Interaction of colicin E7 with the major coat protein (g8p) may confer limited protection on colicinogenic *Escherichia coli* against M13 bacteriophage infection

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Colicin release provides producer strains with a competitive advantage under certain circumstances. We found that propagation of M13 bacteriophage in cells producing colicin E7 is impaired, without alteration in the efficiency of bacteriophage adsorption, as compared with non-producing cells. In contrast to the protective effect of the colicin against M13 bacteriophage infection, the endogenously expressed colicin does not confer limited protection against transfection with M13 bacteriophage DNA. Furthermore, it was found that the translocation-receptor-binding domain and toxicity domain of the colicin are able to interact with the M13 major coat protein, g8p, during bacteriophage infection. Based on these observations, we propose that interaction between colicin E7 and g8p during infection interferes with g8p depolymerizing into the cytoplasmic membrane during bacteriophage DNA penetration, thus resulting in the limited protection against M13 bacteriophage infection.

INTRODUCTION

Colicins are bactericidal protein toxins that are lethal to *Escherichia coli* and related enteric bacteria (Lazdunski *et al.*, 1998; Pugsley, 1984). They are encoded by colicinogenic plasmids (pCol) and these are widely distributed in natural populations of *E. coli* (Riley & Gordon, 1992). Colicins undoubtedly serve a number of functions within the *E. coli* population, but their ecological role is still not clear (Cascales *et al.*, 2007; Riley & Wertz, 2002). It has been previously reported that ColIb and ColV are able to exert an inhibitory effect against T-odd bacteriophage and Me1 bacteriophage, respectively (Duckworth & Pinkerton, 1988; Reakes *et al.*, 1987). *E. coli* containing ColE7-K317 shows limited protection against bacteriophage M13K07 and λ infection (Lin *et al.*, 2004). These findings indicated that bacteriophage defence might be one of colicins' additional functions. The limited bacteriophage resistance might explain why populations of cells containing colicin plasmids are common in the natural environment (Feldgarden *et al.*, 1995). Such limited protection conferred by colicin-producing cells also provides a distinct defence mechanism from other approaches, which include restriction/modification systems (Bannister & Glover, 1968), resistance induction (Sanders & Klaenhammer, 1983), superinfection exclusion

(McGrath *et al.*, 2002) and abortive infection (Duckworth *et al.*, 1981).

M13 bacteriophage is a filamentous *E. coli*-specific virus that only adsorbs on male *E. coli* strains by interacting with the F conjugative pilus (Rasched & Oberer, 1986). This specific binding is mediated by the central domain (N2) of the bacteriophage gene III protein, g3p (Marvin, 1998). After bacteriophage binding, the F-pilus is retracted and the N-terminal domain (N1) of g3p binds to the C-terminal domain of the TolA protein, which is referred to as the coreceptor (Lubkowski *et al.*, 1999; Riechmann & Holliger, 1997). The cooperative effects of the TolA protein help the insertion of g3p into the inner membrane and facilitate the entry of the bacteriophage DNA into the bacterial cytoplasm (Click & Webster, 1997; Karlsson *et al.*, 2003). Furthermore, penetration of bacteriophage DNA during infection is also accompanied by insertion of the major coat protein, g8p, into the cytoplasmic membrane. Functional TolQ, TolR and TolA proteins are required for insertion of g8p into cytoplasmic membrane upon infection (Click & Webster, 1998). The major coat proteins have to be stripped off from the entering DNA molecule in the inner membrane upon bacteriophage disassembly (Stopar *et al.*, 2003). Therefore, normal depolymerization of bacteriophage coat proteins is essential for the translocation of the bacteriophage genome.

