

Use of a Wild-Type Gene Fusion To Determine the Influence of Environmental Conditions on Expression of the S Fimbrial Adhesin in an *Escherichia coli* Pathogen

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S fimbrial adhesins (Sfa) enable pathogenic *Escherichia coli* strains to bind to sialic acid-containing eucaryotic receptor molecules. In order to determine the influence of culture conditions on the expression of the *sfa* determinant in a wild-type strain, we fused the gene *lacZ*, coding for the enzyme β -galactosidase, to the *sfaA* gene, responsible for the major protein subunit of S fimbriae. By using a plasmid which carries an R6K origin, the *sfaA-lac* hybrid construct was site-specifically integrated into the chromosome of the uropathogenic *E. coli* strain 536WT. The expression of *lacZ*, which was under the control of the *sfa* wild-type promoters, was now equivalent to the *sfa* expression of strain 536WT. With the help of this particular wild-type construct, it was demonstrated that the *sfa* determinant is better expressed on solid media than in liquid broth. The growth rate had a strong influence on Sfa expression under aerobic but not under anaerobic conditions. Production of Sfa was further regulated by catabolite repression, osmolarity, and temperature.

Escherichia coli strains are able to cause infections of the bladder and the kidney (urinary tract infections) and cases of newborn meningitis (26, 37). Such extraintestinal *E. coli* strains express different kinds of fimbrial adhesins, which enable bacteria to colonize eucaryotic tissues (11, 19). Fimbrial adhesins can be distinguished by their receptor specificity. Type I fimbriae bind to mannose-containing receptors and are expressed by both pathogenic and nonpathogenic *E. coli* strains (10, 23). P fimbriae, which are predominantly found among uropathogenic *E. coli* strains, are able to recognize α -D-Gal-(1-4)- β -D-Gal moieties of glycolipids located on different cells (4, 22). In contrast, S fimbrial adhesins (Sfa) facilitate binding of bacteria to receptors containing α -sialic acid-(2-3)- β -Gal residues (16, 41). S fimbriae are commonly found on *E. coli* K1 strains causing newborn meningitis and on uropathogenic O6 isolates (18, 26, 38).

The genetic determinant coding for Sfa was cloned from the chromosome of a uropathogenic O6 strain and genetically characterized (16, 18, 35, 39, 46). It was demonstrated that Sfa directly contribute to nephropathogenicity in rats (30). They enable *E. coli* strains to bind to tissue sections of the human urinary tract and of the rat brain (40, 53). Convincing evidence exists that Sfa are not produced constitutively; rather, the expression of Sfa varies during the infectious process and after cultivation in vitro (44). Therefore, it is speculated that different environmental conditions may influence the production of S fimbriae in wild-type strains.

In order to determine the influence of such environmental signals accurately, we have constructed an SfaA-Lac translational wild-type fusion protein. This gene fusion, which is under the control of the *sfa* promoter region on the chromo-

some of the wild-type pathogen *E. coli* 536WT, was used here as a tool for measuring the influence of different environmental signals on the expression of Sfa.

MATERIALS AND METHODS

Bacterial strains used. From the uropathogenic *E. coli* strain 536WT (O6:K15:H31), a *sfaA-lac* wild-type gene fusion was isolated. Strain 536WT has been characterized elsewhere (16a, 24). As a prerequisite for the isolation of an *sfa-lac* wild-type fusion, we isolated a Lac⁻ mutant from the Lac⁺ strain 536WT. Plasmid pRT733, which carries a *TnphoA* element (34, 50), was introduced into strain 536WT. Since pRT733 is unable to replicate in strain 536WT, kanamycin-resistant mutants must carry *TnphoA* inserted into the chromosome. The Lac⁻ mutant 536-9B4 was detected after cultivation on MacConkey agar plates as well as on agar plates containing X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) and IPTG (isopropyl- β -D-thiogalactopyranoside). Chromosomal DNA was isolated from strain 536-9B4 and hybridized with *lacZ*- as well as with Tn5-specific radioactively labeled DNA probes. The hybridizations showed that the *TnphoA* element was inserted directly into the *lacZ* locus. DNA isolated from strain 536-9B4 but not from 536WT specifically reacted after hybridization with a DNA probe which contained Tn5 sequences (data not shown). Strain 536-17B1 was used as an Sfa⁻ control (Ott and Hacker, unpublished results). The *E. coli* K-12 strain SM10 (λ *pir*) was used for conjugation experiments (50).

Plasmids used. Plasmids pJM703-1 (Ap^r) and pRT733 (Ap^r Km^r *TnphoA*), which carry the R6K origin (34, 50), were mobilized into strain 536, which is a Pir⁻ strain. The recombinant plasmids are able to replicate only in the presence of the Pir protein; therefore, they are not able to survive in strain 536WT. Plasmid pDS410 was used for the isolation of Tn5-specific sequences (7); pANN801-13 is an Sfa⁺ plasmid (18); pANN1403-381 carries a site-specific *sfaA-lac* fusion (5, 16, 17; T. Schmoll, J. Morschhäuser, M.

