and YMC-11 *glnA* deletion strains were unable to grow on these media, when these strains contained the recombinant plasmid pJS139 very weak growth was obtained. In comparison, the YMC-10 *glnA*⁺ *ntrB*⁺ *ntrC*⁺ strain grew well under these conditions.

The *E. coli* strains YMC-10 and YMC-11 carry a *Klebsiella aerogenes hut* operon (Tuli *et al.*, 1982) that has a *hutC* mutation resulting in high basal levels of histidase. The results in Table 2 show that this enzyme level was increased in the YMC-10 strain by the *ntrB* and *ntrC* co-regulation of the *hut* operon (Tuli *et al.*, 1982) in low concentrations of glutamine, but that this was not observed with the YMC-11 *glnA ntrB ntrC* or the YMC-11(pJS139) *glnA*⁺ strains. Increased levels of histidase were observed with all strains when cells were suspended in nitrogen-free minimal medium.

The β -lactamase activities of *E. coli* YMC-11(pJS139) grown in minimal medium with and without glutamine (15 mM) were similar (35.1 and 37.8 units mg⁻¹ respectively).

Purification of the cloned GS gene product

The specific activity of GS increased during the purification which resulted in a 280-fold purification of the enzyme (Table 3). GS activity was not precipitated by 4% (w/v) PEG under

Fig. 3. SDS-PAGE of cell extracts and purified, cloned GS from *E. coli* cells. The gel contained 10% (w/v) polyacrylamide and was 0.5 mm thick. After electrophoresis as described in Methods it was stained with Coomassie brilliant blue. The samples were reduced and treated with SDS to remove secondary and tertiary structures. Lane 1, M_r markers; lane 2, *E. coli* YMC-10 cell lysate (20 μ g); lane 3, *E. coli* YMC-11 cell lysate (10 μ g); lane 4, *E. coli* YMC-11(pJS139) cell lysate (20 μ g); lane 5, peak GS activity fraction from Sephacryl S-1000 (~ 1 μ g).

Table 3. Purification of the cloned *B. fragilis* GS enzyme from *E. coli* YMC-11(pJS139)

Total GS activity was determined by the γ -glutamyl transferase assay. The details of the purification and assay procedures are described in Methods. GS activity is expressed as μ mol γ -glutamyl hydroxamate min^{-1} . Fraction 1 and fraction 5 correspond to lanes 3 and 5 of Fig. 3.

Fraction	Vol. (ml)	Total protein (mg)	Total GS activity	Specific GS activity (per mg protein)	Purification (-fold)
1. Ultrasonic extract	25	175	62.5	0.36	1
2. 4% PEG supernatant	25	152.5	59.4	0.39	1.09
3. 6% PEG supernatant	25	131	10.2	0.08	0.22
4. 6% PEG precipitate	1.5	36	133.9	3.72	10.41
5. S-1000 peak	1.0	0.7	71	101.4	284.3

these conditions but was precipitated by 6% (w/v) PEG. It was necessary to increase the pH of the solution in which the 6% PEG pellet was resuspended to pH 8.0 to dissolve the pellet. An increase in total GS activity following 6% PEG precipitation was observed.

SDS-PAGE of cell lysates showed an increase in the M_r 75–90 000 protein content of crude cell extracts of *E. coli* YMC-11(pJS139), but not in extracts from *E. coli* YMC-10 wild-type or YMC-11GlnA⁻ strains. This is the protein which is purified after Sephacryl chromatography (Fig. 3). Electrophoresis in 8% SDS-PAGE showed the apparent M_r of this polypeptide to be approximately 75 000.

Rabbit antibodies to this protein gave a single band in an Ouchterlony plate and showed homology to a single protein in a crude extract of the *E. coli* YMC-11(pJS139) strain, but no cross-reaction with cell extracts from *E. coli* YMC-10 GlnA⁺ or YMC-11 GlnA⁻ was observed.

Western blot analysis showed a weak cross-reactivity between the antiserum prepared against the purified cloned *B. fragilis* GS and a polypeptide of the same M_r as *E. coli* GS.

DISCUSSION

A cloned *B. fragilis* *glnA* gene was shown to function in *E. coli* $GlnA^-$ deletion mutants: this is the first report of the expression of a gene from the medically important, obligate anaerobe, *B. fragilis* in *E. coli*.

The origin of the *glnA* gene was confirmed by DNA hybridization. The gene directed the synthesis in *E. coli* glutamine auxotrophs of a *B. fragilis* GS which was enzymically active.

The cloned GS gene appeared to be expressed from a promoter contained within the inserted *B. fragilis* DNA segment of pJS139 as it is unlikely that a plasmid promoter would be regulated by nitrogen levels. In addition, *E. coli* YMC-11(pJS139) containing pcI_{857} Kan^r and λ -repressor(ts), produced the same levels of GS per mg of protein when incubated in nitrogen-free minimal medium at 23 °C and 39 °C, which indicated that the cloned *B. fragilis* *glnA* gene was not expressed from the rightward λ -promoter present on the vector.

The purified *B. fragilis* GS subunit had an apparent M_r of approximately 75000. Assuming that the enzyme is the typical dodecamer, then the particle M_r for the undissociated GS would be approximately 900000, a value larger than that reported for other bacteria. *E. coli*, *Bacillus subtilis*, *Rhizobium japonicum* and *Vibrio alginolyticus* have subunits with M_r values of 50000, 56000, 60000 and 60000 respectively (Stadtman & Ginsberg, 1974; Deuel *et al.*, 1970; Bhandari *et al.*, 1983; Bodasing *et al.*, 1985).

DNA hybridization experiments indicated that there was less than 50% DNA homology between the *B. fragilis* *glnA* gene and the *E. coli* *glnA* gene. Although no cross-reactivity was detected by the Ouchterlony immunoprecipitation, Western blotting detected weak cross-reactivity between antiserum raised to the purified cloned *B. fragilis* GS and *E. coli* GS. Lack of DNA homology but antigenic cross-reactivity was reported for the *glnA* gene cloned from the Gram-negative chemolithotroph *Thiobacillus ferrooxidans* and the *E. coli* *glnA* gene and GS (Barros *et al.*, 1985, 1986). These observations extend the findings that GS enzymes from Gram-negative bacteria are antigenically related (Tronick *et al.*, 1973).

The *B. fragilis* *glnA* gene was subject to nitrogen repression in *E. coli*. The regulation by nitrogen affected the *glnA* gene and was not due to an increase in plasmid copy number as a result of growth in the different media. The levels of another plasmid gene product, β -lactamase, produced by *E. coli* YMC-11(pJS139) and assayed after growth on the different nitrogen media, did not show any variation [35–38 U (mg protein⁻¹)] in the samples assayed.

The regulation of the cloned *B. fragilis* *glnA* gene differed from the regulation of the *E. coli* *glnA* gene in the wild-type strain: the cloned *B. fragilis* *glnA* gene was repressed by glutamate whereas the *E. coli* *glnA* gene was expressed in the presence of glutamate. Since the *glnA* gene on pJS139 was regulated by nitrogen it is suggested that some nitrogen regulatory activities are located on pJS139 but that these activities are not directly analogous to the *ntrB* and *ntrC* system of the enteric bacteria.

In comparison with the *E. coli* wild-type strain the cloned *B. fragilis* DNA fragment only enabled weak growth of the *E. coli* *glnA ntrB ntrC* deletion mutant on media containing arginine or low concentrations of glutamate as sole sources of nitrogen and it was not able to activate the *Klebsiella hut* operon in *E. coli* YMC-11.

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