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Rapid, Synchronous, and Stable Induction of Type 1 Piliation in *Escherichia coli* by Using a Chromosomal *lacUV5* Promoter

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Type 1 pili are filamentous proteinaceous appendages produced by certain members of the family *Enterobacteriaceae*. In *Escherichia coli*, the adhesive properties of these pili are due to the binding of at least one minor pilus component to mannose, a sugar common to cell surface molecules of many eukaryotic cells. The study of pilus assembly may be benefited by a rapid way of inducing pilus synthesis de novo. We describe herein the construction and characterization of a strain in which piliation can be rapidly induced by the addition of lactose or its analog isopropyl-β-D-thiogalactopyranoside. This was accomplished by placing the chromosomal *fimA* gene (encoding the major structural subunit of pili) under *lacUV5* promoter control. Further experiments suggested that transcription of genes downstream of *fimA*, whose products are required for normal pilus assembly and function, may also be controlled by the *lacUV5* promoter. The construction described herein may have a variety of applications apart from aiding the study of pilus assembly since its adhesive properties can be rapidly and easily turned on and off.

Type 1 pili are filamentous proteinaceous appendages produced by certain members of the family *Enterobacteriaceae*. These pili mediate mannose-inhibitable bacterial attachment to a variety of eukaryotic cells that have surface-exposed mannose-containing molecules (8). Type 1 pili of *Escherichia coli* were initially thought to be rather simple structures composed exclusively of a single repeating subunit, pilin, that formed the hollow helical fiber of the pilus and bound mannose-bearing ligands on eukaryotic cells. We now know that there are at least three minor components, the products of the *fimF*, *fimG*, and *fimH* genes (refer to the top of Fig. 1A for the arrangement of the type 1 pilus gene cluster), incorporated into the pilus fiber (18). One of these products (the product of the *fimH* gene) effects receptor binding (17). Two additional proteins, the products of the *fimC* and *fimD* genes, are required for polymerization of pilin monomers, the product of the *fimA* gene, into pili (15, 23). The expression of *fimA* is controlled at the transcriptional level in an "ON or OFF" fashion by the products of the *fimB* and *fimE* genes acting on a 314-bp invertible DNA segment containing the *fimA* promoter (1, 6, 21). This promoter may be the major *cis*-active element for other genes of the cluster (16). Because of promoter inversion, piliation in the commonly used *E. coli* strains (e.g., K-12 and B) is subject to phase variation (1). Most recent evidence suggests that the invertible segment is maintained chiefly in the OFF orientation in many naturally occurring *E. coli* strains (6) and that its orientation may be influenced by environmental factors (28).

All bacterial pili are assembled from a limited number of components, and the finished product is outside the cell. This process provides an interesting engineering problem for piliated bacteria and a tractable system for investigating the factors involved in producing these and related supramolecular structures. For certain pili (e.g., *pap* pili, associated with *E. coli* causing pyelonephritis), an ordered sequence of events for assembly and incorporation of the minor components has been proposed and a higher-ordered tip structure has been observed (19). For type 1 pili, such a higher-order structure, while suggested (10, 18), has not been demonstrated (27). In no instance has there been a kinetic study of de novo pilus assembly although with some types of pili, whose production is subject to temperature regulation, such experiments may be possible (9).

We decided to use a *fimA* promoter deletion mutant (23, 24, 26) to see whether we could construct a strain in which type 1 piliation could be rapidly and synchronously induced de novo. Evidence for the success of this endeavor is presented as part of our results.

All bacterial strains employed were F⁻ *E. coli* K-12 derivatives. The bacterial strains, bacteriophage, and plasmids used are listed in Table 1. Genetic and recombinant DNA techniques have all been described previously (14, 26, 29) except for DNA sequencing by the dideoxy chain termination method. DNA sequencing was carried out with Sequenase Version 2.0, utilizing double-stranded plasmid DNA, as recommended by United States Biochemical Corporation. Assays for piliation included bacterial agglutination in antipilus antiserum and guinea pig erythrocytes as previously described (11).

Construction of an *E. coli* strain inducible for type 1 piliation (Fig. 1A and B) proceeded from plasmid pORN103 (Fig. 1A), which has a Tn*5* insertion in *fimE* and is deleted for 301 bp of the 314-bp invertible segment containing the *fimA* promoter as determined by DNA sequencing (nucleotides 2605 to 2906 were deleted; coordinates refer to the sequence of the *fimABE* region in GenBank [accession number X00981]). An earlier report stated that 246 bp were deleted, implying more precision than was actually possible without sequencing (26). A subclone, pORN111, containing the deletion and flanked by a portion of IS*50* and *fimA*, was digested with *Pvu*II, and a promoterless *lac* region (*lacZYA*), excised from pORN129 with *Xho*I, was end filled and

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TABLE 1. Strains, bacteriophage, and plasmids

