Use of the xenobiotic extrusion pump, MexAB-OprM, of \emph{Pseudomonas aeruginosa} as a reporter to construct a high throughput screening system for the development of novel antimicrobials

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Recent development of multidrug resistance in several pathogenic bacteria, such as methicillin-resistant \emph{Staphylococcus aureus} (MRSA), \emph{Mycobacterium tuberculosis}, \emph{Pseudomonas aeruginosa}, and \emph{Acinetobacter baumannii}, poses a serious public health concern. To combat these multidrug-resistant pathogens, urgent efforts to develop novel antibiotics must continue. The targets of currently used antibiotics are the metabolic pathways essential for bacterial survival hence the development of the antibiotic resistance is inevitable. On one hand, to establish an infection, bacteria elaborate various virulence factors, which are not necessarily essential for their survival. Therefore, biological systems associated with these virulence factors could be an appropriate target for new antibiotic development. A novel protein translocation system, Tat (twin-arginine translocation), which was identified recently, is such a target since the Tat system appears to be involved in several aspects of bacterial virulence and is not present in mammalian cells. In this report, a novel high throughput screening strategy for the development of Tat inhibitors by employing the multidrug efflux pump, MexAB-OprM, of \emph{P. aeruginosa} is presented.

**Keywords** \emph{Pseudomonas aeruginosa}, multidrug resistance, MexAB-OprM xenobiotic efflux pump, twin-arginine translocation system

1. Introduction

“The antibiotic era” starting from the discovery of penicillin by Fleming, has led to the development of more than 20 novel classes of antimicrobial agents [1]. This arsenal has had a tremendous impact on human and animal health, resulting in the illusion that bacterial infections have been controllable. However, multidrug-resistant pathogens consistently emerge soon after launching new antibiotics into the clinic, and therefore, have been posing a serious public health problem [2, 3]. Indeed, infectious diseases are still the second-leading cause of mortality worldwide and the third-leading cause even in developed countries [4, 5]. In addition, it has been reported that half of the deaths from clinical infections in Europe are caused by multidrug-resistant bacteria [6], particularly by methicillin-resistant \emph{Staphylococcus aureus} (MRSA), multidrug-resistant Gram-negative pathogens such as \emph{Acinetobacter baumannii}, \emph{Escherichia coli}, \emph{Klebsiella pneumoniae}, and \emph{Pseudomonas aeruginosa}, as well as multidrug-resistant strains of \emph{Mycobacterium tuberculosis} [1, 2, 7]. To combat these life-threatening pathogens, efforts to develop new antibiotics must continue [8]. A variety of new antibiotics have been developed after the antibiotic era (1962–present), of which only two, linezolid and daptomycin, are novel classes of systemic antibiotics. The other compounds are modified versions of the existing classes of antibiotics. Most of the antibiotics currently in clinical use target the four essential metabolic processes in bacteria: (i) cell wall biosynthesis, (ii) protein biosynthesis, (iii) nucleic acid (DNA and RNA) biosynthesis, and (iv) folate biosynthesis [9, 10].

There is a pressing need for the development of new antibiotics to keep pace with the rate of emergence of multidrug-resistant bacteria, but the capacity of the world’s pharmaceutical companies to produce novel antibiotics is falling behind the appearance of the superbugs (multidrug-resistant pathogens) [3, 11]. A reason for the lack of discovery of new antibiotic classes may be a limited number of available targets [12]. The explosion of microbial genome sequencing has changed this situation from “target-poor” to “target-rich” areas and has identified many essential genes for bacterial survival, which are regarded as the most attractive genes for antibiotic targets because inhibition of these housekeeping functions results in bacterial cell death. Accordingly, extensive target-based screening approaches employing genomics information have been made, but these strategies are yet to produce novel antibiotics that have been introduced into the clinic [13, 14].

New antibiotics discovered in the future will be nullified by development of resistance to those antibiotics sooner or later, since their targets are essential for bacterial survival. From this aspect in which inhibitors of the essential functions inevitably select the resistant strains of pathogens, bacterial virulence could be a fascinating target for the development of totally new antibiotics [15], because virulence attenuators are thought to be less likely to generate resistance [15, 16].

