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# How bacteria protect themselves against channel-forming colicins

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**Summary** Here we review the mechanisms that bacterial cells use to protect themselves against channel-forming colicins. Four mechanisms are examined: immunity, resistance, tolerance and PacB character. Immunity confers protection to colicinogenic cells against the colicin they produce, since the colicinogenic plasmid bears the genetic determinant for such immunity protein. Resistance is provided by modifications on colicin receptors located on the outer membrane. It prevents colicin adsorption and protects against those colicins sharing a common receptor. Tolerance is achieved by changes in the translocation system. The adsorbed colicin is not translocated toward the periplasmic space. This impedes its insertion into the cell membrane as well as the formation of the transmembrane channel. Tolerance confers protection against colicins that share the same translocation system. Finally, we discuss the PacB character, that confers protection against all known channel-forming colicins. The latter property is encoded by non-colicinogenic plasmids in the H-incompatibility complex.

**Key words** Channel-forming colicins · H plasmids · PacB character · Tellurite · Translocation systems

## General characteristics of colicins

Colicins are a subset of the bactericidal proteins known as bacteriocins, produced by *Escherichia coli* strains harboring colicinogenic plasmids. Unlike most bacterial toxins, they exert their effect against *E. coli* and closely related bacteria. The origin and evolution of colicinogeny are still the subject of controversy. Since colicin production is encoded by plasmids, it has been seen as a dispensable and nonessential function for the survival of coliform bacteria. Nevertheless, the relative abundance of colicinogenic strains (30%–50%) among *E. coli* isolates, and the highly-conserved organization of colicinogenic genes and amino acid sequences, suggests that they could play a positive role in the ecology of coliform bacteria [5, 22, 29]. The prevalence of colicinogenic strains in the gastrointestinal ecosystem could be explained if it were found to benefit the colonization and defense of an ecological niche occupied or shared by non-colicinogenic sensitive microorganisms with similar or identical nutritional requirements.

Colicin synthesis is an inducible system; i.e., it is not produced under normal circumstances because of a strong repressive control. When colicinogenic cells are under stressful conditions or are exposed to physical or chemical agents, colicin synthesis is derepressed, resulting in an increase in transcription rates and colicin synthesis [22]. Once the colicin is produced, it is carried to the extracellular environment, causing a partial lysis and death of the producer microorganism. Therefore, colicin production is known as a "suicide" phenomenon, affecting viability of the induced cell. However, it becomes a selective advantage for the remainder of the colicinogenic population, which is protected by a specific immune mechanism [14].

Colicins are toxins whose molecular sizes range from 29 to 75 kDa. So far, 18 colicins have been identified and classified according to immunity and resistance. They are: colicin A,

B, D, E1 to E9, Ia, Ib, K, L, M and N. Based on their mode of action, colicins have been classified into two major groups: Enzymatic colicins and Channel-forming colicins.

Enzymatic colicins kill sensitive cells through degradation of chromosomal DNA or by protein synthesis inhibition.The following subgroups have been recognized: Colicins E2, E7, E8 and E9 are endodeoxyribonucleases. Colicins E3, E4 and E6 are endoribonucleases. Some colicins, including D and E5, inhibit protein synthesis.

The mechanism of action of the remaining classified colicins identified and characterized is common to all of them. They form ionic channels in the cytoplasmic membrane of the sensitive bacteria [18]. Colicins A, B, E1, Ia, Ib, K and N belong to the well defined group of channel-forming colicins.

Channel-forming colicins promote bacterial death by forming voltage-dependent ionic channels on the cytoplasmic membrane. After pore formation, the transmembrane electrochemical gradient is disrupted; as a result, the proton force is dissipated. The main cause of cellular death is the reduction of the intracellular levels of $K^+$ and $Mg^{2+}$, and the fugue of ATP and phosphorylated metabolic intermediaries, which are major components for the cell [17].