Strain, bacteriophage, or plasmid	Description	Source or reference
Strains		
LE392	*supR supE hsdR galK trpR metB lacY tonA*; used as the recipient in plasmid transformations	Laboratory collection
ORN109	*thr leu proA2 lacY1 galK his argE rpsL supE mtl xyl recBC sbcB* (has Tn*10* between *hsd* and *serB*); Fim$^-$ Tc^r	26
ORN115	*thr-1 leuB thi-1* Δ(*argF-lac*)*U169 malA1 xyl-7 ara-13 mtl-2 gal-6 rpsL tonA2 supE44*	26
ORN192	ORN115 except Δp*fimA fimE*::Tn*5*; Fim$^-$ Kn^r	P1 transduction from ORN109 (Δp*fimA fimE*::Tn*5*)[a]
ORN193	ORN109 except Δp*fimA fimE*::Tn*5*; Fim$^-$ Kn^r Tc^s	P1 transduction from ORN192 to ORN109 (Fig. 1B)
ORN194	ORN193 except IS*50*::*lacUV5 cat*	Linear transformation from pORN157 (Fig. 1B)
ORN195	ORN109 except Δ(*fimBEACDFGH*); Kn^r Tc^s	Linear transformation of ORN191[b] with *Sal*I-cut pORN154
ORN172	ORN115 except Δ(*fimBEACDFGH*); Kn^r	P1 transduction from ORN195 to ORN151 (5, 22)
Bacteriophage		
P1	*vir*	Laboratory collection
Plasmids		
pACYC184	P15A replicon; Cm^r Tc^r	7
pORN123	ColE1 replicon; same as pBR322, only lacking the *Pvu*II site; Ap^r Tc^r	26
pSH2	pACYC184 *fimA-H*; Cm^r	23
pKB252	Source of *placUV5*	4
pKP1013	Source of *cat* gene	20
pORN129	Source of *Xho*I-linked *lac* genes for fusion	26
pORN154	pSH2 except deleted for *fimBEACDFGH*; Kn^r Cm^r	—[c]
pORN103	pSH2 except Δp*fimA fimE*::Tn*5*; Cm^r Kn^r	23, Fig. 1A
pORN111	pORN123 *fimA'* Δp*fimA fimE'*::IS*50'*; Tc^r	29, Fig. 1A
pORN155	pORN111 except Φ*fimA-lacZYA* fusion	Fig. 1A
pORN156	pORN155 with *placUV5* from pKB252 in IS*50*	Fig. 1A
pORN157	pORN156 except a *Xho*I-linked *cat* gene from pKP1013 was inserted beside *plac*UV5	Fig. 1A
pORN158	Same as pORN111 except a *Xho*I-linked *cat* gene was inserted into the *Xho*I site in IS*50*	This study; a portion of this plasmid was used as a probe in Fig. 2.
pORN106	ColE1 replicon carrying *fimC'*, *fimA*, *fimB*, and *fimE*	23

[a] ORN109 (Δp*fimA fimE*::Tn*5*) was constructed by linear transformation of ORN109 with *Eco*RI-cut pORN103. This construction was Kn^r and Tc^r.

[b] ORN191 is the same as ORN109 except that it contains a *tetR* insertion in *fimA* and lacks the Tn*10* between *hsd* and *serB*. The fusion was introduced by linear transformation of ORN117 (26) with *Sal*I-cut pORN146. This plasmid has the *Xho*I-linked *Bgl*II fragment containing the *tetR* gene from Tn*10* (12) inserted in the *fimA* gene. This was accomplished by methods analogous to those for the construction of pORN125 (26).

[c] pORN154 was constructed by deleting the *fimBEACDFGH* genes and replacing that section with the *Sal*I-*Xho*I fragment of Tn*5* containing the *kan* gene from Tn*5* (13). The fragment removed encompassed the area from the *Cla*I site in ca. the middle of *fimB* to a *Pvu*II site ca. 600 bp after the 3' end of *fimH*. Removal was facilitated by the prior individual conversion of each of the five *Pvu*II sites and two *Cla*I sites to *Xho*I sites. This plasmid was used to construct strains ORN195 and ORN172, which were used as negative controls for piliation.

introduced into the unique *Pvu*II site in *fimA* on pORN111. Determination of the orientation of the *lacZYA* gene (by *Eco*RI digestion) in a number of transformants resulted in the isolation of pORN155. The 209-bp *lacUV5* promoter was excised from pKB252 with *Eco*RI, *Xho*I linkers were added, and the resulting fragment was introduced at the unique *Xho*I site in IS*50* in pORN155. The orientation of the *lacUV5* promoter in a number of *lacUV5* probe-positive transformants was determined by sequencing the region of the insertion and resulted in the isolation of pORN156. The addition of the ca. 2.2-kbp chloramphenicol resistance gene (*cat*) was accomplished by partial digestion of pORN156 with *Xho*I followed by the introduction of a *Xho*I-linked, *Pst*I-excised *cat* gene from pKP1013. The location and transcriptional orientation of the *cat* gene were determined by sequencing the insertion region of *lacUV5* probe-positive transformants selected on medium containing chloramphenicol and resulted in the isolation of pORN157.

