Penicillin-binding proteins of pathogenic *Escherichia coli* strains

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Abstract

The penicillin-binding proteins of 11 pathogenic *Escherichia coli* strains, including enteropathogenic, enterotoxigenic, enteroinvasive, enteraggregative, and enterohemorrhagic *E. coli*, were detected in gels following the labeling of isolated cell envelopes with [3H]benzylpenicillin. The electrophoretic profiles, sensitivities to and morphological changes induced by β-lactam antibiotics showed that the penicillin-binding proteins of most pathogenic *E. coli* possess structural and physiological functions similar to those of *E. coli* K12. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Penicillin-binding protein; Pathogenic *Escherichia coli*; β-Lactam antibiotic; Enteropathogenic *Escherichia coli*

1. Introduction

*Escherichia coli* is a member of the normal human intestinal flora but also represents an important cause of diseases such as diarrhea causing thousands of deaths every year, mainly among children in developing countries [1]. Five major categories of diarrheagenic *E. coli* have been defined based on the specific molecular strategies used by these pathogens to cause disease [2]. Enterotoxigenic *E. coli* (ETEC) produces heat-stable and heat-labile enterotoxins and a variety of colonization factors which were responsible for the binding of bacteria to enterocyte receptors. Enteroinvasive *E. coli* (EIEC) invades the intestinal epithelium causing local necrosis and hemorrhagic diarrhea. Enteropathogenic *E. coli* (EPEC), a frequent cause of persistent diarrhea, attaches to enterocytes and promotes the effacement of microvilli. Enterohemorrhagic *E. coli* (EHEC), like EPEC, displays the attachment and effacement phenotype but additionally produces a Shiga-like toxin causing hemorrhagic diarrhea and, in some cases, kidney failure, the hemolytic uremic syndrome. Enteraggregative *E. coli* (EAEC) has a biofilm-based adherence pattern and causes diarrhea through secreted cytotoxins. Besides the great diversity of virulence-associated traits, serological and molecular biology techniques have demonstrated that pathogenic *E. coli* strains show a significant diversity in their genetic composition [3,4].

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The final stages of peptidoglycan biosynthesis are catalyzed by a set of cytoplasmic membrane proteins, some of which represent the killing targets of β-lactam antibiotics, the penicillin-binding proteins (PBPs) [5,6]. PBPs are present in all bacteria with a cell wall but they may vary in number, size, relative amounts, and affinity for β-lactam antibiotics, frequently following taxonomic lines [7,8]. PBPs perform essential physiological roles in the bacterial metabolism such as control of cell shape, septum formation and osmotic pressure resistance [8,9].

So far, studies relating to E. coli PBPs have focused only on K12 laboratory strains, which may differ significantly in genetic and antigenic composition from their pathogenic relatives. In this work we began structural and physiological studies of the PBPs from diarrheagenic E. coli strains.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Eleven diarrheagenic E. coli strains were analyzed in this work. All strains were considered sensitive to the tested β-lactam antibiotics in disk diffusion susceptibility tests carried out in LB agar plates and using E. coli C600 as a reference strain. One representative strain of each serotype, representing the major diarrheagenic E. coli groups, was chosen as follows: serotype O157:H43 (ETEC); serotype O129:H3 (EIEC); O157:H7 (EHEC); O111:H12 (EAEC), and the O127:H6 prototype EPEC strain E-2348/E [10]. Six EPEC strains, belonging to different serotypes of significant epidemiological relevance in Brazil, were also included in this study: O111ab:H2, O86:H34, O55:H6, O142:H34 and O142:H6 [11]. All pathogenic E. coli strains were isolated from hospitalized diarrheic infants in São Paulo, Brazil, except O157:H7 which was from the USA and was kindly given by Dr. T.S. Whittam (University of Pennsylvania, USA). The strains did not produce β-lactamase, as evaluated by the sensitivity to β-lactam antibiotics and lack of reaction with nitrocephin (Oxoid, Hampshire, UK), a chromogenic cephalosporin. Bacteria were grown as aerated cultures in LB medium at 37°C.

2.2. Detection of PBPs and SDS-PAGE

The isolated cell envelopes were suspended in phosphate buffer and aliquots, containing approximately 250 μg of total protein, were labeled with [3H]benzylpenicillin (21 Ci/mmol; Amersham, Little Chalfont, UK) for 20 min at room temperature as previously described [5,12,13]. [3H]Benzylpenicillin was used at a final concentration of 14 μg ml⁻¹ which, in our hands, was enough to saturate all PBPs of E. coli K12 C600 strain. Proteins were fractionated in 10% (w/v) acrylamide gels by use of a Mini Protean II slab gel electrophoresis system (Bio-Rad Laboratories, Richmond, CA, USA). Polyacrylamide gels were treated with 1 M sodium salicylate, dried, and placed against Kodak XAR-5 films at −70°C for 6–10 days. Some E. coli strains were treated with 1 mM sodium metaperiodate in 100 mM sodium acetate (pH 4.5) for 15 min at room temperature in the dark before being subjected to the cell envelope extraction procedure in order to reduce the amount of LPS in the Sarkosyl-soluble fraction.