The primary structure of the channel-forming colicin has allowed polypeptidic chain domains to associate with each of the events needed for their action (Fig. 1). Thus, the N-terminus is involved in translocation through the outer membrane, whereas the C-terminus is the pore-forming domain, and the receptor-binding domain is located in the middle of the protein [2]. Sequence analysis of various channel-forming colicins (A, B, E1, Ia, Ib, N) revealed a conservation of the C-terminal domains [18, 19, 26]. Circular dichroism studies in ColA show that colicin secondary structure is composed mainly of α-helix [18, 19, 26]. Crystal structures of several colicins have been determined. In the case of ColA, ten α-helices were observed, all of which are amphiphilic except for the highly hydrophobic central helices (helices 8 and 9). A model has been proposed to explain the mode of action of colicins, which suggests a first step of electrostatic binding to the membrane surface, followed by the insertion of 8 and 9 helices into the membrane, and insertion of the amphiphilic hairpin helices 5 and 6.

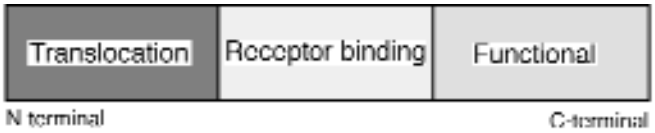


**Fig. 1** The domains of channel-forming colicins

All channel-forming colicins are encoded by plasmids. The organization of genes involved in colicin synthesis is highly conserved. Generally, there are three adjacent genes: the structural gene, the immunity gene and the lysis gene, coding for colicin, immunity protein and lysis protein, respectively. The structural and lysis genes constitute an operon, and the immunity gene is located between these two genes and is transcribed in the opposite direction from its own promoter. In ColB, ColIa and ColIb, there are no lysis genes, but the arrangement of the other two genes is identical to that previously described. The transcription of the operon constituted by the structural gene and the lysis gene is strongly repressed by the binding of the LexA repressor protein. Thus, under normal circumstances, the colicin cannot be synthesized. However, the immunity gene is transcribed at low levels [5, 18, 28]. There is a transcription terminator at the end of the structural gene, which causes the lysis gene to be produced at a much lower level than the structural gene. When the system is induced, the lysis gene causes colicin accumulation within the cell, and colicin exportation begins when the product of this gene reaches significant levels. The product of the lysis gene is a protein that is modified post-transcriptionally to form a lipoprotein that is located in the inner membrane and is partially outside the cell; this lipoprotein activates a phospholipase. The outer membrane becomes permeable, allowing the random release of low molecular weight proteins including the colicin. The increase in the permeability of the outer membrane induces a partial lysis of the colicinogenic cell, leading to its death. Note that the partial lysis of the colicinogenic cell occurs as a result of colicin exportation, and not because of the action of intracellular colicin. Colicin molecules are not able to insert themselves on the inner side of the membrane, presumably because the transmembrane potential polarity is contrary to that required for their correct insertion[18].

## Action of channel-forming colicins on sensitive bacteria

Channel-forming colicins exert their lethal effects following a sequence of events: a) the colicin binds to a specific receptor on the outer membrane of the target cell; b) it is translocated through the cell envelope; and c) finally, it is inserted in the membrane and forms the pore or channel that leads to cell death.

**Binding to the receptor** Receptors are integral membrane proteins, that have several transmembrane domains as well as an external domain exposed to the environment (Table 1). Nutrient receptors are commonly used by colicins ensuring their maintenance without other selective pressures. In general, colicins require a single membrane receptor to be internalized, with the exception of colicin A, which uses two receptors: OmpF y BtuB [5].

**Table 1** Channel-forming colicins. Activity, immunity, receptor proteins, and translocation system

| Colicin | Activity protein* | Immunity protein* | Receptor protein | Translocation system |
|---|---|---|---|---|
| A | 592 | 178 | BtuB/OmpF | Tol system |
| B | 510 | 175 | FepA | Ton system |
| E1 | 522 | 113 | BtuB | Tol system |
| Ia | 626 | 111 | Cir | Ton system |
| Ib | 626 | 115 | Cir | Ton system |
| K | 548 | 96 | Tsx | Tol system |
| N | 387 | 85 | OmpF | Ton system |

* Number of amino acid residues of the open reading frame.

**Translocation through outer membrane** Translocation occurs after the recognition of the colicin outer membrane receptor. Although colicin absorption follows a first-order kinetic, which implies that a single colicin molecule can kill a cell, more than one bound colicin is required to kill a cell. Apparently, not all bound colicins are translocated through the cytoplasmic membrane. According to the translocation system used, channel-forming colicins have been classified into two groups: group A (colicins A, E1, K) requires the Tol system; group B (colicins B, Ia, Ib, N) requires the Ton system [18–20] (Table 1).