Bacteriophage resistance systems have been extensively studied in lactic acid bacteria due to their important role in

A supplementary table showing the limited protection against M13 bacteriophage exerted by various colicin groups after mitomycin C induction is available with the online version of this paper.

the dairy fermentation industry. These natural host-encoded bacteriophage-resistance systems have been divided into four groups based on their mode of action: prevention of bacteriophage adsorption, blocking of bacteriophage DNA injection, restriction/modification systems and abortive infection (Abi) systems (Coffey & Ross, 2002; Dinsmore & Klaenhammer, 1995). In this work we investigated a further mechanism by which harbouring the ColE7-plasmid confers on *E. coli* a limited resistance against M13 bacteriophage infection. Induction of the ColE7 operon does not alter the efficiency of M13 bacteriophage adsorption. Moreover, colicin-producing cells do not show limited protection against transfection by M13 bacteriophage DNA. Most interestingly, we found that the translocation-receptor-binding domain (TR domain) and toxicity domain (T2A domain) of ColE7 are able to interact with the g8p protein of the M13 bacteriophage. Thus, these results suggest a possible mechanism by which limited protection against M13 bacteriophage is conferred on colicin-producing cells.

METHODS

Bacterial strains, media, and construction of plasmids. *E. coli* JM101 F' was used as the host strain for subcloning, expression and bacteriophage infection. The expression plasmid pQE30 (Qiagen) was used for the overexpression of the colicin-immunity protein complex (Col-Im), TR domain, toxicity domain-immunity protein complex (T2A-Im), and immunity protein. The expression plasmid pQE70 (Qiagen) was used for the overexpression of the TR-lys, T2A-Im-lys and Im-lys domains. M13K07 bacteriophage was purchased from the Institute of Food Industry Research and Development, Shin-Chu, Taiwan. Cultures were routinely grown in Luria-Bertani (LB) broth or on plates of LB agar and were supplemented, where required, with ampicillin (100 µg ml⁻¹).

Construction of vectors for expression of the individual functional domains. Clones were constructed using the method described by Lin *et al.* (2004). *Bam*HI and *Hind*III sites were used in the construction of pQE30 clones, and *Sph*I and *Bgl*II sites were used for pQE70 clones. Colicin E7 contains a TR domain consisting of the N-terminal 447 amino acids and a toxicity domain consisting of the C-terminal 133 amino acids (amino acid residues 444 to 576). Respective DNA fragments for genetic modification were amplified by PCR using ColE7-pK317 as template. The primers used for PCR amplification are listed in Table 1. The ColE7 operon was subcloned into pQE70 and genetically manipulated to generate TR-Lys, T2A-Im-Lys and Im-Lys. The amplified DNA fragments were phosphorylated by T4 polynucleotide kinase. After self-ligation using T4 DNA ligase, the plasmids were transformed into *E. coli* JM101.

Quantification of M13 bacteriophage progeny by ELISA. Bacteria (JM101 and JM101-pK317) were grown in LB broth at 37 °C to OD₆₀₀ 0.6. M13 bacteriophage particles were added to the bacterial cells at an m.o.i. of 0.1 and allowed to infect for 15 min at 37 °C. The bacterial culture was centrifuged at 4000 g for 10 min and the supernatant were completely removed. The bacterial pellets were resuspended in the same volume of LB broth and grown at 37 °C. At this point 1 ml of the bacterial culture was removed and centrifuged at 13 000 r.p.m. for 5 min and the supernatant was retained. Aliquots of the supernatant were diluted 100-fold with NaHCO₃ buffer (50 mM, pH 9.6). The supernatant was sampled every 20 min for 3 h and then diluted; 200 µl of the supernatant dilution at each time point was coated onto microtitre plates (Nunc MaxiSorp) overnight at 4 °C.

Table 1. Oligonucleotide primers used for the amplification of the colicin functional domains

Functional domain*	Primer sequence (5'–3')
ColE7	F: ATTTGCATGCGCGGTGGAG R: GATTGCAGATCTCTGCGTTCCAC
Col-Im	F: GGAGGATCCATGAGCGGTGG R: GCATTAAGCTTTCAGCCCTGTT
TR domain	F: GGAGGATCCATGAGCGGTGG R: CTGGAAGCTTCCGTTTACTCTC
T2A-Im	F: TTAGGATCCGAGAGTAAACG R: GCATTAAGCTTTCAGCCCTGTT
Im	F: GGTAGGATCCATGGAACGAAA R: GCATTAAGCTTTCAGCCCTGTT
TR-Lys	F: TGAAATATGAATGCCGGTTG R: CCGTTTACTCTCCTTATC
T2A-Im-Lys	F: GAGAGTAAACGGAATAAGCC R: CATGCTTAATTTCTCCTC
Im-Lys	F: ATGGAAGTAAAAATAGT R: CATGCTTAATTTCTCCTC

*ColE7, Colicin E7 operon; Col-Im, colicin and immunity protein; TR, translocation and receptor binding domain; T2A-Im, toxicity domain and immunity protein; Im, immunity protein; TR-Lys, translocation-receptor binding domain and lysis protein; T2A-Im-Lys, toxicity domain-immunity protein and lysis protein; Im-Lys, immunity protein and lysis protein.