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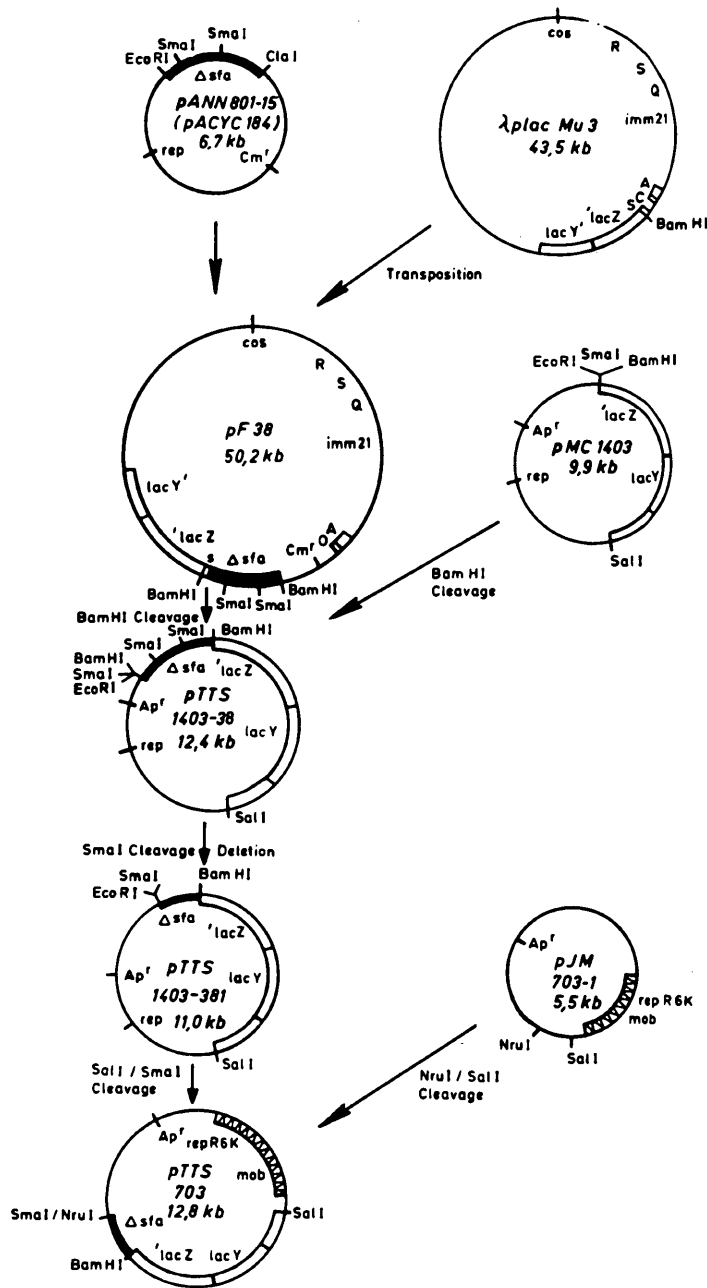


FIG. 1. Construction of plasmid pTTS703, which was used for the site-specific insertion. A λ lac Mu3 fusion with plasmid pANN801-15, containing the proximal part of the *sfa* gene cluster, was constructed. The *sfa*-specific sequences of the resulting construct pF38 were ligated into the *Bam*HI site of pMC1403, leading to plasmid pTTS1403-38. A further deletion of the *sfa*-specific *Sma*I fragment, carrying the *sfa* promoters, generated plasmid pTTS1403-381. The *Sal*I-*Sma*I fragment of this construct was then cloned into the pJM703-1 suicide vector, resulting in plasmid pTTS703, which could then be mobilized into strain 536-9B4 (see Fig. 2).

Ott, B. Ludwig, J. Van Die, and J. Hacker, *Microb. Pathog.*, in press). The recombinant plasmid pTTS703, which consists of the vector pJM703-1 and promoterless *sfa* sequences fused to *lacZ*, was used for a site-specific integration of Δ *sfaA-lacZ* sequences into the chromosomally encoded *sfa* determinant of strain 536-9B4. The construction of plasmid

pTTS703 is indicated in Fig. 1. As a prerequisite, plasmid pF38 was cleaved with *Bam*HI. Plasmid pF38 consists of the phage λ lac Mu-3, and the recombinant DNA pANN801-15 carries the proximal part of the *sfa* determinant (17, 18). A *Bam*HI fragment (2.7 kilobases [kb] in size) of pF38 containing the 5' end of the *sfa* determinant, including its promoter

region, and the structural gene *sfaA* was ligated into the vector pMC1403 to construct plasmid pTTS1403-38 (5). The deletion of an *Sma*I fragment containing the *sfa* promoter sequences from plasmid pTTS1403-38 resulted in plasmid pTTS1403-381. The latter was used to insert the promoterless Δ *sfaA-lacZ*-containing *Sma*I-*Sal*I fragment into the suicide vector pJM703-1.

