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Capsule Shields the Function of Short Bacterial Adhesins

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Bacterial surface structures such as capsules and adhesins are generally regarded as important virulence factors. Here we demonstrate that capsules block the function of the self-recognizing protein antigen 43 through physical shielding. The phenomenon is not restricted to *Escherichia coli* but can occur in other gram-negative bacteria. Likewise, we show that other short adhesins exemplified by the AIDA-I protein are blocked by the presence of a capsule. The results support the notion that capsule polysaccharides sterically prevent receptor-target recognition of short bacterial adhesins. This negative interference has important biological consequences, such as affecting the ability of bacteria to form biofilms.

All members of the *Enterobacteriaceae* are able to elaborate a layer of surface-associated polysaccharides called the capsule. The composition of these capsular polysaccharides is very much strain dependent. In *Escherichia coli* it may be one of the 80 distinct polysaccharides (designated the K antigens) or a polymer derived from the 170 different O antigens. In effect, whereas all polysaccharide K antigens form a capsule structure, the inverse is not always true: not all capsules are composed of K antigens (53).

Capsule polysaccharides provide protection against desiccation and attack from phages, however, they are first and foremost recognized virulence determinants. It has been known for years that some capsular antigens, notably K1 and certain types of O antigen, are strongly associated with extraintestinal infections such as septicemia, meningitis, and urinary tract infection (UTI) (19, 25, 28, 32, 46). *E. coli* strains of serotype K1 represent 80% of all *E. coli* strains isolated from cases of newborn meningitis and sepsis in humans. Also, K1 strains are often implicated in UTI. The K1 capsule is a linear homopolymer of sialic acid residues. The capsule is identical to the polysialic acid found on certain human cells and is poorly immunogenic due to molecular mimicry of host structures (49). Strains causing UTI, meningitis, and sepsis are generally resistant to the bactericidal action of human serum (reviewed in references 34, 53). Furthermore, K1 capsules are antiphagocytic, arguably a trait that would be helpful to a bacterium for survival in blood and tissue.

Another group of virulence factors is constituted by the bacterial adhesins, protein structures that recognize a wide range of molecular motifs and provide targeting of the bacteria to specific tissue surfaces in the host (for a recent review, see reference 24). While most adhesins recognize nonself targets present in the environment (e.g., a specific saccharide), some mediate self-recognition.

Antigen 43 (Ag43) is exceptional in being such a self-recognizing adhesin (15, 21). Ag43 is a surface protein that confers bacterial cell-to-cell aggregation, a trait that can be visualized macroscopically as flocculation and settling of cells from static liquid suspensions; hence, the gene name *flu* was originally coined for the corresponding genetic locus (12). In an independent study, a major *E. coli* outer membrane protein antigen was investigated by virtue of its aggregative properties and termed Ag43 (29). Only later was Ag43 identified as the product of the *flu* gene (15, 18). In line with the gene product name Ag43, the term *agn43* is also frequently used for the corresponding gene instead of *flu*. Ag43 expression confers a characteristic frizzy colony morphology (16). Expression is phase variable with switching rates of $\sim 10^{-3}$ per cell per generation under normal growth conditions due to the concerted action of the Dam methylase (positive regulation) and the OxyR redox sensor (negative regulation) (18, 38, 41, 52). Ag43 is an autotransporter protein, and all information required for traverse of the bacterial membrane system and final routing to the surface resides in the protein itself. This is also supported by the fact that Ag43 can be expressed in a wide range of gram-negative bacteria (20, 21). In *E. coli*, Ag43 is present in ~50,000 copies per cell (30). It is processed into two subunits, α and β, each constituting about half of the protein. The β subunit is an outer membrane component that presumably forms a pore through which Ag43α gains access to the surface. The α subunit remains attached to the cell surface via interaction with the β subunit but can be detached by brief heat treatment (18).

Expression of Ag43 dramatically enhances biofilm formation in bacteria (9, 21, 22, 23), and Ag43 expression in *E. coli* is specifically correlated with the biofilm mode of growth (39). Conversely, lesions in the *flu* gene causing abolishment of Ag43 expression in many cases result in cells with a very limited ability to form a biofilm (9, 22, 39). Biofilm formation is often correlated with bacterial virulence (8, 37). Expression of Ag43 was recently reported to be correlated with biofilm formation by uropathogenic *E. coli* during infection of bladder cells (1). Furthermore, Ag43-mediated cell aggregation was found to protect bacteria against killing agents like hydrogen peroxide (38). Ag43 is widespread in *E. coli*, and it is expressed in many pathogenic strains. A survey of enteropathogenic and UTI strains showed that 77 and 60%, respectively, of these were capable of Ag43 expression (31). Also, many strains possess duplex or multiple copies of the gene, as seems to be the case