After being washed with Tris-buffered saline (TBS)/0.1% Tween-20 once, the plates were blocked for 1 h with 200 µl 2% BSA in TBS-T. The plates were washed and incubated with 100 µl mouse anti-pVIII first antibody (Abcam) at a dilution of 1:5000 in blocking solution for 2 h at room temperature. Following this, 100 µl of secondary antibody (goat anti-mouse) at a dilution of 1:5000 was added, and incubated for another 1 h. Then 100 µl of tetramethylbenzidine (TMB) peroxidase substrate was added to each well, and the plates were incubated for 20 min at 37 °C. Finally, TMB stop solution (100 µl) was added and A₄₅₀ was measured using a microplate reader (Molecular Device). The experiments were performed in triplicate.

Bacteriophage adsorption assay. Bacteria (JM101 and JM101-pK317) were grown in LB broth at 37 °C to OD₆₀₀ 0.8 and induced with mitomycin C (0.5 µg ml⁻¹) for 30 min. Induced or non-induced cells were infected with M13 bacteriophage at an m.o.i. of 0.01. The mixtures were incubated for 10 min at 37 °C to allow binding of the bacteriophage to cell surfaces. After centrifugation at 13 000 r.p.m. for 5 min, the bacteriophage titre in the supernatant was determined by a standard plaque assay. The percentage adsorption was calculated as follows: [(control titre – residual titre of supernatant after cells were removed)/control titre] × 100.

Transfection of M13 bacteriophage ssDNA by heat shock. Bacterial cells containing pQE30 *cea-ceiE7* plasmids were grown in LB broth at 37 °C to OD₆₀₀ 0.4. Colicin expression was induced with 0.1 mM IPTG for 30 min. The bacterial cells were harvested for preparation of competent cells by the calcium chloride method (Sambrook *et al.*, 1989). Bacteriophage ssDNA was isolated from M13 bacteriophage particles using a QIAprep Spin M13 kit (Qiagen). The isolated M13 bacteriophage ssDNA was transformed into the competent cells endogenously expressing colicin. The samples were plated on soft agar LB plates and incubated overnight at 37 °C. The

number of p.f.u. for each of the bacteria was determined by counting the plaques on each plate. The experiments were performed in triplicate.

In vivo cross-linking and pull-down by Ni-NTA beads. *E. coli* cells harbouring various plasmids (pQE30-Col-Im, pQE30-TR, pQE30-T2A-Im or pQE30-Im) were grown to OD₆₀₀ 0.6 and induced with 0.1 mM IPTG for 1 h. Next they were infected with M13 bacteriophage at an m.o.i. of 0.1 for 15 min and cross-linked with 1 % formaldehyde for 30 min at room temperature. The cross-linking reaction was quenched by the addition of 1 M Tris buffer to a final concentration 0.1 M for 20 min. After cross-linking, cells were collected and washed with Tris buffer. The cell pellet was resuspended in 10 ml buffer A (20 mM Tris/HCl, 100 mM Na₂HPO₄, 0.1 % SDS, 8 M urea, pH 7.8) and lysed using a French press. Cell lysates were centrifuged at 15 000 r.p.m. (27 216 g) for 20 min and the supernatant was incubated with 1 ml pre-equilibrated Ni-NTA resin for 1 h at room temperature. Beads were washed with 5 ml buffer A and 8 ml buffer B (buffer A with 50 mM imidazole). Protein was eluted in 6 ml elution buffer (50 mM Na₂HPO₄, 150 mM NaCl, 0.1 % SDS, 0.5 M imidazole, pH 7.0). Eluates were dialysed and concentrated using an Amicon Ultra-4 with a molecular mass cut-off of 3000 (Millipore). The samples were mixed with 4 × sample buffer, boiled at 100 °C for 20 min and analysed by immunoblotting.