Media, chemicals, and enzymes. Bacteria were grown in LB (Luria-Bertani) broth as described before (18). For fermentor experiments, LB broth was supplemented with 5 mM potassium phosphate buffer.

The recombinant clones were cultivated under selective antibiotic pressure with ampicillin (50 μ g/ml), chloramphenicol (20 μ g/ml), and tetracycline (20 μ g/ml). Radiochemicals were purchased from New England Nuclear Corp., and antibiotics were a gift from Bayer, Leverkusen, FRG. Restriction enzymes and T4 ligase were purchased from New England BioLabs, Beverly, Mass.

For the detection of Lac⁺ colonies, MacConkey agar plates or LB-X-Gal-IPTG medium (containing 0.01% X-Gal and 0.05 mM IPTG) was used. All other chemicals were obtained from Merck, Darmstadt, FRG.

Growth conditions and fermentor modes. To study the influence of the specific growth rate (μ) of bacteria on Sfa production, strain 536-9B4/12 was grown in 500-ml continuous chemostat cultures at 37°C in LB medium and at a controlled pH of 7.0 as described before (52). The O₂ level was kept constant, and the experiments were done under selective pressure. The doubling times were similar after aerobic and anaerobic cultivation (35 to 40 min), indicating nearly identical maximal growth rates (μ_{max}) of about 1.0 h⁻¹ after growth under both conditions. The expression of Sfa was measured after 40 to 47 h of incubation at steady-state conditions with specific growth rates (μ) of 0.1 h⁻¹ and after 10 to 15 h, when the cultures were at a μ of 0.6 to 0.7 h⁻¹. The influence of catabolite repression on Sfa production was measured after growing strain 536-9B4/12 on solid LB medium with and without 0.4% glucose and/or 1 mM cyclic AMP. The influence of osmolarity and temperature on Sfa expression was also measured after cultivation of bacteria on LB agar plates.

Recombinant DNA techniques. Isolation of plasmid DNA was carried out as described before (3, 15). Cleavage of DNA was done under conditions described by the manufacturer. Gel electrophoresis was carried out in 0.7 to 1.0% agarose. Isolation and purification of DNA fragments from agarose gels were performed by electroelution (29). *E. coli* strains were transformed by the CaCl₂ method (27). Ligations were carried out under standard conditions (29).

TnphoA mutagenesis. Conjugative crosses between the donor strain SM10(pRT733) (λ *pir*) and the recipient strain 536WT were carried out as described before (34). The mating samples were streaked onto agar medium containing streptomycin (100 μ g/ml) and kanamycin (50 μ g/ml). 536WT variants which exhibited resistance to streptomycin and kanamycin but not to ampicillin (100 μ g/ml) should carry a TnphoA element integrated into the chromosome.

Immunocolony dots. Immunocolony dots were carried out by the method of Van Die et al. (51). Anti-S-fimbriae antibodies were prepared from rabbits inoculated with purified S fimbrial protein eluted from a sodium dodecyl sulfate (SDS)-polyacrylamide electrophoresis gel (35).

Generation of DNA probes. The *sfa*-specific DNA probe was an 8.5-kb *Eco*RV fragment derived from the plasmid pANN801-13 (18). Plasmid pDS410 contains the 3.4-kb *Hind*III fragment of Tn5 in pBR322, which was used as a

probe (7). A *lac*-specific probe was isolated from the vector pMC1403 as a 6.2-kb *Eco*RI-*Sal*I fragment (5). Only probes which were eluted from agarose gels were used in Southern hybridizations.

Nick translation, Southern hybridization, and autoradiography. The DNA probes were labeled by nick translation with a mixture of all four [α -³²P]-labeled deoxynucleoside triphosphates as described previously (42). Southern transfer, washing, and autoradiography of the filters were carried out as described previously (29, 47). The filters were hybridized in 50% formamide at 43°C for 3 days. Stringent conditions were used for the washing procedure: 30 min at room temperature in 2 \times SSC (1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0]) containing 0.1% SDS and then four times for 30 min at 56°C in 0.1 \times SSC-0.1% SDS.

β -Galactosidase test. β -Galactosidase units were determined by the method of Miller (32). Only bacterial populations with equal amounts of cells (OD₆₀₀, 0.8 to 1.0) were compared.

Statistics. Mean values \pm standard deviation were calculated by the method of Cavalli-Sforza (6).