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)	Reference or source
E. coli strains		
1177	UTI isolate (O1:K1:H7)	7
CN1016	1177 *fim*::*kan*	7
MG1655	K-12 reference strain	2
MS427	MG1655 *flu*	33
MS428	MG1655 *fim*	22
MS528	MG1655 *flu fim*	22
MS641	MG1655 *oxyR*::*kan*	This study
DD14	MS427 containing pKKJ128 and pBR322	This study
DD19	MS641 containing pBR322	This study
DD26	MS641 containing pKT274	This study
DD27	MS641 containing pIR100	This study
DD34	MS427 containing pACYC184 and pKT274	This study
DD35	MS427 containing pACYC184 and pIR100	This study
DD36	MS427 containing pACYC184 and pBR322	This study
DD37	MS427 containing pKKJ128 and pKT274	This study
DD38	MS427 containing pKKJ128 and pIR100	This study
MS718	MS428 containing pFCH2235	This study
PK1081	CN1016 containing pFCH2235	This study
K. pneumoniae strains		
C105	K35 capsule	45
C105 NCV	Spontaneous C105 NCV	45
LH71	C105 containing pIB264	This study
LH72	C105 NCV containing pIB264	This study
PK1013	C105 containing pHHA147	This study
PK1014	C105 NCV containing pHHA147	This study
PK1016	C105 containing pBR322	This study
PK1021	C105 NCV containing pBR322	This study
Plasmids		
pFHC2235	IPTG-inducible *dam* gene in pUC19[a]	Gift from F. G. Hansen
pHHA147	*flu* gene from MG1655 in pBR322	38
pIR100	*E. coli* K5 capsule gene operon in pBR322(cos4)	Gift from I. Roberts
pKKJ128	*flu* gene from MG1655 in pACYC184	21
pKT274	*E. coli* K1 capsule gene operon in pHC79	13
pKKJ101	*flu* gene in pUCP22	21
pIB264	AIDA-I autotransporter and glycosylation genes in pBR322	4

[a] IPTG, isopropyl-β-D-thiogalactopyranoside.

with enteropathogenic and enterohemorrhagic subtypes (23, 35, 48). Ag43 exhibits significant homology to several other members of the autotransporter protein family; for example, the primary structure of the AIDA-I adhesin, involved in diffuse adherence of enteropathogenic *E. coli* strains, shows ~31% identity to Ag43 (5).

Both Ag43 and capsules seem to play important roles in bacterial survival and pathogenicity. However, while Ag43 protrudes ~10 nm beyond the outer membrane (23), the capsule may extend 0.2 to 1.0 μm from the bacterial surface, depending on its type and composition. On one hand, the capsule constitutes a somewhat ephemeral structure that might not interfere with the close cellular contact required for intercellular Ag43 contact. On the other hand, it could be that the presence of a capsule would sterically shield Ag43 and abolish Ag43-Ag43-mediated cell aggregation. To resolve this apparent paradox, we investigated possible interference between Ag43 and capsule.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains and plasmids used in this study are listed in Table 1. Cells were grown at 37°C on solid or in liquid Luria-Bertani (LB) medium supplemented with the appropriate antibiotics unless otherwise stated.

Construction of an *oxyR*::*kan* mutant. An *E. coli* MG1655 *oxyR*::*kan* mutant was constructed by using the λ Red recombinase gene replacement system (10). Briefly, the kanamycin gene from plasmid pKD4 was amplified by primers containing 40-nucleotide *oxyR* homology extensions (288, 5′-GGCTGAACACCGC CATTTTCGGCGTGCGGCAGATTCCTGCGTGTAGGCTGGAGCTGC TTC, and 289, 5′-CGCGGATGGCCTCTGCCAGCTGCTCATAGCGGCTGC GCAGCATATGAATATCCTCCTTAG). This product was digested with *Dpn*I and transformed into MG1655(pKD46), and kanamycin-resistant colonies were selected. The correct double-crossover and recombination event was confirmed by PCR and Southern blotting. The λ Red helper plasmid pKD46 was cured by growth at 37°C, and the subsequent strain was designated MS641.

DNA manipulations. Isolation of plasmid DNA was carried out with the QIAprep Spin miniprep kit (Qiagen). Restriction endonucleases were used according to the manufacturer's specifications (Biolabs). PCRs were made as previously described (44). Amplified products were sequenced to ensure fidelity of the PCR by using the ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (PE Applied Biosystems). Samples were run on an ABI PRISM 310 genetic analyzer (PE Applied Biosystems) as described in the manufacturer's specifications.

Immunofluorescence microscopy. Surface presentation of Ag43 was assessed by immunofluorescence microscopy employing a polyclonal rabbit antiserum raised against the α subunit of Ag43. A fluorescein isothiocyanate-labeled anti-rabbit serum was used as a secondary antibody (Sigma). Cell fixation, immunolabeling, and microscopy were carried out as previously described (16).

Colony morphology. Colony morphology was assayed by employing a Carl Zeiss Axioplan epifluorescence microscope, and digital images were captured with a 12-bit cooled slow-scan charge-coupled device camera (KAF 1400 chip; Photometrics, Tucson, Ariz.) controlled by the PMIS software (Photometrics).