In this report, we describe a novel screening method targeting a recently identified novel protein translocation system, the twin-arginine translocation (Tat) system, which appears to be involved in several aspects of bacterial virulence [17], by using the xenobiotic extrusion pump, MexAB-OprM, of \emph{P. aeruginosa} [18].
2. A novel protein secretion system, Tat, as a potential antibiotic target

Protein transport across the biological membranes is a pivotal function of all living cells, where the transport machinery catalyzes the translocation of a variety of substrate proteins without disturbing membrane integrity [19]. The well-known translocation system, exemplified by the bacterial protein export pathway, is a general protein secretion (Sec) pathway [20]. A substrate protein to be translocated by the Sec pathway contains a signal sequence at its N-terminus [21]. The general feature of the signal sequence (average length, 23 amino acid residues) is a tripartite structure, which comprises a positively-charged N-terminal region, a central region with hydrophobic amino acids, and a C-terminal hydrophilic region [21]. Another key feature of the Sec system is that it operates by a “threading” mechanism, in which the substrate protein is translocated in an unfolded state using energy derived from ATP hydrolysis [20].

Meanwhile, a novel protein secretion pathway, Tat, was found originally in chloroplasts as a system driven solely by proton motive force, but not by ATP hydrolysis [22]. A striking feature of the Tat system is that it translocates folded proteins across the membranes [21, 23]. Proteins to be translocated via the Tat pathway contain a consensus signal sequence (average length, 37 amino acid residues) with an “SRRXFLX” motif, in which the consecutive arginine residues (RR) are almost invariably conserved [21]. Since the overall features of the signal sequences for the Tat and Sec systems are similar, the Sec substrate protein can be translocated via the Tat pathway if its own signal sequence is replaced with the Tat-targeting signal sequence [23, 24].

The Tat system is distributed widely in the microbial world and in plants [25]. In *E. coli*, the Tat system consists of the TatA, TatB, and TatC proteins [19, 21, 25]. However, the composition of the Tat system is varied in other bacteria, where the TatB subunit does not seem to be essential because several bacteria, such as *M. tuberculosis*, *S. aureus*, and *Bacillus subtilis*, do not have TatB homologues [25]. TatC, the most conserved subunit of the Tat machinery, is thought to play a role in the initial recognition of the Tat substrate signal sequence in conjunction with the TatB subunit [19, 21].

Although the Tat pathway was originally found to transport a group of proteins that bind cofactors in the cytoplasm, in which folding occurs before translocation across the cytoplasmic membrane, a wide range of proteins have now been shown to be Tat-dependent substrates [25, 26], indicating that a variety of cellular processes are influenced by the Tat system. Indeed, recently, lines of evidence have been accumulating and point to an important role of the Tat system in the establishment of bacterial infection in animals and plants [17, 27, 28]. In addition, the Tat pathway is not present in mammalian cells [17, 27], which meets an important criterion for antibiotic development. Therefore, the Tat system attracts increasing attention as a novel antibiotic target. In order to develop Tat system inhibitors, it is necessary to evaluate the Tat biological function, protein translocation, by a highly sensitive, as well as simple, reporter assay system.

3. MexAB-OprM xenobiotic extrusion pump of *P. aeruginosa* is involved in intrinsic and multidrug resistance

*P. aeruginosa* is an opportunistic pathogen, which shows resistance to a variety of structurally dissimilar antibiotics [29]. Furthermore, the pathogen easily acquires multidrug resistance after being exposed to a certain type of antibiotic. Thus, infection caused by *P. aeruginosa* has led to a problematic situation. The intrinsic antibiotic resistance has been thought to derive from its low outer membrane permeability [30]. However, energy-dependent multidrug efflux pumps encoded on the chromosome of this bacterium appear to play a major role in intrinsic and acquired multidrug resistance in conjunction with the low outer membrane permeability [31, 32]. The most important efflux pump, MexAB-OprM, consists of two inner membrane proteins, MexA and MexB, and an outer membrane antibiotic exit channel, OprM [31, 32]. A single-knockout mutant deficient in MexA, MexB, or OprM shows hypersensitivity to a variety of antibiotics [33], indicating that a tripartite assembly of these subunits is essential to extrude antibiotics out of the cells. It also indicates that the efflux pump plays a primary role in intrinsic antibiotic resistance. Furthermore, a mutation that leads to over-expression of the operon encoding the MexA, MexB, and OprM pump subunits provides *P. aeruginosa* with the multidrug resistance phenotype [34].