RESULTS

Construction of the wild-type *sfaA-lac* fusion 536-9B4/12. The construction of the translational fusion strain 536-9B4/12 is shown in Fig. 2. First, the recombinant plasmid pTTS703, which was unable to replicate in strain 536-9B4, was transferred into the Lac⁻ mutant. In order to select variants with pTTS703 inserted into the chromosomally encoded *sfa* determinant, ampicillin-resistant transconjugants were tested in an immunocolony dot assay with a monospecific polyclonal antiserum raised against the S fimbrial subunit protein (Table 1). Two of 12 ampicillin-resistant 536-9B4 variants tested were negative in the immunocolony dot assay. This result indicates that pTTS703 had integrated into the *sfa* gene cluster.

To determine whether pTTS703 was integrated into the *sfaA* gene of strain 536-9B4, DNA from the Sfa⁻ variant 536-9B4/12 together with DNA from strains 536WT and 536-9B4 was probed with a radioactively labeled *sfa*-specific DNA fragment. As demonstrated in Fig. 3, the strains 536WT and 536-9B4 (Lac⁻) hybridized with two *Eco*RI fragments of the chromosomal DNAs. The smaller *Eco*RI fragment (4.8 kb in size) represents the 5' end of the *sfa* determinant and includes the structural gene *sfaA*, coding for the major fimbrial subunit (38, 46). This fragment was absent in the Sfa⁻ strain 536-9B4/12. However, the appearance of two *Eco*RI fragments of 7.0 and 14.4 kb indicates a site-specific integration of pTTS703 into the *sfaA* gene of strain 536-9B4/12.

Influence of solid and liquid media on the expression of *sfa*. *E. coli* 536WT and 536-9B4/12, which carries the *sfaA-lac* fusion on the chromosome, were grown on solid medium and in a noncontinuous liquid culture. As shown in Fig. 4, the *lacZ* gene of 536-9B4/12 (which is under the control of the *sfa* promoter) was expressed four times better after cultivation on solid medium than after growth in liquid broth (449 versus 125 U of β -galactosidase activity). The expression of the *lac* operon of 536WT induced by IPTG (0.05 mM) was not influenced by the alteration of these growth conditions.

Influence of the specific growth rate under aerobic and anaerobic conditions on the expression of *sfa*. Strain 536-9B4/12 was grown under aerobic and anaerobic conditions in continuous cultures. β -Galactosidase activity was measured in cells cultured under steady-state conditions. As demon-

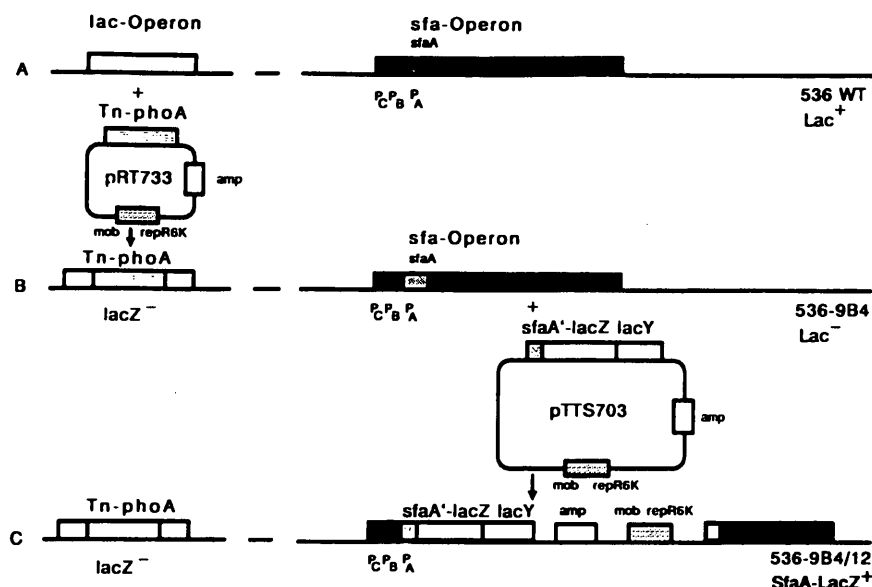


FIG. 2. Generation of the *Lac*⁻ derivative of the wild-type strain 536-9B4 by insertion of a *TnphoA* element into the *lac* gene cluster of strain 536WT (left part) and construction of the site-specific wild-type fusion 536-9B4/12 by integration of plasmid pTTS703 carrying the *lacZ* gene fused to *sfaA* into the chromosome of strain 536-9B4 (right part). The *sfa*-specific sequences are shown as black bars, with exception of the *sfaA* region, which is shown as a stippled box. The promoters *P_A*, *P_B*, and *P_C* are indicated below. Designations of strains are given, with the respective *Lac* phenotypes on the right side. Plasmids used for genetic manipulation are shown between the indicated genome structures of strains.