Infection experiments. Bacteria were grown in LB broth at 37 °C to OD₆₀₀ 0.6. Protein expression was induced with 0.1 mM IPTG for 1.5 h. Thereafter, M13 bacteriophage particles were added to the bacterial cells at an m.o.i. of 0.01 and incubated for 15 min at 37 °C. The samples were plated on soft agar LB plates and incubated overnight at 37 °C. The number of p.f.u. for each of the bacteria was determined by counting the plaques on each plate. The p.f.u. of non-induced *E. coli* cells was taken as 100 % and the p.f.u. of induced *E. coli* cells was expressed as a percentage of this. The experiments were performed in triplicate.

Immunoblotting analysis. Rabbit polyclonal antibodies against the receptor binding domain (R domain), toxicity domain (T2A) and immunity protein (Im) were produced for this study. Anti-R, Anti-T2A and Anti-Im antibodies were used to detect the TR domain, the T2A domain and the immunity protein, respectively. Rabbit polyclonal antibody against 6 × His was purchased from Bethyl Laboratories. Mouse monoclonal antibody against GroEL was purchased from Sigma. Individual samples were resolved by Tricine-SDS-PAGE (Schägger & von Jagow, 1987). The gel was then transferred to a nitrocellulose membrane using a wet transfer apparatus. After blocking, the membrane was probed individually with the various antibodies. Protein bands were visualized with NEN Western blot chemiluminescence reagent plus.

RESULTS

Reproduction, adsorption and DNA transfection of bacteriophage in colicin-producing cells

First, we examined the multiplication of M13 bacteriophage in colicinogenic *E. coli* cells as compared with non-colicinogenic cells. The relative number of bacteriophage progeny was determined by ELISA quantification of the major coat protein, g8p. The virion number was lower and the multiplication rate of bacteriophage progeny was slower in colicin-producing cells than in non-producing cells (Fig. 1). The propagation of M13 bacteriophage in colicin-producing cells was thus being impeded. In order to clarify the mechanism by which the colicin-producing cells exert this limited protection, we first

Fig. 1. Production of M13 bacteriophage progeny in colicin-producing cells (JM101-pK317, ○) and non-producing cells (JM101, ●). M13 bacteriophage collected from culture supernatant at each time point were coated on ELISA plates. The relative amount of M13 bacteriophage progeny was determined by detecting the amounts of g8p. The OD₄₅₀ was measured every 20 min after M13 bacteriophage infection and the results are expressed as mean ± SD of triplicate measurements (most of the error bars are smaller than the symbols).

examined whether the ability to adsorb bacteriophage was altered. Adsorption assays showed that M13 bacteriophage adsorbed to colicin-producing cells with a similar efficiency to that of colicin-non-producing cells, and the results were the same for the mitomycin C-induced group (Table 2). Therefore, expression of the ColE7 operon would seem to have no influence on bacteriophage attachment.

The colicin-mediated limited protection seemed to act after bacteriophage adsorption to the cell and therefore we next examined whether the colicin interferes with the subsequent translocation of bacteriophage DNA into the cytoplasm of *E. coli* cells. Transfection of M13 bacteriophage DNA into colicin-containing cells would effectively permit the bacteriophage to bypass the adsorption and DNA penetration parts of the infection process. Following transfection of M13 bacteriophage ssDNA into colicin-containing cells, the number of p.f.u. of colicin-containing cells was not reduced as compared with non-induced cells (Fig. 2). Based on the above results, it would seem that bacteriophage adsorption was not altered and that colicin-containing cells do not exert any protection against transfected M13 bacteriophage ssDNA. This suggests that colicin-mediated limited protection against M13 bacteriophage may operate by interfering with bacteriophage DNA penetration into the cell.

Interference with penetration of M13 bacteriophage DNA into cells

The minor coat protein, g3p, is essential for recognition of the F-pilus and binding to the coreceptor TolA and may

Table 2. Effect of colicin production on adsorption of M13 bacteriophage to *E. coli*

The experiments were performed in triplicate; results are means $\pm$ SD.