Interestingly, the *fimA-lacZYA* fusion in the pORN157 construction was active regardless of the presence of the *lacUV5* promoter. Deletion analysis suggested that this activity was likely due to the coincidental formation of an active promoter at the *fimA-lacZYA* fusion site. This unexpected activity was used as a tool to provide supporting evidence that a double crossover occurred in the subsequent step of the construction.

In the final construction (Fig. 1B), *Bgl*II-cut pORN157 was introduced into strain ORN193, a *recBC sbcB* mutant of *E. coli* K-12, via linear transformation. One hundred Cm^r Kn^r Tc^s Lac$^-$ transformants were screened for the inducible piliation phenotype. Five of the transformants tested had the expected inducible piliation phenotype, although 99 had evidently received the *cat* gene through a double-crossover event. Genetic mapping of one of the five (ORN194) by P1 transduction revealed that *cat* was 93% linked (100 transductants scored) to inducible piliation in this strain. This linkage is in line with the theoretical 100% expected if the genes were immediately adjacent to one another.

The initial low level of linkage (5%) seen in the ORN193 transformants remains unexplained but may be due in part to

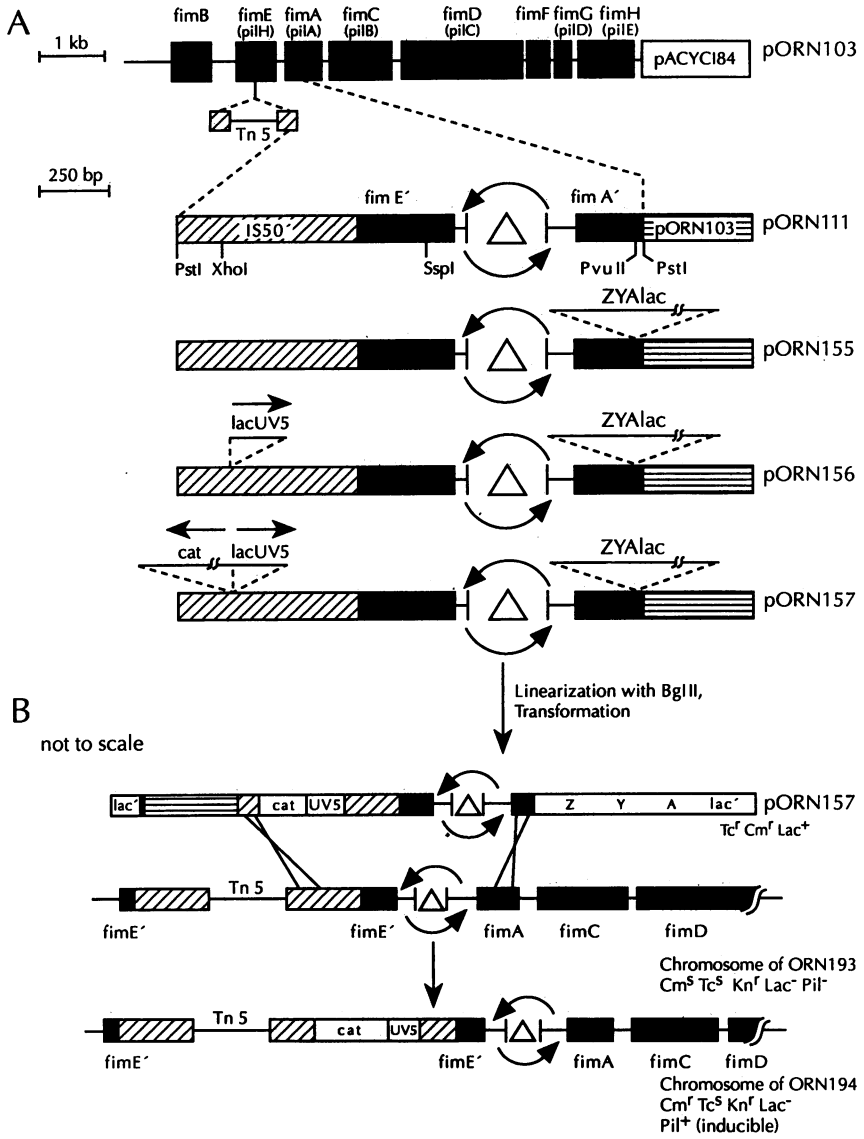

FIG. 1. (A) In vitro construction of plasmid pORN157, which was used to introduce the *lacUV5* promoter into the chromosome. Plasmid pORN103 shows the genes of the *fim* region. Transcription is from left to right for all the genes (black rectangles). Flanking and intercistronic regions are not drawn to scale. The upper mnemonics are those used by Bachmann for *E. coli* K-12 in the most recent edition of the *E. coli* genetic map (3). The mnemonics in parentheses are those of Orndorff and Falkow (25). The *fimE* gene was also designated as *hyp* in early descriptions (24). The pORN103 plasmid was used as a source of a *PstI-PstI* fragment containing the last 680 bp of IS*50* (2), the terminal two-thirds of the *fimE* gene, the deleted *fimA* promoter region, and the 5' half of the *fimA* gene. Additions to pORN111 are described in the text. The Tn*5*, *cat*, and *lacZYA* segments are not drawn to scale. Arrows denote the direction of transcription of the *lacUV5* promoter and the *cat* gene. (B) *BglII*-cut linear pORN157 was introduced into strain ORN193 by transformation and Cm^r transformants were screened as described in the text. Strain ORN194 was one such transformant.