strated in Fig. 5, in one series of experiments, the specific growth rate (μ) was set at 0.09 to 0.1 h⁻¹. After growth under aerobic conditions, only 15 U were measured. In contrast, under anaerobic conditions, 160 U were detected. After strain 536-9B4/12 was grown at a specific growth rate of 0.6 to 0.7 h⁻¹, the *sfa* determinant was expressed under aerobic as well as under anaerobic conditions (160 versus 210 U). These data indicate that the specific growth rate has a strong influence on Sfa expression under aerobic conditions. Under anaerobic conditions, the specific growth rate had only a small effect on Sfa production.

sfa determinant is regulated by catabolite repression. In order to examine whether the production of Sfa is regulated by catabolite repression, β -galactosidase production by strain 536-9B4/12 was measured after cultivation in the presence or absence of 0.4% glucose. As demonstrated in Fig. 6, the presence of glucose had a negative effect on the expression of the wild-type *Sfa* gene cluster. While 565 U of β -galactosidase were measured after incubation of 536-

9B4/12 on a glucose-free medium (Fig. 6A), 45 U were produced after growth on a medium containing 0.4% glucose (Fig. 6B). Production of β -galactosidase was restored after 1 mM cyclic AMP was added to the medium (Fig. 6C), indicating that the dramatic effect of glucose on Sfa expression depends on catabolite repression. Glucose and cyclic AMP also had a strong effect on the expression of the *lac* determinant of the wild-type strain 536WT, which was induced by IPTG (0.05 mM).

Influence of osmolarity on the expression of *sfa*. To study the influence of osmolarity on Sfa expression, strains 536-9B4/12 and 536WT were grown on LB plates containing different amounts of sodium chloride. As shown in Fig. 7, the *sfa* gene cluster was optimally expressed after cultivation on plates with 43 mM NaCl. The production of β -galactosidase by strain 536-9B4/12 decreased with increasing amounts

TABLE 1. Production of Sfa and β -galactosidase by *E. coli* 536WT and mutants^a

Strain	Phenotype	
	Sfa ⁺	Lac ⁺
536WT	+	+
536-9B4	+	-
536-9B4/12	-	+ ^b
536-17B/1 (control)	-	+

^a Sfa⁺ was tested in an immunocolony dot assay with anti-SfaA antiserum. Lac⁺ was tested after cultivation of strains on MacConkey and X-Gal-IPTG plates.

^b β -Galactosidase production depended on the regulatory signals of the *sfa* gene cluster.

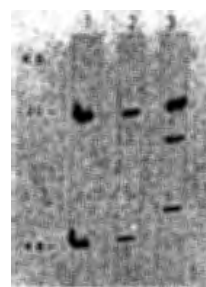


FIG. 3. Southern blot hybridization patterns of *EcoRI*-cleaved genomic DNAs of *E. coli* strains 536WT (Sfa⁺ Lac⁺, lane 1), 536-9B4 (Sfa⁺ Lac⁻, lane 2), and 536-9B4/12 (*sfaA-lac* Sfa⁻ Lac⁺, lane 3). The DNAs were hybridized to nick-translated DNA probe specific for *sfa* (5, 7, 18) (see text). The respective alterations in the fragment pattern are marked (X).

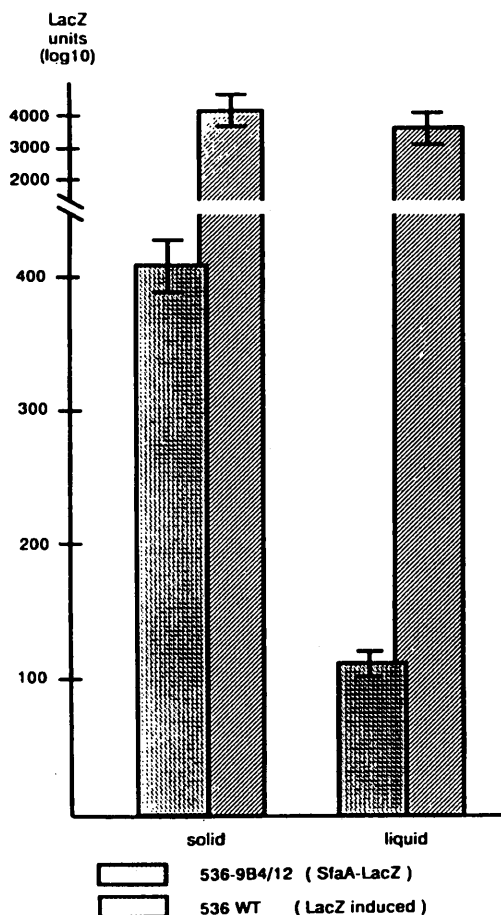


FIG. 4. β -Galactosidase (LacZ) production, given as LacZ units on a logarithmic scale for wild-type strain 536WT and the fusion strain 536-9B4/12 grown on solid or liquid medium under aerobic conditions. LacZ production by 536WT was induced by addition of 0.05 mM IPTG. Mean values \pm standard deviation are given.

of NaCl. The expression of the *lac* wild-type determinant induced by IPTG (0.05 mM) was only slightly affected by the differences in the NaCl content of the medium.