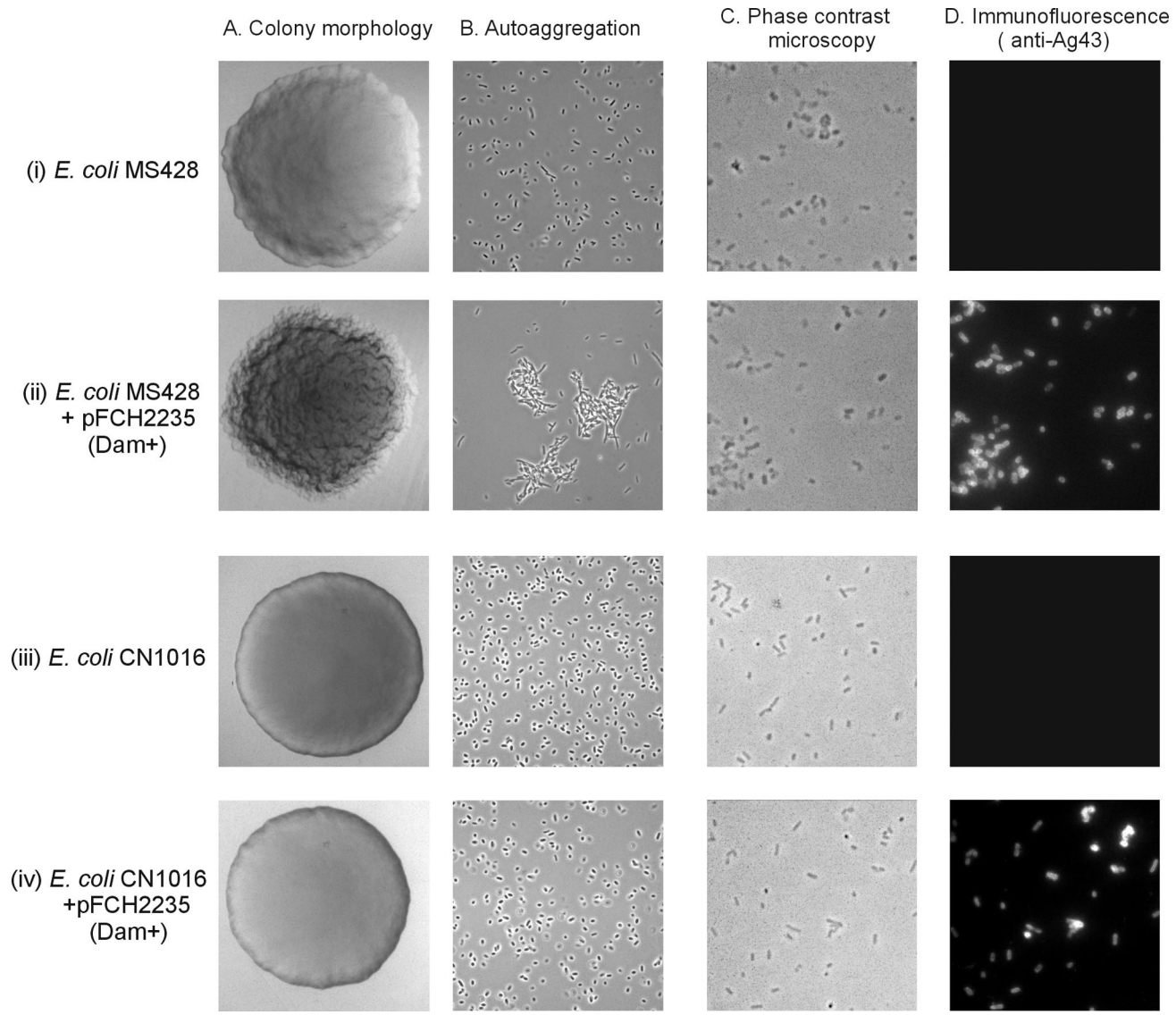

FIG. 1. Colony morphology (A), cell-cell autoaggregation (B), phase-contrast microscopy (C), and immunofluorescence microscopy with anti-Ag43 serum (D) of (i) *E. coli* MS428 (MG1655 *fim*), (ii) *E. coli* MS428 harboring pFCH2235 (induced with isopropyl-β-D-thiogalactopyranoside [IPTG] for Dam overexpression), (iii) *E. coli* CN1016 (1177 *fim*), and (iv) *E. coli* CN1016 harboring pFCH2235 (induced with IPTG for Dam overexpression). The frizzy colony morphology and cell-cell aggregation imparted by Dam-induced expression of Ag43 are not observed in the K1 capsulated UTI *fim* mutant strain CN1016.

Biofilm assay. Biofilm formation on plastic surfaces was monitored in 96-well polystyrene microtiter plates. Cells were grown statically for 24 h in LB medium at 37°C, washed to remove unbound cells, and stained with crystal violet as previously reported (40). Quantification of bound cells was performed by addition of acetone-ethanol (20:80) and measurement of the dissolved crystal violet at an optical density of 600 nm.

Cell adhesion assay. Adherence of bacteria to mammalian HT29 colon cancer cells was performed as follows. A monolayer of cells was grown to 95% confluence in standard tissue culture medium. Exponentially growing bacterial cells were harvested, washed, and resuspended in phosphate-buffered saline (containing 0.5% methyl-α-D-mannopyranoside to rule out any contribution from type 1 fimbriae) at a concentration of 10^8 cells per ml. Bacteria were incubated together with the HT29 cells for 2 h at 37°C, and afterwards, nonadhered bacteria were removed by extensive washing with phosphate-buffered saline. The cells were then fixed with 70% ice-cold methanol for 15 min and examined by phase-contrast microscopy.

RESULTS

Ag43 function is inhibited in a wild-type encapsulated uropathogenic *E. coli* strain. Expression of Ag43 is positively regulated by Dam methyltransferase. We have previously found that the presence of type 1 fimbriae sterically blocks intercellular Ag43-Ag43 interaction (15) and also affects its expression (41, 42). Accordingly, when the *E. coli* K-12 strain MS428, a *fim* derivative of the K-12 reference strain MG1655, was transformed with the high-copy-number plasmid pFHC2235 (encoding the *dam* methyltransferase gene) we expected it to produce high levels of Ag43 by virtue of the enhanced level of the Dam protein. In line with this tenet, the MS428 (pFHC2235) transformant produced colonies with a frizzy