The Ton system has three major proteins: TonB, ExbB and ExbD. TonB is an energy transducer, and uses the transmembrane electrochemical gradient to take up a variety of nutrients such as iron and vitamin $B_{12}$. It was proposed that ExbB and ExbD might allow TonB to achieve an energized conformation, which would open translocating channels of the outer membrane. The interactions of TonB with the outer membrane receptors occurs through a highly-conserved region in their N-terminus called the TonB box. [15, 17, 20].

The Tol system proteins are located in the sites where inner membrane and outer membrane are in contact [2]. Four genes, *tolQ*, *tolR*, *tolA* and *tolB*, encode these proteins. This non-energy-dependent system seems to be implicated in the integrity and preservation of the cell envelope [25]. Nevertheless, it has been shown that this system is casually used by colicins and phages to translocate them through outer membrane [20, 39]. Some components of the Tol system and the TonB system are homologous.

**Insertion into the cytoplasmatic membrane** Channel-forming colicin activity is located at the C-terminus, which is able to attach itself electrostatically to the membrane surface and spontaneously insert itself into it. Models have been proposed to explain colicin insertion into the cytoplasmic membrane [5, 27]. According to the umbrella model, the hydrophobic helix insertion is perpendicular to the membrane. In the Penknife model, helices are in a diagonal position. Once the hydrophobic helices are inserted, the colicins refold themselves in a way that allows a channel through the membrane to be formed. Three factors control the insertion kinetics. First, colicins are strictly dependent on the lipid-to-protein ratio; secondly, they depend on pH, and finally they depend on the proportion of negatively charged lipids. The second and third factors appear to modify the formation of an insertion-competent state more than the insertion itself [17]. A structural study of pore formation has been difficult to carry out due to its poor stability when it is not in the correct voltage. Neither direct detection methods, such as Nuclear Magnetic Resonance (NMR) and X-ray diffraction, nor indirect methods, such as light spectroscopy, can be applied to the system. Therefore, only a hypothetical model could be drawn based on biochemical and biophysical data.

## Mechanism of protection against channel-forming colicins

Four protection mechanisms against lethal action of channel-forming colicin have been reported in bacteria. Three of them, immunity, resistance and tolerance, have long been recognized and are well characterized. We ourselves have described the fourth, PacB, which is currently under study.

**Immunity** The lethal effect of a channel-forming colicin is efficiently neutralized by the immunity protein coded by the same colicinogenic plasmid. The immunity proteins constitutively expressed by natural colicinogenic plasmids are membrane proteins with three or four transmembrane domains. They confer protection against the colicin molecules absorbed by colicinogenic bacteria, by binding to and inactivating cytotoxic domains. Intracellular colicin does not have any effect on the cell that produces it. According to their size, immunity proteins have been classified into two groups. Type E1 includes colicins E1, Ia and Ib, which have approximately 113 residues,whereas type A includes colicins A, B and N, which are larger than E1 type, having approximately 170 residues (Table 1). Both groups present a high-density charge central region, and a highly hydrophobic C-terminus. The smallest homology between immunity proteins is found at their N-terminal end [36]. It has been reported that neutralization occurs through a highly specific interaction between the immunity protein and the C-terminus of the colicin [9, 18]. The first of these reports implies the presence of colicins Ia and Ib, both of which could have been originated from a common ancestral protein. They are related both structurally and functionally, and show 60% homology at their C-terminus. Nevertheless, cells bearing plasmid ColIb show immunity against ColIb, and are sensitive to ColIa. More evidence of the specificity of the immunity system has been shown through research with hybrid colicins ColA and ColE1 [2]. Six hybrid colicins were constructed, using combinations of ColA and ColE1 domains (the functional, receptor-binding and translocation domains). Hybrid colicins bearing the ColA pore-forming domain were inactive against cells producing ColA

immunity protein. In addition, hybrid colicins bearing the C-terminal domain from ColE1 were innocuous to cells that produced ColE1 immunity protein.