Influence of temperature on the expression of *sfa*. Strains 536-9B4/12 and 536WT were grown at different temperatures. As shown in Fig. 8, optimal expression of β -galactosidase, representing Sfa production, was obtained at 37°C (471 U). At 20°C, no production of β -galactosidase was detected. After a temperature shift from 37 to 43°C, the values dropped significantly to 23 U. As a control, the β -galactosidase production of 536WT induced by IPTG (0.05 mM) was estimated; it was not significantly influenced by temperature.

DISCUSSION

In this report we describe the construction and characterization of a pathogenic *E. coli* wild-type strain carrying a gene fusion of the fimbrial determinant *sfa* and the *lacZ* indicator gene. The *sfa* gene cluster codes for Sfa, which enable extraintestinal *E. coli* strains to bind to sialic acid-containing receptor structures (26, 41). The wild-type construction 536-9B4/12 is identical to the uropathogenic strain 536WT with the exception of a *TnphoA* insertion in the

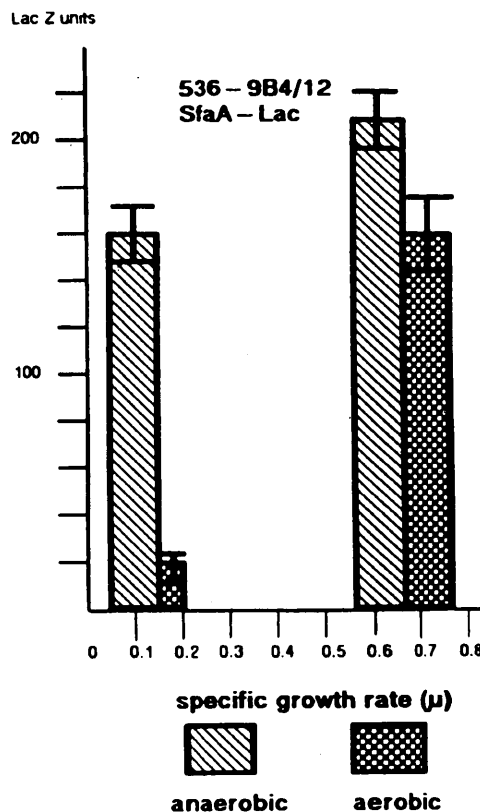


FIG. 5. β -Galactosidase (LacZ) production, given as LacZ units, of the fusion strain 536-9B4/12 grown in a continuous culture under aerobic and anaerobic conditions at different specific growth rates (μ). Mean values \pm standard deviation are given.

original *lacZ* gene and the fusion of a second *lacZ* locus to the *sfa* gene cluster.

Compared with gene fusions constructed in multicopy systems (1, 14) or in the *E. coli* K-12 chromosome (4), or with immunological approaches with pathogenic strains (9, 43), the wild-type gene fusion shows several advantages. (i) An undesirable multicopy effect, which could counteract the regulatory events under question in plasmid systems, is excluded. (ii) *E. coli* wild-type strains, such as strain 536WT, but not *E. coli* K-12 isolates may carry more than one fimbrial adhesin determinant, which may have the capacity to *trans*-complement each other (13, 16a, 19). Such complementation processes may influence the expression of adhesin gene clusters. (iii) Pathogenic *E. coli* wild-type isolates and *E. coli* K-12 laboratory strains differ in their genome structure (16a, 19). It has been shown that pathogenic strains carry large DNA regions (pathogenicity DNA islands) harboring virulence determinants. These DNA regions, which are absent in *E. coli* K-12, may carry additional *trans*-regulatory genes influencing the expression of virulence gene clusters (24). (iv) The cell surfaces of wild-type strains in several aspects, including the presence of capsules and O-repeating units, which are present in pathogenic but absent in K-12 strains. These constituents of the cell surface influence the uptake of ions and other substances which are known to have an effect on gene expression (48). (v) Environmental stimuli may influence the expression of genes as well as the