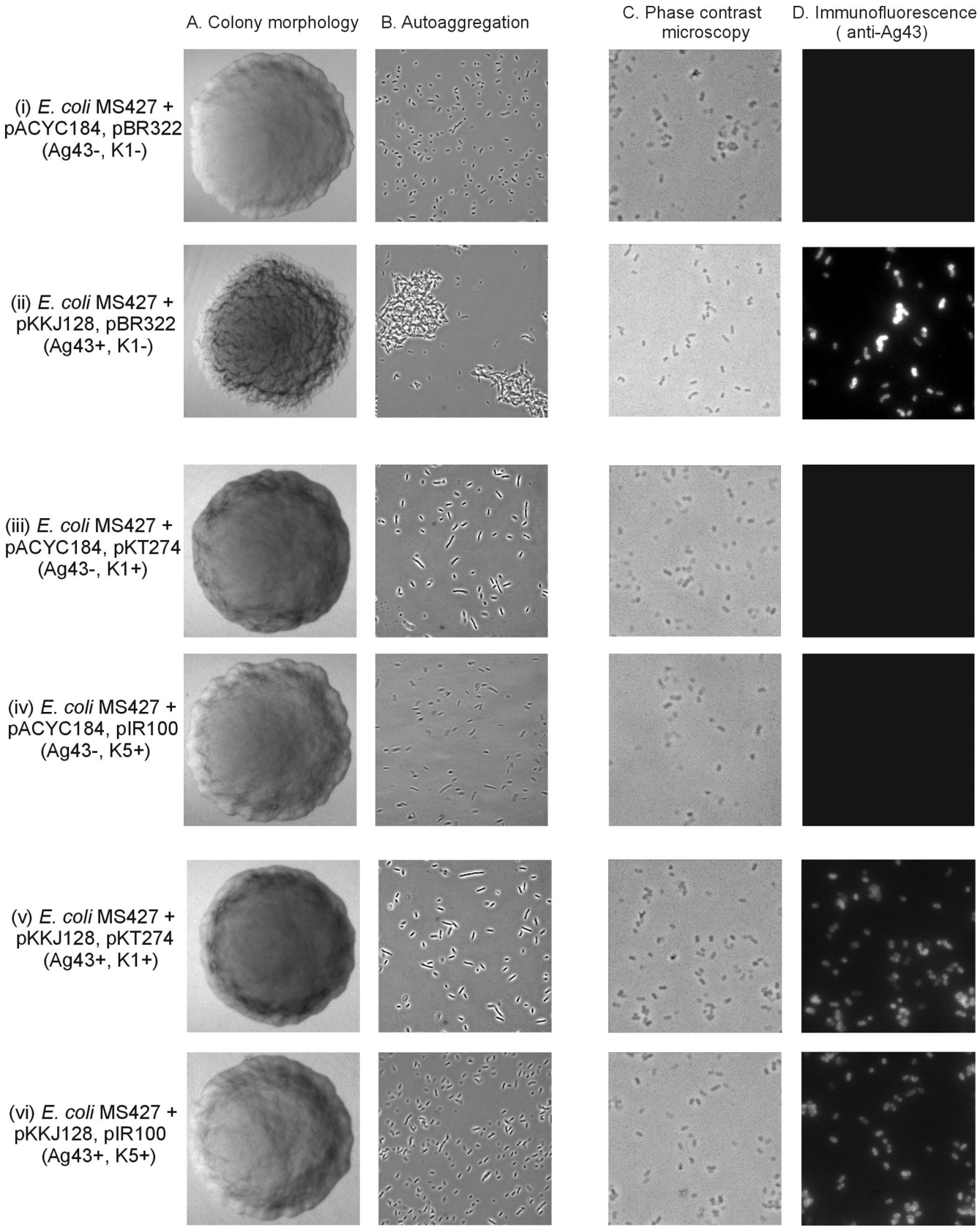

FIG. 2. Colony morphology (A), cell-cell autoaggregation (B), phase-contrast microscopy (C), and immunofluorescence microscopy with anti-Ag43 serum (D) of *E. coli* MS427 (MG1655 Δ*flu*) harboring (i) pACYC184 and pBR322 (control), (ii) pKKJ128 and pBR322 (Ag43+), (iii) pACYC184 and pKT274 (K1+), (iv) pACYC184 and pIR100 (K5+), (v) pKKJ128 and pKT274 (Ag43+ K1+), or (vi) pKKJ128 and pIR100 (Ag43+ K5+). The frizzy colony morphology and cell-cell aggregation imparted by Ag43 are prevented by the concomitant surface expression of either a K1 or K5 capsule.