4. Rationale for a screening method targeting the Tat system

To search efficiently for new compounds that inhibit the Tat function, a simple method is needed to facilitate the evaluation of the Tat biological function of protein translocation using a high-sensitive reporter system. For this purpose, we employed the inner membrane component, MexA, of the MexAB-OprM xenobiotic extrusion pump of *P. aeruginosa*. The MexA-deficient mutant, TNP070, showed restoration of the function of the efflux pump as assessed by MIC determination when TNP070 cells were provided with the wild-type mexA gene that had been cloned on a multicopy plasmid, pMEXA1 (Table 1) [35].

Since MexA has a canonical signal sequence with 23 amino acid residues for the integral membrane or secreted proteins, it is most likely that MexA is translocated across the inner membrane via the Sec system. Hence, if the signal
sequence of MexA is replaced with that of a Tat pathway substrate protein, it can be predicted that the resulting chimeric MexA protein would be translocated via the Tat system. If this is the case, the MexAB-OprM efflux pump would assemble and thus function in a Tat-dependent manner when the recombinant gene encoding the chimeric mexA gene is expressed in cells of the chromosomal mexA gene-deficient host, TNP070. Consequently, the transformant can restore the antibiotic resistance to the same level as that of wild-type cells. If a compound that inhibits the function of the Tat machinery is present, the chimeric MexA would not be translocated across the inner membrane, and thus the transformant would become antibiotic-susceptible.

5. MexA can function in a delipidated soluble form

MexA has a consensus sequence named a lipobox or lipoprotein box in its signal sequence, which means that MexA is likely to anchor in the inner membrane through the lipid chains modified at its N-terminus [36]. To address this possibility, we carried out a [3H]palmitic acid incorporation experiment to show whether or not MexA is a lipoprotein. Fluorography showed that P. aeruginosa TNP071, which has a wild-type mexA gene in the chromosome, exhibited a radiolabeled protein band corresponding to the authentic MexA, the molecular mass of which is 42 kDa (Fig. 1a, lane 2). In contrast, radiolabeling in the MexA-deficient mutant, TNP070, was undetectable (Fig. 1a, lane 1). To further confirm this result, we replaced the cysteine (C) residue with phenylalanine (F) or tyrosine (Y) in the lipobox and determined the incorporation of radiolabeled palmitic acid into the modified MexA, C24F-MexA and C24Y-MexA. As expected, no radiolabeling in both of the modified MexA was detected (Fig. 1b), indicating clearly that MexA is the lipoprotein that has an acyl modification at the cysteine residue in the lipobox. The MexA-deficient mutant, TNP070, transformed with either one of the recombinant plasmids, pMexA(C24F) or pMexA(C24Y) expressing modified MexA - C24F-MexA or C24Y-MexA, respectively - exhibited restored susceptibility to aztreonam and chloramphenicol to the same level as that of their parent, PAO4290 (Table 1), indicating that lipid modification is not essential for the MexA function.