the fact that recombination can occur within either IS*50* of Tn*5* in strain ORN193 and that secondary insertion sites may exist elsewhere in the chromosome. Regardless of the reason for the initial low level of linkage, the aforementioned P1 mapping of ORN194 and the physical mapping of this strain (Fig. 2) indicated a construction consistent with that shown in Fig. 1B.

Piliation in ORN194, having the *lacUV5* promoter, was evident after growth on MacConkey lactose but not MacConkey glucose or xylose agar (sugar concentrations, 1.0%). Also, piliation was measurable after the addition of the lactose analog isopropyl-β-D-thiogalactopyranoside (IPTG) to logarithmically growing cultures in L broth (final IPTG concentration, 8.4 mM). Cultures of the parental strain (ORN193), lacking both the *fimA* promoter and the *lacUV5* promoter, were devoid of pili in the presence or absence of lactose or IPTG (Fig. 3).

The kinetics of pilus appearance in strain ORN194 after IPTG addition revealed that pili were rapidly and uniformly produced in the induced culture but not the uninduced parallel culture (Fig. 4) as measured both functionally (by hemagglutination) and physically (as viewed with the electron microscope). Although the experiment was carried out for 6 h (with the induced and uninduced cultures maintained

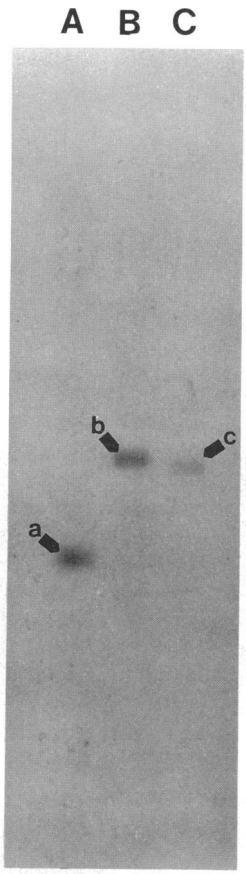

FIG. 2. Southern blot of *Pst*I-digested DNA from the parental strain ORN193 (not having the ca. 2.5-kbp *lacUV5-cat* construction); ORN194, which contains the *lacUV5-cat* construction; and pORN158, which is the same as pORN111 except that it has the ca. 2.2-kbp *cat* gene inserted in the *Xho*I site (refer to Fig. 1A for diagrams of the strains and plasmids). The *Pst*I fragment of pORN158 is ca. 4.0 kbp, the same size as would be predicted for the chromosomal ORN194 insertion strain but ca. 200 bp less because pORN158 lacks the *lacUV5* promoter. A ca. 0.8-kbp radiolabeled *Pvu*II-*Ssp*I fragment from pORN111, which encompasses the terminal portion of *fimE* and the 5′ half of *fimA*, was used as the probe DNA. Lane A contains DNA from the parental strain ORN193 and has a band (a) migrating at ca. 1.8 kbp. Lane B contains DNA from the insertion strain, ORN194, and has a band (b) migrating at ca. 4.2 kb, a size consistent with the addition of the ca. 2.4-kbp *lacUV5-cat* construction. As predicted, lane C, containing the pORN158 DNA, has a band (c) migrating ca. 0.2 kbp less than band b.