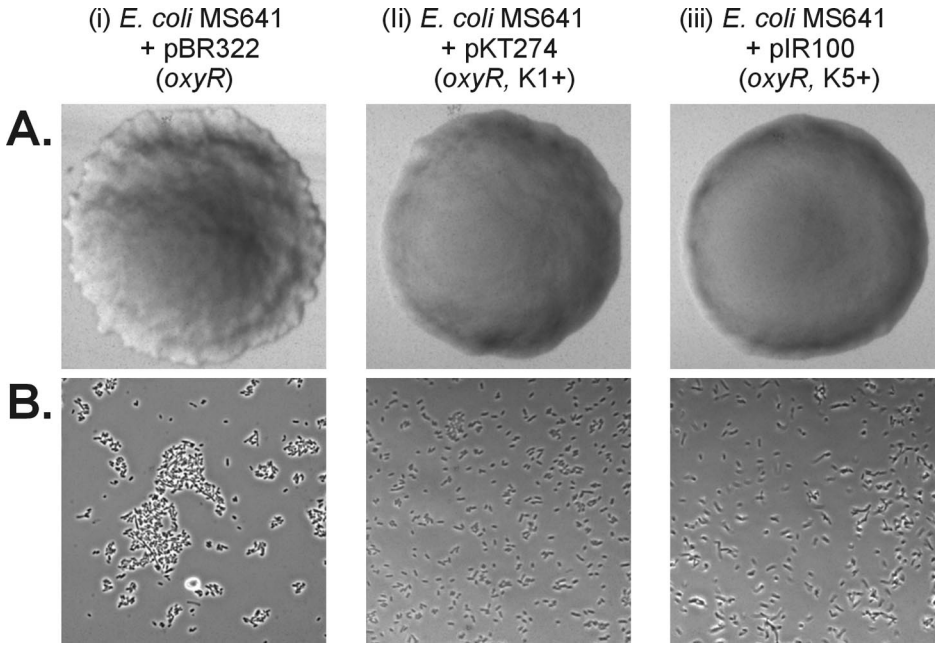

FIG. 3. Colony morphology (A) and phase-contrast microscopy (B) of *E. coli* MS641 (MG1655 *oxyR*::*kan*) harboring (i) pBR322 (control), (ii) pKT274 (K1+), or (iii) pIR100 (K5+). Derepression of Ag43 expression in the absence of OxyR results in a frizzy colony morphology and clumping phenotype that is prevented by the concomitant surface expression of a capsule.

morphology and aggregating cells that settled from static liquid solutions, i.e., traits that were indicative of high levels of Ag43 production (Fig. 1).

The uropathogenic *E. coli* strain 1177 of serotype O1:K1:H7 was previously studied, and a derivative, CN1016, deficient in expression of type 1 fimbriae was constructed (7). Strain 1177 has at least one copy of the *flu* gene on the chromosome (our unpublished data). When the pFHC2235 plasmid was introduced into these strains, we expected it to induce production of high levels of Ag43 in line with the K-12 control. However, the frizzy colony morphology, aggregation, and cell settling profiles we expected (at least in the case of the Fim-negative CN1016 [pFHC2235] strain) were not observed (Fig. 1). We therefore speculated whether a surface feature other than type 1 fimbriae could interfere with the expression or function of Ag43. Although this strain is also able to produce P-fimbriae, overexpression of the Dam protein prevents transition from the phase on to the phase off state (6), and we observed that only a small proportion (less than 5%) of the colonies produced these organelles. A good candidate therefore seemed to be the K1 capsule of 1177 and derivatives.

Ag43 function is blocked upon capsule expression in *E. coli* K-12. Unlike many wild-type strains, *E. coli* K-12 strains are normally incapable of producing extended surface-associated polysaccharides and produce rough colonies; the polysaccharide has a complete core but no O antigen due to the insertion of an IS*5* element in the *rfb* gene cluster controlling O antigen biosynthesis (27). This makes K-12 a perfect test bed for monitoring potential interference of capsular polysaccharides with Ag43. When plasmids carrying the gene cluster required for either K1 or K5 capsule production were introduced in strain MG1655, colonies with a characteristic smooth appearance resulted (Fig. 1). It should be noted that we also used a version of MG1655 (i.e., strain MS427) where the *flu* gene was deleted from the chromosome (33). Such cells do not aggregate or settle from liquid suspension. Meanwhile, expression of Ag43 in MS427 resulted in cells which formed frizzy colonies, aggregated, and settled from static liquid suspensions (Fig. 2). When cells were forced to concomitantly express both surface factors, i.e., capsule and Ag43, the capsule phenotype was dominant and the cells were unable to aggregate. This could either be due to sterical shielding of Ag43 by the capsule or capsule production somehow interfering with synthesis or transport of Ag43 to the cell surface. To differentiate between these possibilities, immunofluorescence microscopy was employed (Fig. 2). This demonstrated that the cells did express Ag43 on the surface and that the signal strengths in capsulated and noncapsulated pairs of strains were similar, indicative of similar levels of Ag43 on the surface (Fig. 2). Western blotting of whole-cell lysates also indicated that similar amounts of Ag43 were present irrespective of encapsulation (data not shown). When settling assays were performed with these cells, they were incapable of aggregation (data not shown). Taken together, the results suggest that a capsule blocks the close cell contact needed for intercellular Ag43-Ag43 interaction.

We also performed the same experiments with an MG1655 *oxyR*::*kan* host. In this host, transcription of the *flu* gene is driven from its natural promoter and the absence of OxyR causes constitutive Ag43 expression. The results indicated that Ag43 was produced but that its function was blocked by the capsule (Fig. 3). Arguably, unless OxyR is involved, capsule production does not seem to influence transcription of the *flu* gene and transport of Ag43 to the cell surface. The data support the notion that a capsule sterically shields Ag43-Ag43 interaction.