Fig. 1  a) [3H]palmitic acid incorporation into MexA. P. aeruginosa cells were grown in the presence of [3H]palmitic acid (1.48 x 10^6 Bq/ml) overnight, and MexA was immunoprecipitated with anti-MexA antibody. Lane 1, TNP070 (MexA-deficient mutant of wild-type PAO4290); lane 2, TNP071(MexB-deficient mutant of PAO4290, which contains wild-type mexA gene). b) [3H]palmitic acid incorporation into modified MexAs. P. aeruginosa TNP070 cells harboring pMexA(C24F) or pMexA(C24Y) were grown in the presence of [3H]palmitic acid (3.7 x 10^6 Bq/ml) and 2 mM IPTG for 6 h, and the MexA was immunoprecipitated with anti-MexA antibody. Lane 1, TNP070 harboring pMexA(C24F); lane 2, TNP070 harboring pMexA(C24Y); lane 3, TNP070 harboring pMEXA1; and lane 4, TNP070. The lower panel shows the same gel visualized by Coomassie Brilliant Blue. c) Western blot analysis of delipidated MexA. Whole cell lysate (10 µg of protein) of P. aeruginosa cells expressing wild-type MexA (lane 2), C24F-MexA (lane 3), or C24Y-MexA (lane 4) were subjected to SDS-PAGE (12% gel) and visualize by anti-MexA antibody. Lane 1 shows molecular markers in kDa. Mature and unprocessed form of MexAs are indicated by black and white arrowheads, respectively. Adapted with permission from the American Society for Biochemistry and Molecular Biology [36].
When the recombinant *mexA* gene was expressed in TNP070 and analyzed by the immunoblotting method using the anti-MexA antibody [36], we found an extra band(s) in addition to that corresponding to the mature form of the wild-type MexA in both C24F-MexA and C24Y-MexA (Fig. 1c). This extra band with a higher molecular mass than the mature form of MexA is most likely the MexA protein with an unprocessed signal sequence, hence this unprocessed MexA should be integrated in the inner membrane through the hydrophobic region of the signal sequence. In contrast, the mature forms of the modified MexA derived from C24F-MexA and C24Y-MexA should reside in the periplasm in a soluble form because they do not have an acyl modification as described above. Thus, a question arises about which form of MexA retains the activity. This is of primary importance and should be clarified before constructing a novel screening system targeting the Tat system using modified MexA as a reporter, because the Tat signal sequence to be used for construction of a chimeric *mexA* gene does not contain the lipobox. To address this issue, we constructed the recombinant *mexA* gene possessing a signal sequence of azurin, a periplasmic protein of *P. aeruginosa*, instead of its own signal sequence [36]. When the resulting recombinant *mexA* gene, *azu-mexA*, was expressed in TNP070, a single band corresponding to the mature MexA was detected only in the whole cell lysate (Fig. 2, lane 5) but not in either the inner or outer membrane fractions (Fig. 2, lanes 6 and 7). Therefore, it can be concluded that the Azu-MexA protein was processed properly, and the mature region of MexA became soluble in the periplasm. We then determined the MICs of aztreonam and chloramphenicol for the MexA-deficient TNP070 harboring pAzu-MexA, which expresses the *azu-mexA* gene (Table 1). Consequently, the transformant showed the same antibiotic resistance as the TNP070 cells harboring pMEXA1, which expresses the wild-type *mexA* gene. Therefore, it was concluded that the soluble form of MexA retains the function (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>MIC (µg/ml)</th>
<th>Aztreonam</th>
<th>Chloramphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAO4290</td>
<td></td>
<td>3.13</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>TNP070</td>
<td>pMEXA1</td>
<td>0.2</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>TNP070</td>
<td>pMMB67HE (vector)</td>
<td>1.56</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>TNP070</td>
<td>pMexA(C24F)</td>
<td>3.13</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>TNP070</td>
<td>pMexA(C24Y)</td>
<td>3.13</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>TNP070</td>
<td>pAzu-MexA</td>
<td>1.25</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Adapted with permission from the American Society for Biochemistry and Molecular Biology [36].

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6. **Chimeric MexA possessing the Tat signal sequence retains its activity**

The chimeric MexA with the signal sequence of the periplasmic protein, azurin, was found to translocate across the inner membrane and retain its activity in a soluble form. Accordingly, we were encouraged to design a chimeric *mexA* gene that directs the translocation of MexA through the Tat pathway instead of the Sec pathway by employing the
signal sequence of a Tat system substrate protein. Phospholipase C (PlcH), a virulence factor of \emph{P. aeruginosa}, is such a protein that has been shown recently to be transported via the Tat system\cite{38}. Therefore, we constructed the fusion gene (\emph{plc-mexA}) between the signal sequence of \emph{plcH} and the mature region of \emph{mexA} by the overlap extension method\cite{18}. When the recombinant plasmid, pPleMexA, harboring the chimeric gene was introduced into TNP070 lacking the \emph{mexA} gene on the chromosome, the transformant showed the aztreonam and chloramphenicol MICs of 2 \(\mu\)g/ml and 32 \(\mu\)g/ml, respectively (Table 2). The MICs were 8 and 4 times, respectively, higher than those in the host cells lacking MexA (TNP070) and were comparable to the MICs in cells expressing wild-type MexA, TNP070(pMEXA1).