In order to explain immunity protein specificity, studies of their topology on the membrane have been carried out, and a model of colicin A immunity protein organization in the plasmatic membrane has been suggested. These studies showed that the modifications that most influence the immune activity are those located in the periplasmic domain of the polypeptide. Song and Cramer [36] suggested that immunity protein specificity was probably due to an interaction with the translocating apparatus, driving the formation of a ternary complex between the translocating system. Modification of hydrophobic amino acids located in the transmembrane regions impedes the insertion of the immunity protein into the membrane.

The models proposed suggest that there is a constant lateral diffusion of the immunity protein in the two-dimensional space of the cytoplasmic membrane [9, 43]. As soon as an inserted colicin is recognized, an interprotein helix complex is formed in which at least one immunity protein transmembrane helix and two hydrophobic colicin helices interact. This model, which was applied to the cases of colicins A and B, appears to be the most important factor in colicin recognition of the immunity protein.

**Resistance** The specificity of the colicin-receptor association has been known for many years. Any alteration of the receptor precludes the recognition of the colicin, rendering the cell insensitive to the colicin's lethal action. This phenomenon, described in the 1960s, was termed "resistance" [5, 14, 22]. Resistance has been studied by means of microbiological and molecular analysis of spontaneous and laboratory mutant strains that have lost their sensitivity to colicin action. These studies have led to the elucidation of the colicin mode of action, and have been used as a model for the study of the cell-envelope proteins. They have also allowed the identification of receptor proteins that are associated with other functions such as translocation. Amino acid modifications associated with colicin resistance are located in areas of the protein exposed to the extracellular media, and are involved in the recognition process. These changes cause modifications in the receptor's three-dimensional conformation and alterations in the colicin-receptor interactions that convey resistance phenotype. Experiments in *E. coli* strains permit classification of resistant strains into two groups: those with alterations at the colicin-binding site, and those with mutations in the receptor motif involved in the colicin translocation.

Colicin resistance caused by alterations in the receptor may or may not be related to deficiencies in the receptor's natural substrate recognition and assimilation. The areas of the receptor that participate in both activities are highly related, thus associating the resistance phenotype with deficiencies in substrate incorporation [13]. Nevertheless, there are reports of mutants of protein FepA (receptor of colicin B) where its receptor activity for enteroqueline is not affected. This suggests that both mechanisms can be separated [1].

By using bypass treatment, Fourel et al. [10] restored ColA sensitivity in mutants that did not produce OmpF protein. This is an evidence of the production of other porines, which permit the internalization of the colicins under osmotic shock. Fourel et al. [11] isolated four types of OmpF mutants resistant to colicin N. The *ompF* DNA sequence showed alterations in the receptor amino acid sequence in these mutants. These are functional changes in the effective binding of the colicin and, therefore, generate a resistance phenotype. Colicin A and N use the same receptor, porine OmpF. In vitro studies [8] using heat and sodium dodecylsulfate denaturalization produce an unfolded monomeric form, incapable of binding either to ColA or to ColN, thus ruling out the possibility that the colicin binding site could be a lineal sequence of the monomer.

Group B colicins and Ton system-dependent receptors present eight highly conserved amino acids at their N-terminal end called "TonB box". Point mutations in the colicin, or in TonB receptor box lead to the binding, but not to the translocation, of either the natural substrate or the colicin [24]. Therefore, the latter type of mutant was redefined as one of those that were mutated in the receptor areas responsible for interaction with the translocation system. Group B colicins N-translocation occurs due to the participation of proteins of the Tol genes group. Therefore, an interaction is required between the receptor and these proteins.

When alterations occur in the area associated to translocation, the resistance could be related to a lower assimilation or even to the lack of assimilation of the substrate when the natural substrate is translocated by the same system as the colicin.

Finally, resistance to several colicins can be proven when they share the same receptor. Nevertheless, in channel-forming colicins, resistance is a limited protection mechanism, which sometimes can be more effective than immunity.