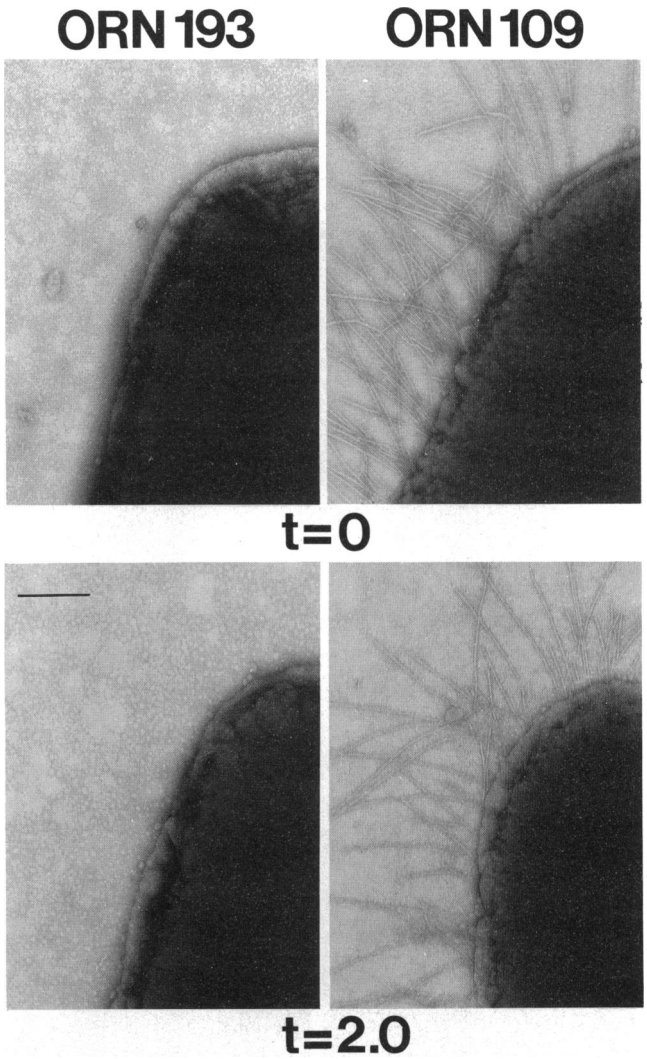

FIG. 3. Effect of IPTG addition on piliation in the parental strain, ORN193, lacking the *fimA* promoter. The parent of ORN193, ORN109 (26), which has a normal *fimA* promoter, is also shown. In logarithmically growing L broth cultures, no pili are seen with ORN193 prior to (t=0) or after (t=2.0) IPTG addition, which is consistent with the absence of the *fimA* promoter. ORN109 is, as expected, normally piliated regardless of IPTG induction. Time (t) is in hours. The bar equals 0.25 μm.

in logarithmic growth by periodic dilution and assayed every hour at nearly identical turbidities), essentially no change in phenotype could be detected in the last 4 h.

Electron microscopic examination of hundreds of cells in the uninduced culture at every time point revealed that, at most, 1 cell per 100 may have had a short protrusion resembling a pilus. In contrast, within 30 min of IPTG addition, virtually all cells examined exhibited a few short pili (Fig. 4) whose numbers per cell increased for up to 2.0 h postinduction but whose lengths did not appreciably increase over this time period (Fig. 4).

Evidence that the *lacUV5* promoter also effected transcription of genes 3′ to *fimA* (transcriptionally downstream) was furnished by introducing a multicopy plasmid (pORN106 [23]) which contains an intact *fimA* gene under its own promoter control and produces pilin constitutively (23, 26). In the uninduced state, no pili were seen despite the expression of *fimA* on the pORN106 plasmid (23). However, after induction, pili appeared with much the same kinetics as those shown in Fig. 4, but the pili were noticeably longer (Fig. 5).

The results presented herein indicate that it is possible to induce type 1 piliation in *E. coli* K-12. The normal invertible *fimA* promoter was removed, and a *lacUV5* promoter was inserted nearby. Induction resulted in the rapid, synchronous, and uniform appearance of functionally normal pili. This is the first instance in which piliation was induced de novo on a time scale amenable to biochemical and gross morphological analysis.

The pilus appearance kinetics suggested that (i) the amount of at least one of the accessory components (encod-