### Table 2: MICs of aztreonam and chloramphenicol for transformants expressing chimeric MexA.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>MIC ((\mu)g/ml)</th>
<th>Aztreonam</th>
<th>Chloramphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAO4290</td>
<td></td>
<td>4.0</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>TNP070</td>
<td></td>
<td>0.25</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>TNP070</td>
<td>pMEXA1</td>
<td>2.0</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>TNP070</td>
<td>pPleMexA</td>
<td>2.0</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>TNP080</td>
<td></td>
<td>0.13</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>TNP080</td>
<td>pMEXA1</td>
<td>2.0</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>TNP080</td>
<td>pPleMexA</td>
<td>0.25</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>TNP080</td>
<td>pTatPleMexA</td>
<td>2.0</td>
<td>32</td>
<td></td>
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</tbody>
</table>

The results suggest that the \emph{plc-mexA} gene had been expressed and that the chimeric MexA had been translocated across the inner membrane via the Tat pathway; hence the antibiotics were extruded by the reconstituted MexAB-OprM efflux pump. We next analyzed the whole-cell lysate of the transformant harboring pPleMexA by immunoblotting and found that there were two anti-MexA antibody-reactive bands in the whole-cell lysate (Fig. 3, lane 3). The lower band corresponded to the mature form of MexA, and thus, the upper band was most likely to have corresponded to the unprocessed form of MexA. If this is the case, it can be hypothesized that the mature form of MexA (lower band) would be localized in the periplasmic aqueous phase. To confirm this prediction, the periplasmic materials obtained by the cold osmotic shock method were analyzed by the immunoblotting method. The electrophoretogram showed only a single major band reactive with the anti-MexA antibody, which corresponded to mature MexA (Fig. 3, lane 8). This result suggests that the chimeric MexA was translocated across the inner membrane most likely through the Tat pathway. In contrast, wild-type cells, which produce fatty acid-modified MexA, did not generate the anti-MexA antibody-reactive band in the periplasm (Fig. 3, lane 6), confirming the previous result (Fig. 1).

### Fig. 3 Western blot analysis of periplasmic fractions extracted from \emph{P. aeruginosa} cells expressing chimeric \emph{mexA} genes. Whole cell lysate (4 \(\mu\)g of protein) (lanes 1 to 5) and periplasmic fractions (1 \(\mu\)g of protein) (lanes 6 to 10) were subjected to SDS-PAGE (12\% gel) and visualized with anti-MexA antibody. Lanes 1 and 6, PAO4290; lanes 2 and 7, TNP070; lanes 3 and 8, TNP070(pPleMexA); lanes 4 and 9, TNP080(pPleMexA); lanes 5 and 10, TNP080(pTatPleMexA). The black and white arrowheads indicate mature and unprocessed forms of MexA. Adapted with permission from the American Society for Microbiology\cite{18}.