**Tolerance** This phenomenon is referred to as the insensitivity of a non-colicinogenic cell to colicin action. Tolerance is produced by alterations in the translocation system. Studies with colicin-tolerant strains have contributed to the understanding of the organization of *E. coli* cell envelope. In this bacteria, Ton and Tol translocation systems facilitate colicin translocation. The Ton system consists of Ton B, ExbB and ExbD proteins, which are integral membrane proteins with periplasmic domains. The Tol system is formed by four proteins (Tol ABQR), which are probably involved in the cell envelope maintenance, since no transport function has been associated [41]. Both systems show functional resemblance and high homology in the TolQR and ExbBD amino acid sequence at the transmembrane region [4, 7, 39]. Mutations in Ton or Tol system proteins prevent the internalization of the adsorbed colicin, generating a tolerance phenotype.

Two colicin-tolerant cell types have been identified: those that are mutated in the Ton system and are tolerant to group B

colicins, and those mutated in the Tol system and are tolerant to group A colicins. In both translocation mechanisms, the internalization process is similar, but each colicin has its particular molecular elements.

The outer membrane of *E. coli* does not have an energy source, therefore substrate translocation occurs by energy transduction. Ton system proteins are involved in this process. It has been suggested that TonB should be activated when complexed with ExbD and ExbB. The interaction of the N-terminal domain with ExbB stabilizes TonB.

Traub et al. [37] identified TonB functional domains, and located ColIa and ColIb tolerance between residues 181 and 239. A physical interaction area between TonB and the receptors was also found. In this sense the structural requirements of colicins Ia and B are, to a certain point, different. Traub et al. also established that variations surrounding TonB 160 residue could generate patterns of tolerance to colicins Ia and B. Traub and Braun [38] found that the area located between residues 13 and 32 was also relevant for colicin B and Ia translocation.

For the Tol translocation system, tolerance patterns with respect to group A colicins were also determined. Vianney et al. [39] carried out a mutational analysis of TolQ protein to determine its role in substrate assimilation and cell-envelope integrity. Different Tol mutants were found to be tolerant to A and E1 colicins.

Bacteria with mutations in *tolQ*, *tolR* or *tolB* genes display a colicin tolerant phenotype that can be overcome by increasing the colicin concentration ($10^3$–$10^4$ times). Nevertheless, Tol A mutants need to eliminate concentrations $10^6$ times higher than those needed to eliminate a wild-type cell population. This means, apparently, that TolA is the major protein in the translocation of group A colicins.

Based on these results, Levengood-Freyermuth et al. [21] analyzed the structure and cell localization of TolA protein. In this research it was impossible to determine with precision the role of TolA in the internalization of group A colicins. However, it was proposed that the TolA third domain interacts either with the outer membrane periplasmic proteins or with the colicin-receptor complex in order to complete the translocation process. A bridge may form between the outer membrane and the plasmatic membrane, allowing the transport of molecules through the cell envelope. This hypothesis fits in with the results reported by Bénédetti et al. [3], showing that colicins A and E1 interact with protein TolA in vitro. Moreover, those results explain why mutations in TolA protein promote such colicin-tolerant phenotypes.

When mutations occur in the accessory proteins of the translocation mechanism, partial or total tolerance could appear. This tolerance acts as a protective mechanism against channel-forming colicins, but, like resistance, it confers only limited protection.

**PacB character** The last of the protective mechanisms reviewed here is a property that Rodríguez Lemoine calls "PacB character" (**p**rotection **a**gainst **c**olicin **B**) [30]. This new property appears to be unique; it is not a result of colicinogenic plasmid gene products (immunity proteins) nor of alterations to structural or functional chromosomal genes (receptor or translocation genes). Instead, PacB is encoded by non-colicinogenic plasmids, and restricted to the conspicuous and well-defined H Incompatibility Complex [27, 30–35].

Bacterial sensitivity to lethal action of colicins is routinely detected by measuring the size of growth-inhibition haloes formed around a colicinogenic colony. This method was used to assess the sensitivity of *E. coli* K12 strains harboring a set of colicinogenic plasmids. It was found that strains harboring a set of colicinogenic plasmids of the H incompatibility complex were less sensitive to the lethal action of colicin B. However, they maintained their susceptibility to colicins having other modes of action [31–33].