Strain	Adsorption (%)
JM101	98.89 ($\pm$ 0.05)
JM101-pK317	98.89 ($\pm$ 0.03)
JM101/MMC*	98.91 ($\pm$ 0.03)
JM101-pK317/MMC*	98.95 ($\pm$ 0.03)

*MMC, mitomycin C induction.

form a channel for passage of the bacteriophage DNA. Membrane insertion of the major coat protein, g8p, is coupled to the translocation of the DNA into the cytoplasm. One possibility is that colicin E7 interferes with M13 bacteriophage infection by disturbing how the coat protein functions. To this end, we performed *in vivo* cross-linking experiments with formaldehyde to examine whether the colicin is able to interact with coat protein in *E. coli* cells infected with M13 bacteriophage. The immunoblotting analysis indicated that the major coat

protein, g8p, was able to interact with colicin molecules (Fig. 3a).

The structure of colicin E7 is divided into two functional domains, the TR domain and the T2A domain. The latter is lethal to the producing cells, so the immunity protein (Im) is coexpressed to block this domains DNase activity by forming a protein complex (T2A-Im). The TR domain and T2A domain of colicin E7, but not the immunity protein, are involved in binding to g8p (Fig. 3b, c, d). Importantly, g8p was not detected in the control groups without protein expression or when they were devoid of M13 bacteriophage particles (Fig. 3b). g8p was also not detected in infected cells not treated with formaldehyde; this would rule out the possibility of non-specific binding of M13 bacteriophage particles to beads. Thus, these experimental results support the hypothesis that the transport of M13 bacteriophage DNA across the cell membrane is impeded to some extent in colicin-producing cells, which results in these cells having limited protection against M13 bacteriophage infection.

Limited protection against M13 bacteriophage by individual functional domains of colicin E7

Since the functional domains of colicin E7 are involved in binding to g8p, we next investigated whether these domains are required for the protective effects. The protective effects of colicin-producing cells against M13 bacteriophage were determined using the number of p.f.u. as a standard (Lin *et al.*, 2004). As compared with the non-induction groups, IPTG induction of *E. coli* JM101 (TR, T2A-Im and Im) clearly showed that both the TR domain and the T2A-Im complex contributed to the limited protection against M13 bacteriophage but not the immunity protein (Im) alone. The p.f.u. of induced TR-domain-producing cells were reduced to 43 % compared with the non-induced cells. Similarly, the p.f.u. of induced T2A-Im complex-producing cells were reduced to 40 % (Fig. 4a). It is noteworthy that induction of immunity protein and the control group (empty vector) showed no effect on p.f.u. Immunoblotting analysis of the cell extracts verified the presence of the induced proteins (Fig. 4b). The observed limited protection of the TR domain and the T2A-Im complex was indeed related to their protein expression level. These results indicated that the functional domains involved in binding to the major coat protein, g8p, are important to the limited protection against M13 bacteriophage.

Fig. 2. Effect of limited protection during transfection of M13 bacteriophage ssDNA into *E. coli* cells containing endogenous colicin. (a) 'Vector' indicates the control group without colicin expression. Total p.f.u. of the non-induced cells was taken as 100 % (black bars). The p.f.u. of the *E. coli* cells with endogenous colicin is shown as a percentage with respect to the total number of p.f.u. of non-induced cells (white bars). (b) The endogenous colicin levels were analysed by immunoblotting: +, with IPTG induction; -, without IPTG induction. GroEL was used as an internal control.

The importance of lysis protein in the colicin-producing cells for the limited protection against M13 bacteriophage infection

The lysis protein is essential for colicin secretion, and high levels of lysis protein will result in quasilysis. In order to examine the effect of lysis protein on limited protection, the lysis protein was coexpressed with the various different functional domains. The p.f.u. of the induced groups

Fig. 3. *In vivo* cross-linking of colicin in M13 bacteriophage-infected *E. coli* cells. The expression of Col-Im (a), TR domain (b), toxicity domain and immunity protein (T2A-Im) (c) and immunity protein (d) is indicated. *E. coli* cells infected with M13 bacteriophage (+) or uninfected (-) were subsequently incubated with formaldehyde. Cells were lysed and various domains were purified under denaturing conditions using Ni-NTA beads. Then the eluates were analysed by immunoblotting using anti-His-tag and anti-g8p antibodies. Marker, g8p coat protein from denatured M13 bacteriophage particles.