### 7. Translocation of the chimeric MexA occurs via the Tat pathway

The results described above could not determine definitively whether the chimeric MexA was translocated via the Tat pathway or the Sec pathway (Fig. 4a). To address this issue, we employed a genetic approach in which the Tat machinery malfunctioned due to the introduction of a mutation in an essential component, TatC, of the Tat system in TNP070 lacking the chromosomal \emph{mexA} gene. If the chimeric MexA is secreted via the Tat system, this protein cannot reach the periplasm when the chimeric \emph{mexA} gene is expressed from the plasmid in the double mutant lacking both the \emph{tatC} and \emph{mexA} genes; therefore, the MexAB-OprM extrusion pump should not function leading to an antibiotic hypersensitive phenotype (Fig. 4b). If the chimeric MexA is secreted via the Sec system, this protein can be translocated...
across the inner membrane in the double mutant, hence the transformant harboring the chimeric mexA-bearing plasmid would show an antibiotic resistant phenotype (Fig. 4c). We thus disrupted the tatC gene by single-crossover mutagenesis in the MexA-deficient mutant to generate the double-knockout mutant, TNP080, which lacked both the tatC and mexA genes. Malfunction of the Tat machinery of the double mutant was determined by measuring pyoverdine production in the culture medium, since it is known that production of this compound is dependent on the Tat system [39]. Though the culture supernatant of the wild-type cells (PAO4290) and the MexA-deficient mutant (TNP070) exhibited the typical absorption spectra of pyoverdine (Fig. 5a and 5b), the double mutant cells (TNP080) produced an undetectable level of pyoverdine (Fig. 5c), indicating that the Tat system was not functional. The loss of pyoverdine production in the double mutant is consistent with a previous report [39]. We further confirmed this by cloning the wild-type tatC gene and introducing the resulting recombinant plasmid, pTatPlcMexA, into TNP080 cells. The transformant, TNP080(pTatPlcMexA), restored the ability to produce pyoverdine in the medium (Fig. 5d), indicating again that the function of the Tat machinery in the double mutant was disrupted.

We next investigated how inactivation of the Tat machinery affects the function of the MexAB-OprM efflux pump by determining the MICs of antibiotics for the double mutant expressing the chimeric MexA from pPlcMexA. The transformant, TNP080(pPlcMexA), showed aztreonam and chloramphenicol MICs of 0.25 µg/ml and 4.0 µg/ml, respectively, which were values comparable with those in the MexA-deficient mutant TNP070 (Table 2). This result suggested strongly that the chimeric MexA remained in the cytoplasm or was stacked at the inner membrane. Indeed, a significant amount of unprocessed MexA was detected in the whole-cell lysate of TNP080 harboring pPlcMexA (Fig. 3, lane 4), whereas almost no processed form of MexA was detected in the periplasmic aqueous phase (Fig. 3, lane 9). These results clearly indicated that the chimeric MexA was translocated across the inner membrane exclusively via the Tat pathway.

8. Plasmid-born tatC gene restores the chimeric MexA translocation via the Tat pathway

To substantiate the results as described above, we designed an experiment to test whether restoration of the Tat machinery by cloning the healthy tatC gene can result in antibiotic resistance in TNP080 and to determine how it affects localization of the chimeric MexA. We constructed the recombinant plasmid, pTatPlcMexA, carrying the tatC gene in addition to the chimeric mexA gene. TNP080 cells transformed with pTatPlcMexA produced pyoverdine in the culture medium to a level similar to that observed in the wild-type PAO4290 and MexA-deficient TNP070 (Fig. 5d), indicating that the Tat machinery was reconstituted properly in intact cells. Functional restoration of the reconstituted Tat machinery was further assessed by determining the antibiotic susceptibility. TNP080(pTatPlcMexA) cells showed...
aztreonam and chloramphenicol MICs of 2.0 µg/ml and 32 µg/ml, respectively, and these values were comparable to those for PAO4290, TNP070(pMEXA1), TNP070(pPlcMexA), and TNP080(pMEXA1) (Table 2), indicating again that the chimeric MexA had been translocated exclusively via the Tat pathway. Consistent with this finding, the processed form of the chimeric MexA protein was observed in the periplasmic fraction of the double mutant TNP080 harboring pTatPlcMexA (Fig. 3, lane 10).

9. Conclusion

In this report, we presented several lines of evidence demonstrating that the chimeric MexA with the Tat-directed signal sequence is exclusively translocated across the inner membrane via the Tat pathway. Therefore, this novel screening system, which is a combination of mexA-deficient host cells and the chimeric mexA-bearing plasmid that has a Tat-directed signal sequence, can be a high-sensitive screening system for Tat pathway inhibitors.

It should be noted that this system can also screen out the MexAB-OprM efflux pump inhibitors, because the strategy uses the function of the efflux pump as a reporter for the biological function of the Tat pathway.

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References