This phenotype, described as the PacB character, has been found exclusively among plasmids of the H incompatibility complex, specifically in groups IncHI (subgroups HI2 and HI3) and IncHII [31–33]. It appears to be highly conserved among the IncHI2 plasmids, with the exception of pR476b, a plasmid isolated from *Serratia marcescens*. Nevertheless, the PacB character is absent in plasmids in the IncHI1 subgroup. None of the IncHI1 plasmids kept at the Venezuelan Center for Culture Collections (Centro Venezolano de Colecciones de Microorganismos, CVCM) protects *E. coli* K12 against colicin B.

An additional property has also been demonstrated: *E. coli* K12 strains harboring IncH plasmids exhibited reduced sensitivity not only to colicin B, but also to all channel-forming colicins A, E1, Ia, Ib, K and N [33]. The protection effect does not neutralize the cellular lysis caused by colicins M and V [5]. From these results, PacB character has been redefined as a property encoded by certain IncH plasmids, which confers protection to *E. coli* K12 against all channel-forming colicins [33].

After further research, this character was found to be clearly associated with the Phi phenotype (reduced efficiency on lytic plaques formation by bacteriophages as T1, T5, T7 and φ80) and with resistance to tellurium oxianions [33–35]. Since these properties are proper of plasmids of the H complex, they have been used as markers for the preliminary identification of plasmids of this complex. Although detection of both PacB and tellurite resistance phenotypes leads to the identification of H plasmids, it does not permit discrimination between groups IncHI (subgroups HI2 and HI3) and IncHII. Plasmids of subgroup IncHI1 are not detected because they do not encode determinants for PacB and/or $Te^r$ properties [26, 34].

The PacB character appears to operate by a mechanism not related to those of colicinogenic plasmids, which confer highly-specific immunity protection against a particular colicin molecule. PacB confers protection against all channel-forming colicins, regardless of the fact that these colicins are neutralized by a specific immune protein, or that they use different receptors to adsorb to outer membranes and/or are translocated into the cytoplasmic membrane by different translocation systems.

Although the mode of action of PacB is not known, it is thought to be closely related to mechanisms for tellurite resistance [35, 40]. Therefore, strategies to explain their mode of action must take into account linkage of both phenotypes.

Resistance to tellurium compounds is unusual among Enterobacteriaceae, but whenever present, it has been associated with plasmids [40, 42]. Nevertheless, cryptic chromosomal genes encoding low-level resistance have also been reported. Tellurite resistance ($Te^r$) has been examined in the IncH plasmids MER610 and R478 (IncHI2), but not in plasmids from other H-incompatibility subgroups [35]. Although the regions of these plasmids that code for tellurite resistance ($Te^r$) have been cloned and sequenced, their biochemical resistance mechanism remains unknown [42].

In order to understand the PacB character mode of action, a region of the plasmid Mip233 (IncHI3), encoding the phenotypes PacB and $Te^r$, has been cloned and studied [35, 40]. Recombinant clones, resistant to ampicillin and potassium tellurite, have been selected, purified and subcloned. A $PacB^+Te^r$ recombinant (pB22) containing a 2200 bp insert, was cloned. This insert is the smallest DNA segment able to express a resistance to channel-forming colicins (A and B) with an efficiency equivalent to that shown by the host strain carrying plasmid Mip233. PacB and $Te^r$ are physically and genetically linked, forming an operon induced by prior exposure at subtoxic tellurite levels [35, 40], or by channel-forming colicins (unpublished results). Linkage of PacB and $Te^r$ has also been established in plasmid R478 [42]. Through sequence analysis of MER610 and R478 clones, up to seven open reading frames (ORFs) have been reported, all of which are necessary to express the $Te^r$ function. These clones are larger than clone pB22. Figure 2 shows the restriction map of pB22 clone.

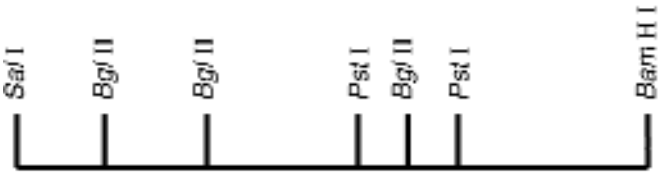


**Fig. 2** The restriction map of pB22 clone

To exert their bactericidal effect, potassium tellurite and colicin molecules must be translocated from the outer membrane to the cytoplasmic membrane. Therefore, research on the expression of both properties has been carried out on *E. coli* strains with alterations in Ton and Tol translocation systems. Preliminary results indicate that for the expression of the PacB character, the intact product of *tol*C gene is required, since TolC mutants carrying pB22 are unable to acquire the phenotype PacB-$Te^r$ [40].