showed that TR-Lys and T2A-Im-Lys, but not Im-Lys, conferred a protective effect. The p.f.u. of the induced TR-Lys group and T2A-Im-Lys group were reduced to 38 % and 44 %, respectively (Fig. 5a). The ratio of p.f.u. in these groups was similar to that in groups devoid of lysis protein (Fig. 4a). Immunoblotting analysis of the cell extracts verified the expression of the induced protein in each group (Fig. 5b). These results indicate that the presence of the lysis protein in the cell membrane does not impede M13 bacteriophage infection. Taken together, the results suggest that the structural gene (*ceaE7*) in the ColE7 operon is mainly responsible for the limited protection against M13 bacteriophage infection.

DISCUSSION

Colicins, encoded by Col plasmids, are known for their killing activity against sensitive Enterobacteriaceae. We previously observed that the expression of colicin is able to provide the producing cells with a limited protection against M13 bacteriophage (Lin *et al.*, 2004). In this report we have further demonstrated that the production of M13 bacteriophage progeny in colicin-producing cells is less effective than that in non-producing cells (Fig. 1). The slower reproduction rate of bacteriophage progeny in colicin-producing cells revealed that the propagation of M13 bacteriophage is partially impeded. In addition, the limited protection against M13 bacteriophage was also exerted by other Col plasmids. Induction of the ColE2, ColE8 or ColE9 operon resulted in respectively 41.7 %, 29.5 % and 44.6 % reduction in p.f.u. as compared with a non-colicinogenic strain (see Supplementary Table S1, available with the online version of this paper).

The adsorption assay showed that the efficiency of bacteriophage adsorption is not affected in colicin-producing cells, indicating that the expression of colicin did not interfere with the normal functioning of the F-pilus

Fig. 4. Limited protection of *E. coli* cells expressing the different domains of colicin against M13 bacteriophage. (a) Total p.f.u. of the non-induced cells with the respective domains was taken as 100 % (black bars). The p.f.u. of the IPTG-induced cells is expressed as a percentage with respect to the total number of p.f.u. of the cells without IPTG induction (white bars). TR domain (TR), toxicity domain and immunity protein (T2A-Im) and immunity protein (Im) are indicated. The experiments were performed in triplicate, and the number of p.f.u. for each is shown as mean $\pm$ SD. *, $P < 0.001$ (versus non-induction control) in Student's *t* test. (b) Immunoblotting of cell extracts was performed to examine the expression levels of each domain by detecting the fused His-tag on each protein: +, with IPTG induction; -, without IPTG induction. GroEL was used as an internal control.

Fig. 5. Effects of limited protection in *E. coli* cells by coexpression of truncated colicin and lysis protein (Lys). (a) Coexpression of individual functional domains (TR domain alone, toxicity domain with immunity protein, and immunity protein alone) and lysis protein is indicated as TR-Lys, T2A-Im-Lys and Im-Lys. Total p.f.u. of the non-induced cells with respective domains was taken as 100% (black bars). The p.f.u. of the IPTG-induced cells is expressed as a percentage with respect to the total number of p.f.u. of the cells without IPTG induction (white bars). The experiments were performed in triplicate and the number of p.f.u. of each is shown as mean $\pm$ SD. *, $P < 0.001$ (versus non-induction control) in Student's *t* test. (b) Immunoblotting of cell extracts was performed to examine the expression levels of each functional domain using respective antibodies. The lysis protein was examined by detecting the fused His-tag: +, with IPTG induction; -, without IPTG induction. GroEL was used as an internal control.

(Table 2). Subsequently, using transfection of bacteriophage DNA into colicin-producing cells, it was shown that there was no limited protection against infection in these circumstances (Fig. 2), which implies that the later steps in the life cycle of M13 bacteriophage were not affected by the colicin. These results indicated that colicin-mediated limited protection might be a result of the blocking of DNA injection. Interestingly, we found that the TR domain and the toxicity domain of colicin E7 are able to interact with the major coat protein, g8p, and this interaction made the most significant contribution to the limited protection afforded to colicin-producing cells (Figs 3 and 4). These results indicated that the colicin might interfere with the translocation of bacteriophage DNA into the cytoplasm of infected cells by interacting with g8p.