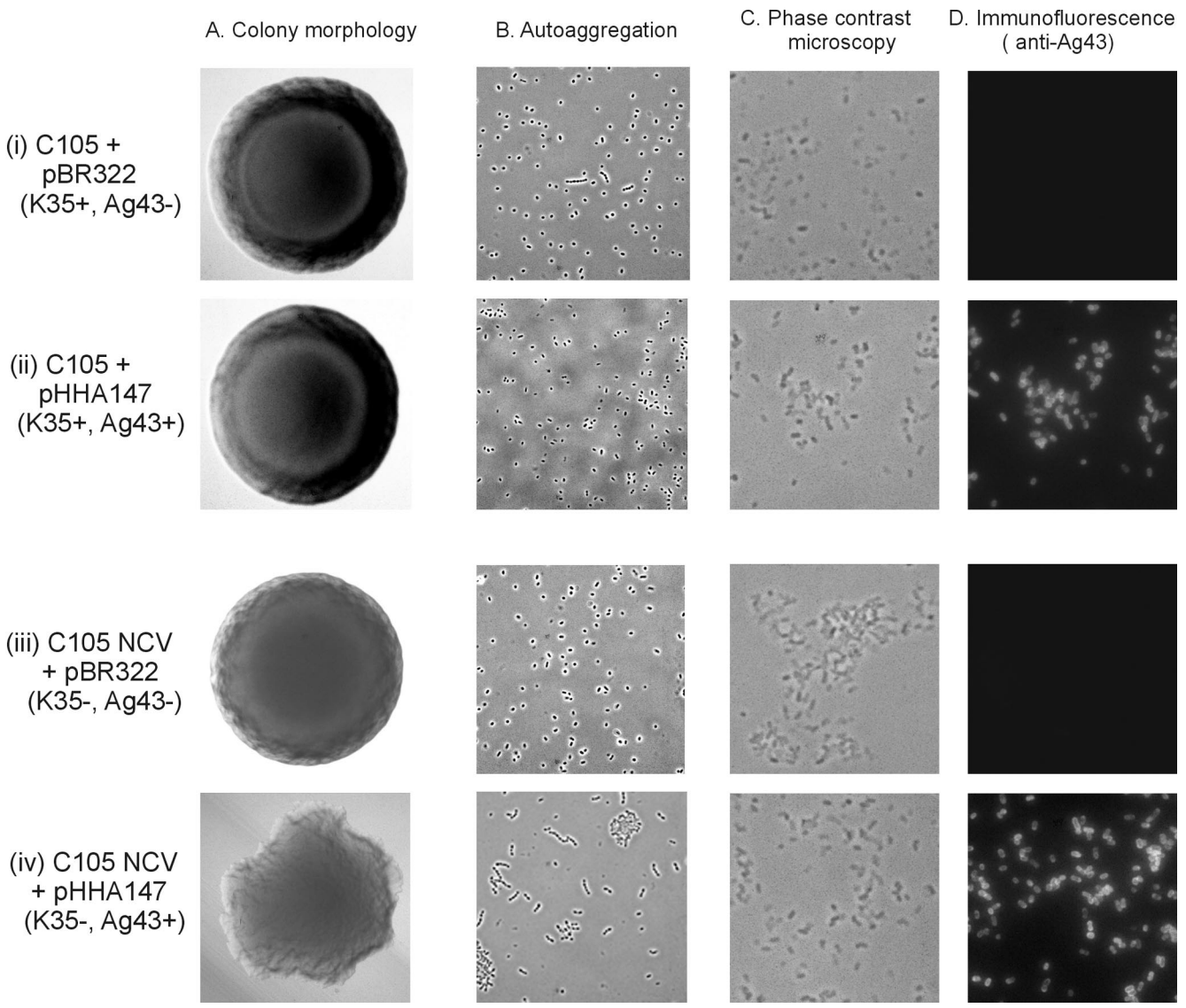

FIG. 4. Colony morphology (A), cell-cell autoaggregation (B), phase-contrast microscopy (C), and immunofluorescence microscopy with anti-Ag43 serum (D) of the capsule-producing *K. pneumoniae* strain C105 containing either (i) pBR322 (control) or (ii) pHHA147 (Ag43+) and the capsule-negative strain C105 NCV containing either (iii) pBR322 (control) or (iv) pHHA147 (Ag43+). The frizzy colony morphology and cell-cell aggregation imparted by Ag43 is prevented by the concomitant surface expression of the *K. pneumoniae* K35 capsule.

Ag43 function can be restored by capsule loss in *Klebsiella pneumonia*. The vast majority of *K. pneumoniae* strains express a large polysaccharide capsule. However, noncapsulated variants (NCVs) arise spontaneously. An isogenic pair of *K. pneumoniae* strain C105 clones, differing only in their ability to express K35 capsule antigen, was recently characterized (45). When this C105/C105NCV pair was transformed with plasmid pHHA147, harboring a constitutively expressed *flu* gene, a characteristic frizzy colony morphology resulted in the case of strain C105NCV(pHHA147), whereas little or no difference in colony morphology was seen in strain C105(pHHA147) (Fig. 4). Microscopic observation of C105NCV(pHHA147) revealed the presence of cell aggregates, whereas no aggregates were observed with C105(pHHA147) cells (Fig. 4). Additionally, we introduced Ag43-expressing plasmids into a number of other gram-negative bacteria from the institute strain collection with a known ability to produce capsules, viz., *Enterobacter cloacae*, *Serratia liquefaciens*, *Burkholderia cepacia*, and *Pseudomonas aeruginosa*. Although Ag43 was expressed on the cell surface, none of the transformed strains were capable of aggregating or settling from standing overnight cultures (data not shown). These observations further bolster the notion of negative interference between capsule and Ag43; they also take it from being a phenomenon restricted to *E. coli* to being a general phenomenon valid in a range of gram-negative bacteria.