At present, neither the genetic product(s) of pB22 are known nor have the mechanisms of action of such product(s) been elucidated. Nucleotide sequencing of clone pB22 is currently under way, aimed at establishing the nature of the products responsible for both phenotypes. Based on preliminary results, a model is suggested in which genetic product(s) responsible for the PacB phenotype could have reductase-like activity acting at the membrane level (Fig. 3).

## Concluding remarks

To survive in natural environments where colicins and other toxin-like products are present, sensitive bacteria must develop

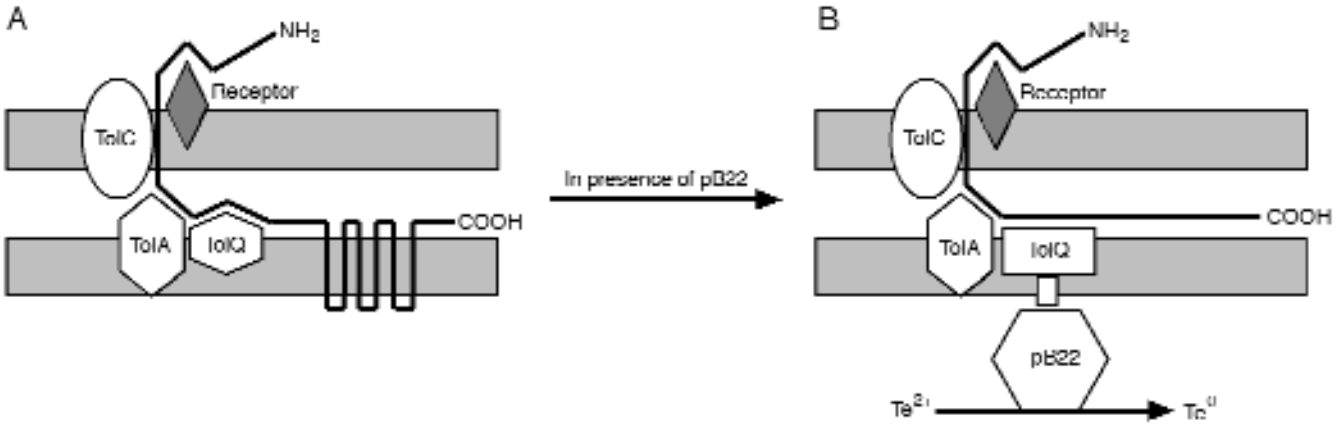


**Fig. 3** Model for PacB-$Te^r$ resistance mechanism. (A) Colicin molecule binds its receptor and translocates to the inner membrane in which it is inserted to form a transmembrane channel. (B) The PacB protein (pB22 product) interacts with the cytoplasmic membrane and/or the Tol translocation system proteins, affecting the colicin C-terminal domain and the ability to produce channels. Its reductase-like activity converts $Te^{2+}$ to $Te^0$. The region between the outer membrane (OM) and the inner membrane (IM) is the periplasmic space

mechanisms to protect themselves against these lethal products. Otherwise, non-colicinogenic bacteria would be at a disadvantage when facing those bearing the colicinogenic determinants. Spontaneous alterations of chromosomal genes leading to modification of colicin receptor proteins (resistance), or of translocation systems (tolerance), are among the naturally selected protective mechanisms. Both resistance and tolerance exhibit a limited range of protection against channel-forming colicins. PacB, on the other hand, is a property that confers protection to *E. coli* against channel-forming colicins and against tellurite oxianions. Both are properties encoded in large plasmids, which are transferable to a wide range of bacteria [32]. PacB-Te$^r$ plasmids frequently code for additional functions, that contribute indirectly to the maintenance and dissemination of these properties in natural populations [26, 32, 35]. Thus, the PacB character could be seen as a major factor for the survival of cells sharing an ecological niche with channel-forming colicinogenic strains.

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