TolQ, TolR and TolA are required for membrane insertion of the major coat proteins of bacteriophage M13 and they mediate the depolymerization of g8p into the cytoplasmic membrane. DNA translocation and membrane insertion of g8p are closely coupled, although the details of the bacteriophage DNA translocation mechanism are still unclear (Click & Webster, 1998). Thus, our results indicated that colicin E7 is able to interfere with depolymerization of g8p into the cytoplasmic membrane. Recently, Stopar *et al.* (2003, 2006) reported that individual amino acid residues or domains of amino acid residues with similar properties play important roles in the protein-protein and protein-lipid interactions of g8p during the infection of *E. coli* by M13 bacteriophage (Stopar *et al.*, 2003, 2006). The proper insertion and solubilization of g8p during bacteriophage disassembly is followed by transfer of bacteriophage DNA. Thus it seems that the delicate process needs to be correctly carried out. Our study shows that the interaction between colicin and g8p may be responsible for the limited protection against M13 bacteriophage infection afforded by having a colicin plasmid present in the cell.

Several studies have demonstrated the blocking of bacteriophage DNA penetration, and the mechanism was first described for various bacteriophage-resistance systems (Garvey *et al.*, 1996; Hofer *et al.*, 1995; Lu *et al.*, 1993; McGrath *et al.*, 2002). In some cases (Hofer *et al.*, 1995; Lu *et al.*, 1993; McGrath *et al.*, 2002), this involved bacteriophage-mediated superinfection exclusion while in other cases it involved host-encoded bacteriophage defence systems (Garvey *et al.*, 1996). Either interaction with the bacteriophage DNA pilot protein or modifying/masking of the proteins required for bacteriophage DNA injection will result in the blocking of DNA injection. It should also be noted that colicin E7 is able to interact with g8p that is localized in the inner membrane, which is where the Tol protein facilitates membrane insertion of g8p. Therefore we are unable to exclude the possibility that colicin may compete for some components needed for infection such as Tol proteins. Hence, the detailed pathway during colicin export needs to be further investigated. Nevertheless, this is to our knowledge the first description of a colicin-mediated bacteriophage-resistance system that acts by inhibiting bacteriophage DNA penetration.

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REFERENCES

- Bannister, D. & Glover, S. W. (1968). Restriction and modification of bacteriophages by R^+ strains of *Escherichia coli* K12. *Biochem Biophys Res Commun* **30**, 735-738.