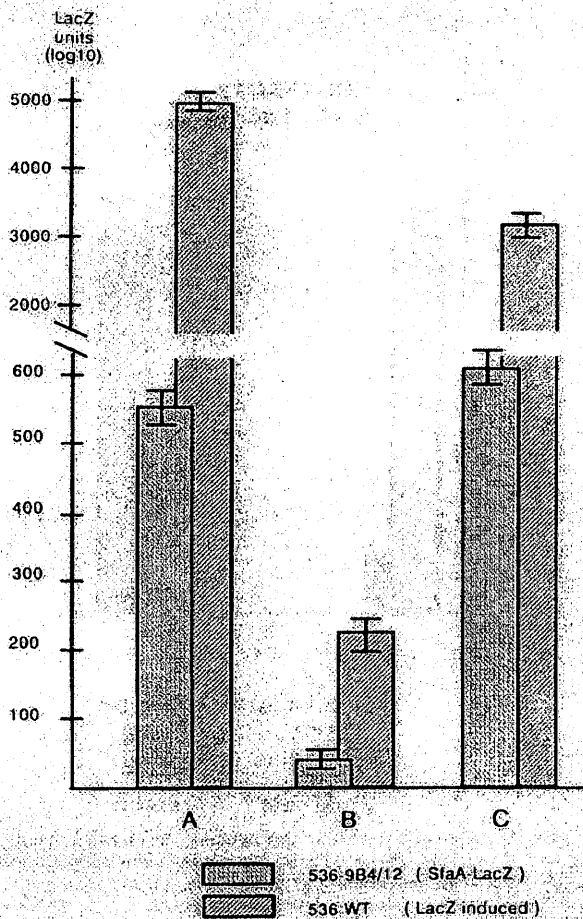


FIG. 6. β -Galactosidase (LacZ) production, given as LacZ units on a logarithmic scale, by the wild-type strain 536WT and the fusion strain 536-9B4/12 grown on solid medium without glucose (A), with 0.4% glucose (B), and with 0.4% glucose plus 1 mM cyclic AMP (C) under aerobic conditions. LacZ production by 536WT was induced by addition of 0.05 mM IPTG (see text). Mean values \pm standard deviation are given.

transport and assembly processes of subunit proteins into fimbrial structures (20, 43). With the help of the translational fusion used here, however, the direct effects of physiological signals on the expression of the Sfa in the wild-type isolate can be measured.

In general, the expression of bacterial pathogenicity determinants is affected by multiple physiological signals (8, 11). The production of Sfa directly depends on the specific growth rate (μ) of strain 536WT. Significant differences were observed between aerobic and anaerobic growth conditions. While at high μ the expression under both conditions was high, at low μ the *sfa* gene cluster was expressed only under anaerobic conditions. The influence of oxygen on *sfa* expression, however, seems to be restricted to steady-state conditions and was not observed after growing the wild-type strain in batch cultures (Hacker, unpublished results). It is interesting that the expression of Sfa is further induced after cultivation of strains on solid medium. Such an induction of genes by surface cultivation was recently observed for the lateral flagella gene cluster of *Vibrio parahaemolyticus* (2). It can be speculated that in both cases an attachment of

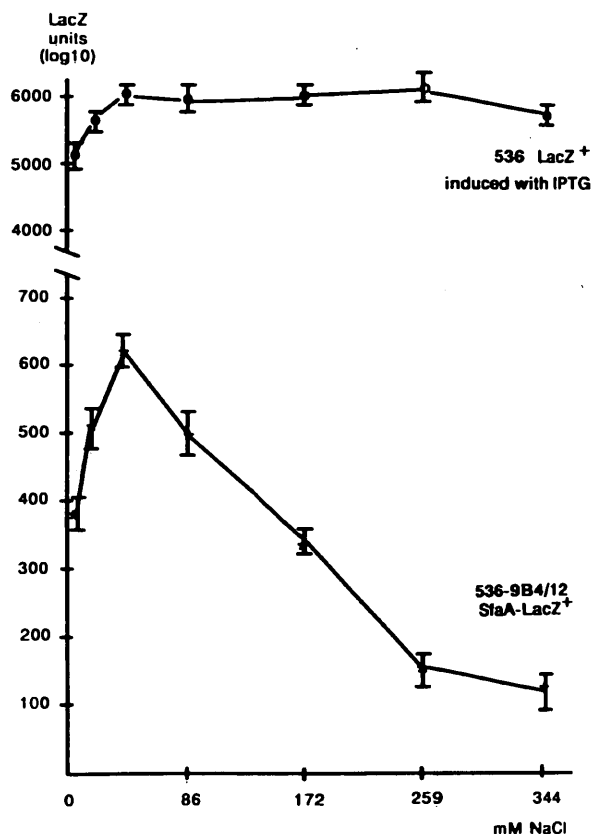


FIG. 7. Influence of the NaCl content of the medium on β -galactosidase (LacZ) production, given as LacZ units on a logarithmic scale, by the wild-type strain 536WT and the fusion strain 536-9B4/12 grown on solid medium under aerobic conditions. LacZ production by 536WT was induced by addition of 0.05 mM IPTG (see text). Mean values \pm standard deviation are given.

organisms, which is a prerequisite for the formation of microcolonies, triggers full expression of these gene clusters, which are necessary for settlement of the corresponding organisms on surfaces.