Biofilm aspects. We and others have demonstrated that Ag43 expression confers excellent biofilm forming properties upon a variety of bacteria (9, 21, 22, 23, 38, 39). Since we have demonstrated here that the presence of a capsule virtually abolishes Ag43 functionality, we surmised that encapsulated cells expressing both capsule and Ag43 would be impaired in biofilm formation compared to noncapsulated cells. To test

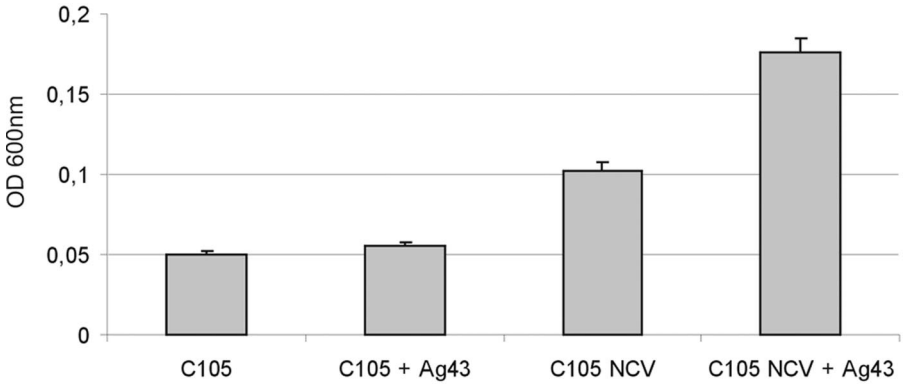

FIG. 5. Biofilm formation by the capsule-producing *K. pneumoniae* strain C105 containing either (i) pBR322 (control) or (ii) pHHA147 (Ag43+) and the capsule-negative strain C105 NCV containing either (iii) pBR322 (control) or (iv) pHHA147 (Ag43+). Surface expression of Ag43 promoted an enhanced biofilm formation phenotype only in the absence of any capsular material. Strains were grown in LB media on polystyrene microtiter plates. Adhered cells were stained with 0.1% crystal violet, and the absorbance was measured at 600 nm. Shown are the averages of readings from 3 experiments (± standard deviation).

this hypothesis, a set of strains differing in their ability to produce capsule and Ag43 were investigated for their ability to form biofilms on an abiotic surface (in this case polystyrene microtiter plates). Our results revealed that in capsulated cells expression of Ag43 did not improve biofilm formation, whereas in a noncapsulated strain a significant improvement was observed when Ag43 was expressed (Fig. 5). It should also be noted that when the capsulated and noncapsulated pair of strains were compared, the noncapsulated variant appeared to be a slightly better biofilm former. This is probably due to capsule shielding of other (non-Ag43) factors that improve biofilm formation.

The action of other autotransporter adhesins similar to Ag43 is abolished by capsule shielding. The AIDA-I autotransporter is a potent adhesin involved in the adherence of enteropathogenic *E. coli* strains to various mammalian cells (4, 5). The AIDA-I adhesin and Ag43 are predicted to have the same overall tertiary structure. We speculated that, since capsulation obstructs the activity of Ag43, it might also interfere with the activity of similar-sized proteins such as AIDA-I. To test this, we introduced a plasmid, pIB264, encoding the AIDA-I locus (4) into a pair of host cells differing in their ability to produce a capsule, viz., C105 and C105 NCV. The ability of these strains to adhere to human intestinal cell line cells (HT29 cells) was assayed. Bacteria producing both AIDA-I and capsule were unable to adhere, whereas bacteria producing AIDA-I, but not capsule, bound in large numbers (Fig. 6).

DISCUSSION

Bacteria express a number of surface structures that enable them to interact with the environment, e.g., flagella for swimming and adhesins for attachment. These surface structures have a highly diverse size spectrum, and it must be implicit that sometimes they must interfere physically with each other in such a way that the function of some will be obstructed by the presence of other (more extended) structures. As an example, it was previously demonstrated that fimbriae, which extend ~1 μm from the bacterial surface, physically block the action of the much shorter Ag43 in bacteria (15). Based on this observation, we predict that fimbriae will also block the action of other adhesins similar in size to Ag43. Such surface structure interference must have important consequences for the interplay of bacteria with the environment. Here, we have studied the interference between nonfimbrial adhesins and capsule.

Several lines of evidence indicated that Ag43 function was sterically blocked by extended polysaccharides. In a uropathogenic *E. coli* strain of serotype K1, Ag43 could be expressed but it was inactive. In *E. coli* K-12 strains expressing Ag43, aggregation was abolished when these strains were made to express either K1 or K5 capsules. Furthermore, in a well-characterized strain set of *K. pneumoniae*, differing only in capsulation, Ag43 activity was only observed in the NCV. In line with this, Ag43 activity was blocked in a range of bacterial strains, arguably due to capsule interference. All phenotypic traits associated with Ag43 were affected: cell aggregation, settling from static liquid suspension, biofilm formation, and in most cases, colony morphology. The interference between capsule and Ag43 was physical in nature rather than by genetic cross talk. Negative interference between capsule and Ag43 seemed to be a general phenomenon in a range of gram-negative bacteria and was not isolated to *E. coli*. Bacterial capsules might provide various degrees of blockage depending on their thickness and quantity and perhaps also on their charge.