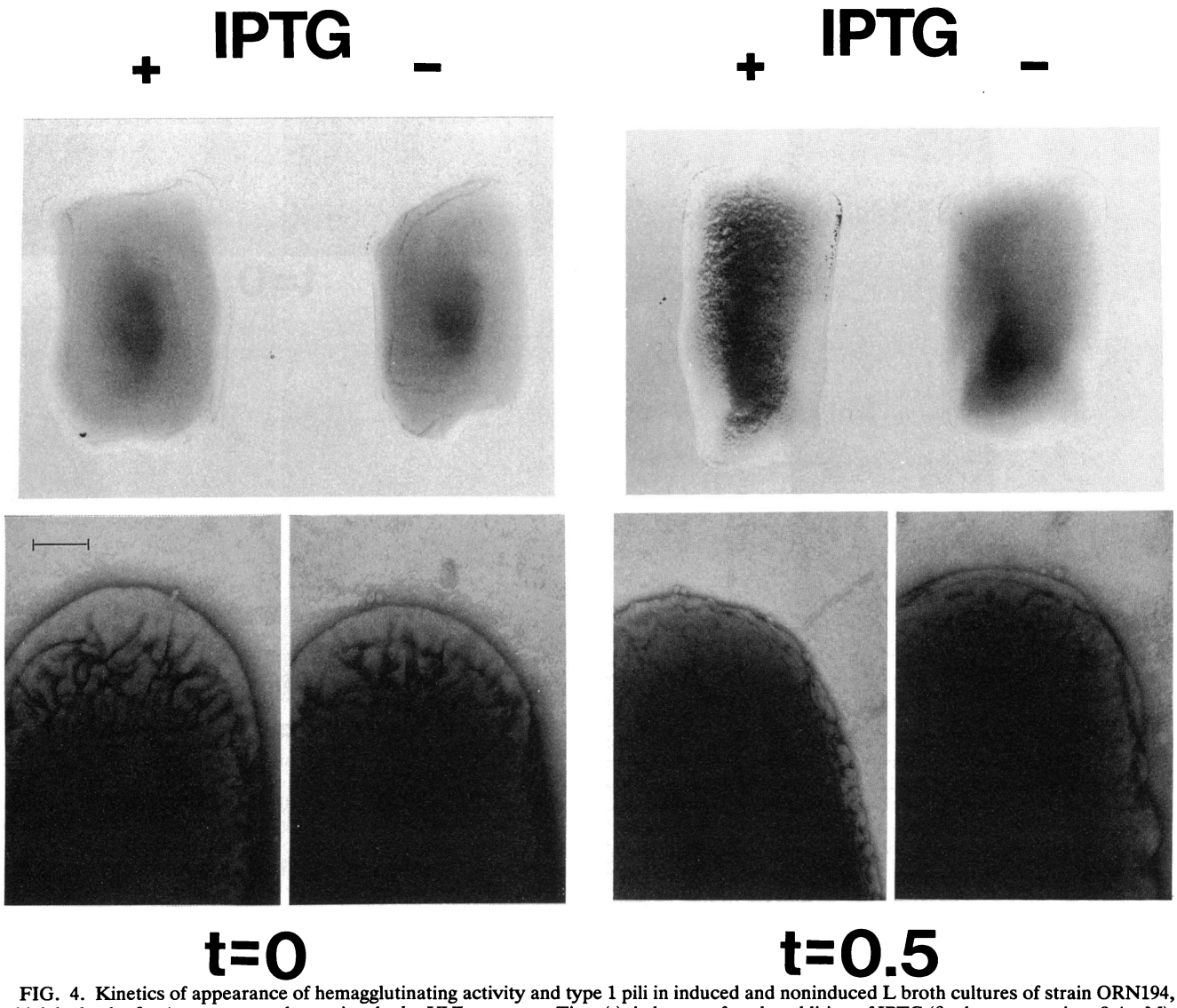

FIG. 4. Kinetics of appearance of hemagglutinating activity and type 1 pili in induced and noninduced L broth cultures of strain ORN194, which lacks the *fimA* promoter and contains the *lacUV5* promoter. Time (*t*), in hours, after the addition of IPTG (final concentration, 8.4 mM), is shown at the bottom of each set of photographs and micrographs for the six illustrated time points. Both IPTG induced (+) and noninduced (−) cultures are shown at each time point. In the upper part of each illustration, hemagglutination reactions are shown. Twenty microliters of the culture was mixed with 20 μl of 3% guinea pig erythrocytes in phosphate-buffered saline on a clear glass slide. These slides were photographed with transmitted light. In the lower portion of each panel, electron micrographs show the piliation exhibited by a representative member of the induced and noninduced cultures. Cultures were kept at nearly identical turbidities and in logarithmic growth by dilution. The bar in the *t*=0 micrograph equals 0.25 μm.