Osmolarity represents another physiological parameter influencing the expression of S fimbriae in a wild-type organism. It has been demonstrated that the salt content of the medium also affects the expression of virulence factors of *Vibrio cholerae*, such as cholera toxin, adhesin and colonization factors, and outer membrane proteins (34). The salt concentration may monitor the adaptation of the bacteria either to the hostile environment or to living conditions outside the host organisms. Another physiological trigger by which microorganisms discriminate between different natural environments is temperature (21, 31). It has been shown that the expression of the Sfa is strictly adapted to the temperature of the human body. Such a stringent temperature adaption to 37°C was also shown for other adhesins of pathogenic *E. coli*, like P fimbriae (14) and adherence factors produced by intestinal *E. coli* strains (8, 54). On the other hand, a new type of a fibronectin-binding adhesin termed curly is expressed but not efficiently assembled at 37°C, but is produced at 26°C (36). It is speculated that curly adhesins, in contrast to the S fimbriae, may be adapted to a nonhostile environment for *E. coli* strains.

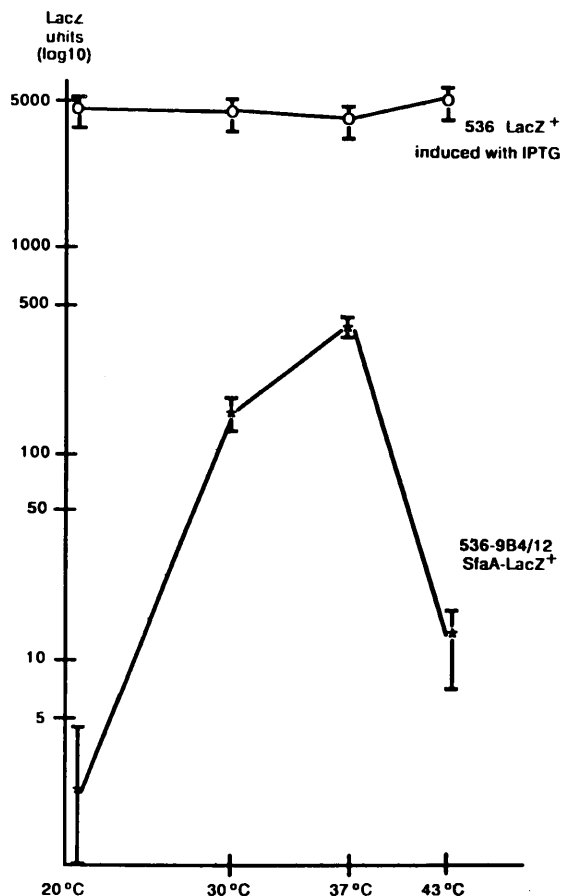


FIG. 8. Influence of growth temperature on β -galactosidase (LacZ) production, given as LacZ units on a logarithmic scale, by the wild-type strain 536WT and the fusion strain 536-9B4/12 grown on solid medium under aerobic conditions. LacZ production by 536WT was induced by addition of 0.05 mM IPTG (see text). Mean values \pm standard deviation are given.

It was further demonstrated that the presence of glucose negatively influenced the expression of the *sfa* gene cluster. This repression could be reversed by the addition of cyclic AMP to the culture. Therefore, the expression of *sfa* shows the characteristic features of catabolite repression (28). It is interesting that a fairly good binding site for the catabolite repression protein exists within the *sfa* promoter region of strain 536WT (Schmoll et al., unpublished data). Since *E. coli* P fimbrial gene clusters are also controlled by catabolite repression (12), this mode of regulation might be a basis of linking the expression of a set of virulence-associated genes in a coordinate way. Such *trans*-regulatory networks are found in different bacterial pathogens. For example, several virulence genes of *Vibrio cholerae* are coordinately regulated by the factor ToxR, a transmembrane protein which is able to bind to particular DNA regions upstream of virulence-associated genes (33, 50).

While ToxR-specific regulation depends on one regulatory element, the virulence genes of *Bordetella pertussis*, the causative agent of whooping cough, are regulated by a two-component system. One regulatory protein, located in the periplasmic space, receives stimuli from the environ-

ment, and following interaction with this component, a second factor acts directly as a DNA-binding protein on the promoter sequences of virulence genes (25, 33).

Other two-component systems have been described for the regulation of invasiveness of *Salmonella typhimurium*, the induction of plasmid transfer from *Agrobacterium tumefaciens* into plants, and the osmoregulation of *E. coli* outer membrane proteins (33, 49). In general, the *trans*-regulation of pathogenicity gene clusters, which brings unlinked gene clusters under the control of regulatory elements, represents an effective mechanism to adapt bacterial pathogens to certain environments and to distribute cellular energy in an optimal manner.

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