An important aspect of this study was the finding that capsulation not only interfered with the function of Ag43 but also shielded and blocked the action of another autotransporter adhesin, namely AIDA-I. Both adhesins have been predicted to protrude ~10 nm from the bacterial surface. The implications of these findings are far-ranging because it means that an entire class of short nonorganelle bacterial adhesins and invasins are nonfunctional in the presence of a capsule. This class encompasses not only the autotransporter adhesins and invasins such as TibA, AIDA-I, and Ag43 from enterics but also autotransporters from a wide spectrum of bacteria, including pertactin P.69 and TcfA from *Bordetella*, Hsf from *Haemophilus influenzae*, and UspA1 from *Moraxella catarrhalis* (for a review, see reference 17). Additionally, there are numerous other nonfimbrial adhesins and invasins exemplified by Afa-I and intimin (reviewed in references 24 and 43), which would be candidates for capsule shielding. In line with our data, several other studies have actually reported that capsulated bacteria

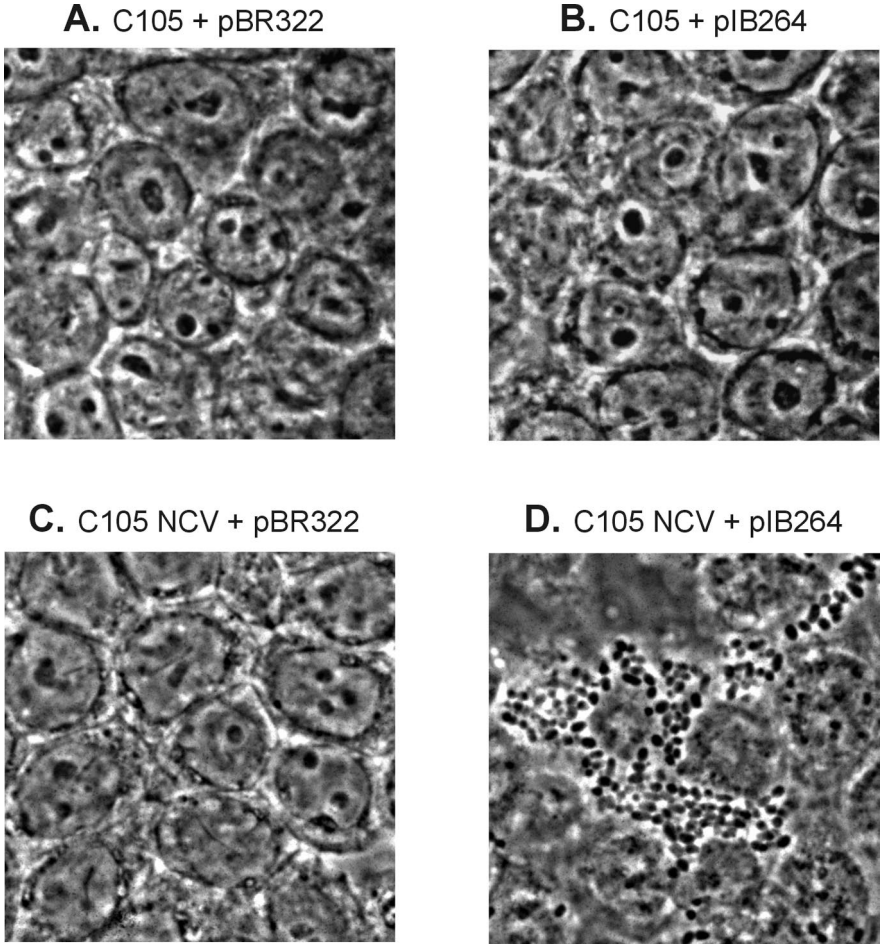

FIG. 6. Adherence of the capsule-producing *K. pneumoniae* strain C105 containing either pBR322 (control) (A) or pIB264 (AIDA-I) (B) and the capsule-negative strain C105 NCV containing either pBR322 (control) (C) or pIB264 (AIDA-I) (D) to HT29 colon cancer cells. Adherence mediated by the AIDA-I autotransporter protein was blocked by the concomitant expression of a surface capsule layer.

adhere poorly to epithelial cells and exhibit severe reductions in their abilities to invade compared to their capsulated counterparts (14, 36). In *Neisseria meningitidis*, the adhesive Opc protein was reported to be active only in acapsulated strains (51). A study of *E. coli* P-fimbrial adhesins expressed in a nonorganelle context revealed their activity could be blocked by extensive O antigens (50). Finally, the synthesis of lipopolysaccharide by enterohemorrhagic *E. coli* O157:H7 was shown to interfere with adherence to HeLa cells in vitro (47).

The capsule shielding concept leaves the bacteria with an obvious dilemma. They cannot adhere or invade without the assistance of adhesin proteins, but at the same time, the capsule provides protection against many of the countermeasures at the disposal of a mammalian host such as phagocytosis or complement, etc. Arguably, bacteria that use short adhesins and invasins would have to coordinate their expression with that of capsules. An alternative solution would be to express adhesins that can penetrate and reach beyond the capsule, viz., fimbriae.

At present, it is hard to say whether bacteria actually coordinate production of capsules and nonorganelle adhesins, and to our best knowledge, no reports to this end are available from the literature. The regulation of capsule gene expression is complex, with overlapping regulatory circuits. Capsule expression has in many cases been reported to be phase variable, for example, in *Bacteroides fragilis* (26), *N. meningitidis* (11), and *Campylobacter* (3). In *K. pneumoniae*, some types of capsule production were reported to be influenced by environmental conditions (14). Arguably, differential expression of capsules will intermittently result in a noncapsulated state where nonorganelle adhesins like Ag43 and AIDA-I would be exposed.

Ag43 promotes bacterial biofilm formation and aggregation, both of which are traits closely associated with bacterial virulence (1, 8). Capsulation protects bacteria that live in close association with a mammalian host against several defense mechanisms. The future challenge is to decipher how the bacteria orchestrate the expression of these two important types of surface structures to make use of their full survival and virulence capabilities.

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