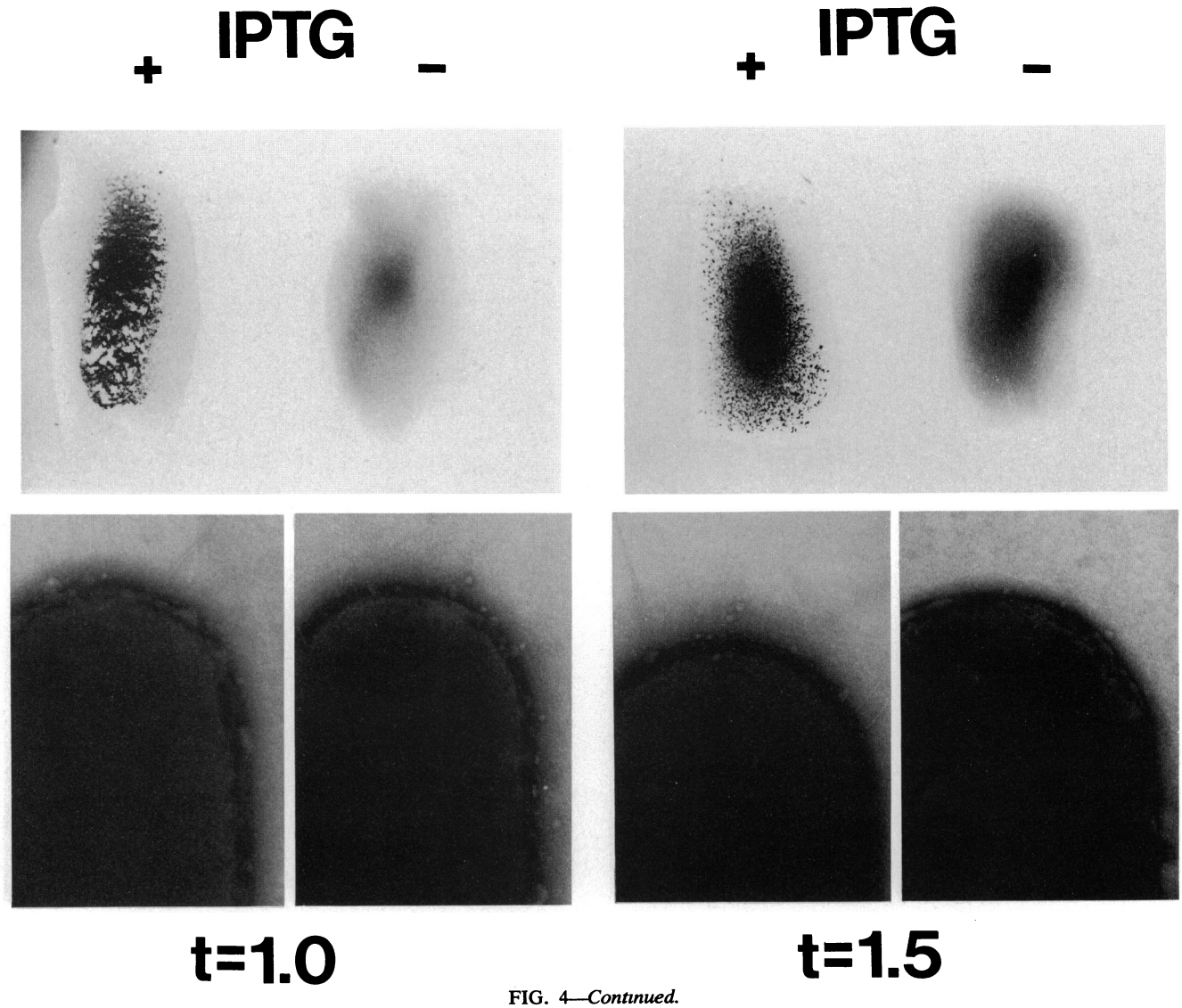

FIG. 4—*Continued.*

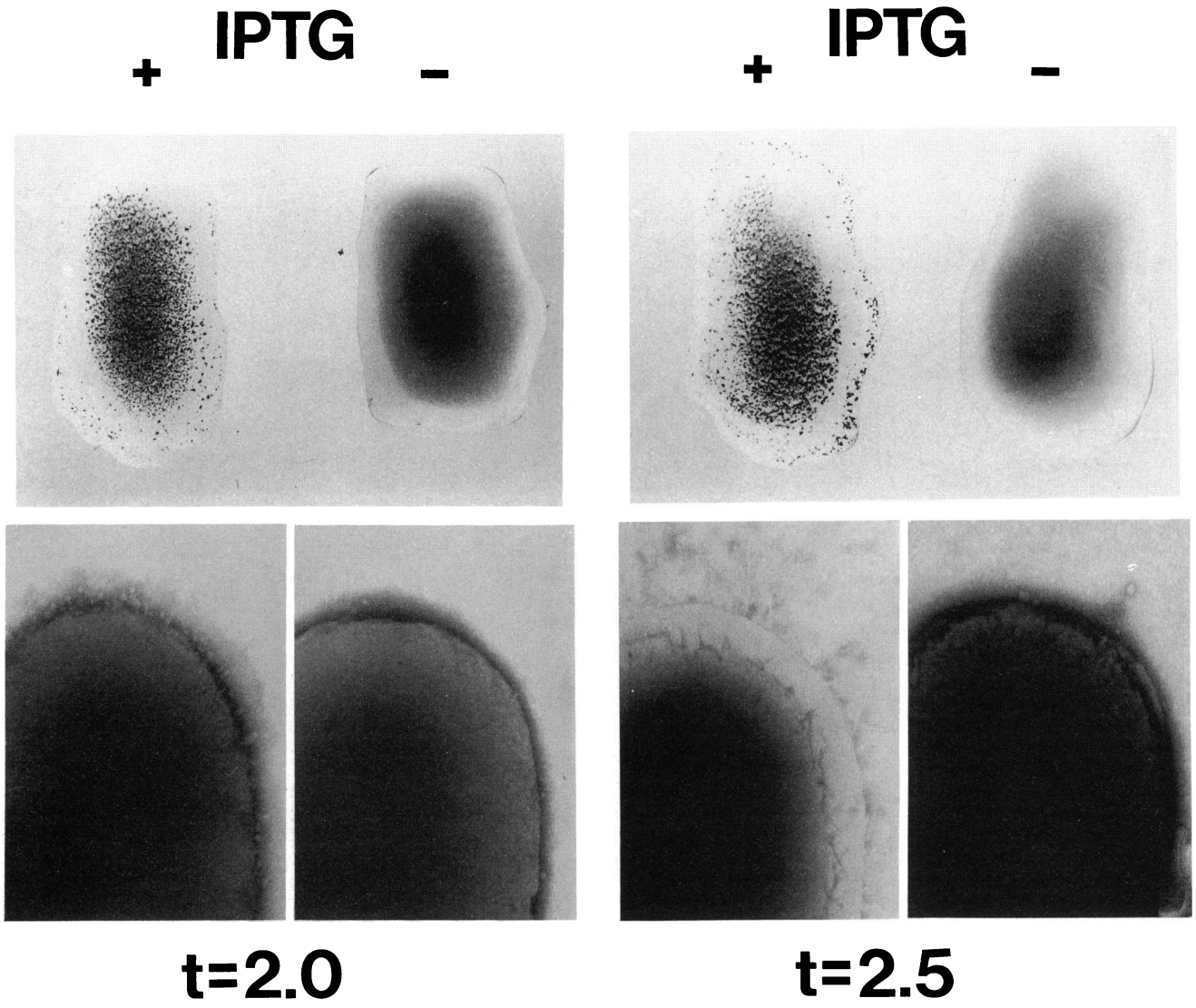

FIG. 4—*Continued.*

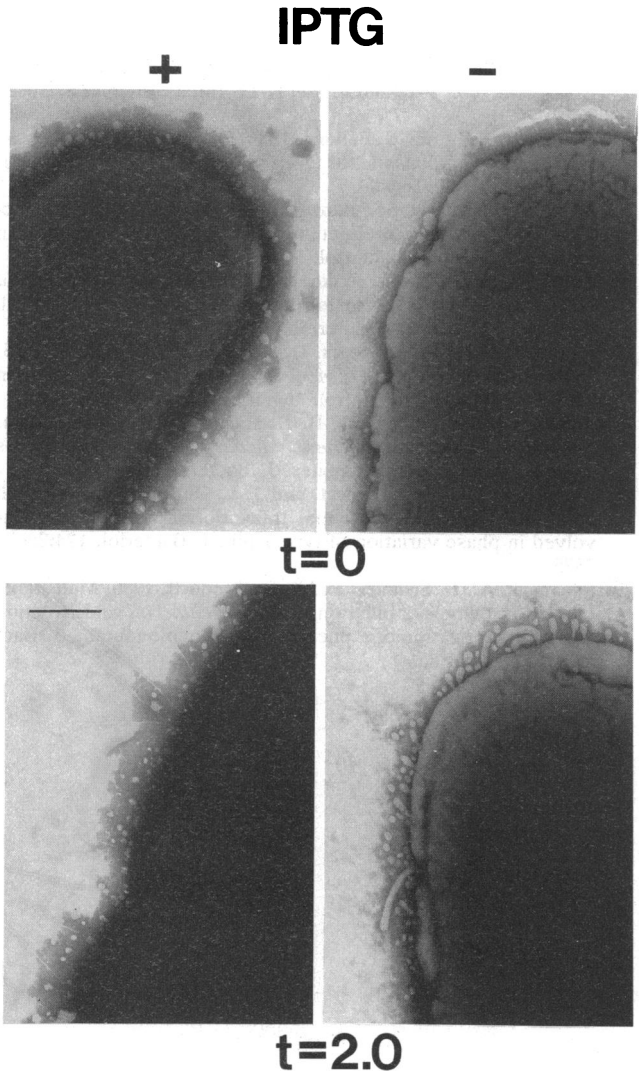

FIG. 5. Effect of IPTG addition on pilus appearance in strain ORN194 harboring plasmid pORN106. Strain ORN194 lacks the *fimA* promoter and has the *lacUV5* promoter in the chromosome. Plasmid pORN106 produces the *fimA* gene product constitutively. Pili are produced in ORN194/pORN106 after (+) IPTG induction (t=2.0) but not prior to (t=0) or in the absence of (−) induction. Time (t) is in hours. The bar equals 0.25 μm.

ed by genes 3′ to *fimA*) needed to initiate pilus assembly was rate limiting prior to induction and (ii) pilin became rate limiting soon thereafter. This follows from the observation that pilus number rather than pilus length increased over time. That is, if components involved in initiation were present in excess prior to induction, then we would expect that numerous short pili would appear synchronously after induction and that their numbers per cell would not increase with time. If pilin did not become rate limiting soon after induction, then we would have expected pilus length to increase over time. Both of these points were additionally supported by the observation that longer pili were made in the *fimA* constitutive strain ORN194/pORN106 only after induction (Fig. 5). Also, the results with this strain suggest that the *lacUV5* promoter regulates the genes 3′ to *fimA* as well as *fimA* itself.

Both pilus number and length remained noticeably below wild-type magnitude after induction of strain ORN194 (compare Fig. 3 with Fig. 4). This may simply be due to the position and strength of the *lacUV5* promoter. We positioned the promoter some distance from the start of the pilin gene in part to help ensure that, in the uninduced state, cells would be devoid of pili.

The ability to induce piliation should aid in the examination of pilus assembly since populations can now be synchronized to produce pili essentially de novo. This may allow us to address many questions regarding the steps in pilus assembly in a more kinetic fashion. Also, the ability to alter the adherence properties of this strain, or strains containing the described construction in the chromosome, may have a number of applications, notably in industrial processes. Finally, the *lacUV5-cat* construction in IS*50* may facilitate the movement of this selectable promoter to any site in which Tn*5* or its derivatives are positioned.

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