- Cascales, E., Buchanan, S. K., Duche, D., Kleanthous, C., Lloubes, R., Postle, K., Riley, M., Slatin, S. & Cavad, D. (2007). Colicin biology. *Microbiol Mol Biol Rev* **71**, 158–229.
- Click, E. M. & Webster, R. E. (1997). Filamentous phage infection: required interactions with the TolA protein. *J Bacteriol* **179**, 6464–6471.
- Click, E. M. & Webster, R. E. (1998). The TolQRA proteins are required for membrane insertion of the major capsid protein of the filamentous phage ϕ 1 during infection. *J Bacteriol* **180**, 1723–1728.
- Coffey, A. & Ross, R. P. (2002). Bacteriophage-resistance systems in dairy starter strains: molecular analysis to application. *Antonie van Leeuwenhoek* **82**, 303–321.
- Dinsmore, P. K. & Klaenhammer, T. R. (1995). Bacteriophage resistance in *Lactococcus*. *Mol Biotechnol* **4**, 297–314.
- Duckworth, D. H. & Pinkerton, T. C. (1988). Collb plasmid genes that inhibit the replication of T5 and T7 bacteriophage. *Plasmid* **20**, 182–193.
- Duckworth, D. H., Dunn, G. B., Pinkerton, T., Rose, K. & Walia, S. K. (1981). Colicin activity and abortive infection of T5 bacteriophage in *Escherichia coli* (Collb). *J Virol* **37**, 916–921.
- Feldgarden, M., Golden, S., Wilson, H. & Riley, M. A. (1995). Can phage defence maintain colicin plasmids in *Escherichia coli*? *Microbiology* **141**, 2977–2984.
- Garvey, P., Hill, C. & Fitzgerald, G. F. (1996). The lactococcal plasmid pNP40 encodes a third bacteriophage resistance mechanism, one which affects phage DNA penetration. *Appl Environ Microbiol* **62**, 676–679.
- Hofer, B., Ruge, M. & Dreiseikelmann, B. (1995). The superinfection exclusion gene (*sieA*) of bacteriophage P22: identification and overexpression of the gene and localization of the gene product. *J Bacteriol* **177**, 3080–3086.
- Karlsson, F., Borrebaeck, C. A., Nilsson, N. & Malmberg-Hager, A. C. (2003). The mechanism of bacterial infection by filamentous phages involves molecular interactions between TolA and phage protein 3 domains. *J Bacteriol* **185**, 2628–2634.
- Lazdunski, C. J., Bouveret, E., Rigal, A., Journet, L., Lloubes, R. & Benedetti, H. (1998). Colicin import into *Escherichia coli* cells. *J Bacteriol* **180**, 4993–5002.
- Lin, Y. H., Liao, C. C., Liang, P. H., Yuan, H. S. & Chak, K. F. (2004). Involvement of colicin in the limited protection of the colicin producing cells against bacteriophage. *Biochem Biophys Res Commun* **318**, 81–87.
- Lu, M. J., Stierhof, Y. D. & Henning, U. (1993). Location and unusual membrane topology of the immunity protein of the *Escherichia coli* phage T4. *J Virol* **67**, 4905–4913.
- Lubkowski, J., Hennecke, F., Pluckthun, A. & Wlodawer, A. (1999). Filamentous phage infection: crystal structure of g3p in complex with its coreceptor, the C-terminal domain of TolA. *Structure* **7**, 711–722.
- Marvin, D. A. (1998). Filamentous phage structure, infection and assembly. *Curr Opin Struct Biol* **8**, 150–158.
- McGrath, S., Fitzgerald, G. F. & van Sinderen, D. (2002). Identification and characterization of phage-resistance genes in temperate lactococcal bacteriophages. *Mol Microbiol* **43**, 509–520.
- Pugsley, A. P. (1984). The ins and outs of colicins. Part I: production, and translocation across membranes. *Microbiol Sci* **1**, 168–175.
- Rasched, I. & Oberer, E. (1986). Ff coliphages: structural and functional relationships. *Microbiol Rev* **50**, 401–427.
- Reakes, C. F., Gann, A. A., Rossouw, F. T. & Rowbury, R. J. (1987). Abortive infection by bacteriophage Me1 of *Escherichia coli* K12 strains bearing the plasmid ColV, I-K94. *J Gen Virol* **68**, 263–272.
- Riechmann, L. & Holliger, P. (1997). The C-terminal domain of TolA is the coreceptor for filamentous phage infection of *E. coli*. *Cell* **90**, 351–360.
- Riley, M. A. & Gordon, D. M. (1992). A survey of Col plasmids in natural isolates of *Escherichia coli* and an investigation into the stability of Col-plasmid lineages. *J Gen Microbiol* **138**, 1345–1352.
- Riley, M. A. & Wertz, J. E. (2002). Bacteriocins: evolution, ecology, and application. *Annu Rev Microbiol* **56**, 117–137.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sanders, M. E. & Klaenhammer, T. R. (1983). Characterization of phage-sensitive mutants from a phage-insensitive strain of *Streptococcus lactis*: evidence for a plasmid determinant that prevents phage adsorption. *Appl Environ Microbiol* **46**, 1125–1133.
- Schägger, H. & von Jagow, G. (1987). Tricine–sodium dodecyl sulfate–polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* **166**, 368–379.
- Stopar, D., Spruijt, R. B., Wolfs, C. J. & Hemminga, M. A. (2003). Protein–lipid interactions of bacteriophage M13 major coat protein. *Biochim Biophys Acta* **1611**, 5–15.
- Stopar, D., Spruijt, R. B. & Hemminga, M. A. (2006). Anchoring mechanisms of membrane-associated M13 major coat protein. *Chem Phys Lipids* **141**, 83–93.

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