2.3. Identification of β-lactam lethal targets

The lethal targets of β-lactam antibiotics were determined by competition binding assays between non-radioactive β-lactam antibiotics (penicillin G, mecillinam and cephalexin) and [3H]benzylpenicillin to the PBPs of pathogenic E. coli strains, as previously described [5]. Non-radioactive β-lactams were tested at their minimal inhibitory concentrations (MIC).

2.4. Microscopic observations

The morphological effects induced by different β-lactam antibiotics on exponential phase E. coli cells were observed in a Zeiss phase contrast microscope.

2.5. Determination of MICs

The MIC for each β-lactam antibiotic was determined in microdilution broth susceptibility tests using 96-well microtiter plates containing two-fold serial dilutions of the tested antibiotics in LB medium (200 μl) and seeded with approximately 10⁸ cells (10 μl) of each strain. Plates were visually inspected for...
bacterial growth following overnight incubation at 37°C. Strains were tested in duplicate in at least two independent experiments.

3. Results

The PBP electrophoretic profiles of five pathogenic E. coli strains, representing five major diarrheagenic groups for humans, were determined in fluorograms after labeling cell envelopes with [3H]benzylpenicillin. Strains 48/63 (O129:H3, EIEC), E2348/69 (O127:H6, EPEC), 30 (O157:H43, ETEC), 4A (O157:H7, EHEC), and 132 (O111:H12, EAEC) possessed similar PBP profiles when compared to the E. coli K12 C600 strain (Fig. 1). Five major bands with molecular masses ranging from 94 to 42 kDa, corresponding to the major E. coli K12 PBPs (PBP1a and 1b and PBP5 and 6 were resolved as single bands in minigels), were detected in all strains. Except for the O157:H7 strain, which showed weakly [3H]benzylpenicillin-labeled bands corresponding to PBP2 and PBP3, all pathogenic E. coli strains had PBP levels similar to those found in E. coli C600. A sixth band, probably corresponding to PBP7, was also detected in most of the isolates (not shown). Several pathogenic E. coli strains expressed large amounts of lipopolysaccharide (LPS) which were released during the Sarkosyl solubilization treatment and interfered with the resolution and molecular mass determination of co-migrating PBPs in SDS-PAGE. Therefore, most of the strains were subjected to sodium metaperiodate treatment before isolation of cell envelopes.

The morphological effects induced by the β-lactam antibiotics showed that some PBPs of pathogenic E. coli strains have physiological functions similar to those described for E. coli K12 strains. Therefore,

![Image](https://example.com/image.png)

**Fig. 1.** The PBP electrophoretic profiles of pathogenic E. coli strains. The PBP profiles of different E. coli strains were determined in fluorograms following labeling with [3H]benzylpenicillin. The samples were as follows: 1, strain C600 (K12); 2, strain 48/63 (O129:H3, EIEC); 3, strain E2348/69 (O127:H6, EPEC); 4, strain 30 (O157:H43, ETEC); 5, strain 4A (O157:H7, EHEC); 6, strain 132 (O111:H12, EAEC). The position of PBPs and molecular mass markers (phosphorylase B, 97.4 kDa; bovine serum albumin, 68 kDa; and ovalbumin, 43 kDa) are indicated on the left and right sides of the figure, respectively.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Strain</th>
<th>Penicillin G MIC (µg ml⁻¹)</th>
<th>Penicillin G ME</th>
<th>Mecillinam MIC (µg ml⁻¹)</th>
<th>Mecillinam ME</th>
<th>Cephalexin MIC (µg ml⁻¹)</th>
<th>Cephalexin ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>K12</td>
<td>C600</td>
<td>10</td>
<td>F,B</td>
<td>0.25</td>
<td>R</td>
<td>5</td>
<td>F</td>
</tr>
<tr>
<td>EIEC</td>
<td>48/63</td>
<td>20</td>
<td>F,B</td>
<td>0.25</td>
<td>R</td>
<td>5</td>
<td>F</td>
</tr>
<tr>
<td>EPEC</td>
<td>O127:H6</td>
<td>E2348/69</td>
<td>20</td>
<td>F,B</td>
<td>1</td>
<td>R</td>
<td>10</td>
</tr>
<tr>
<td>ETEC</td>
<td>O157:H43</td>
<td>EHEC</td>
<td>30</td>
<td>F,B</td>
<td>0.25</td>
<td>R</td>
<td>5</td>
</tr>
<tr>
<td>EHEC</td>
<td>O157:H7</td>
<td>4A</td>
<td>10</td>
<td>F,B</td>
<td>0.1</td>
<td>R</td>
<td>2.5</td>
</tr>
<tr>
<td>EAEC</td>
<td>O111:H12</td>
<td>132</td>
<td>10</td>
<td>F,B</td>
<td>0.25</td>
<td>R</td>
<td>5</td>
</tr>
</tbody>
</table>

*Laboratory strain collection reference code.

*MIC (µg ml⁻¹), based on growth of strains in liquid medium following overnight incubation at 37°C.

*ME: morphological effects induced by the tested antibiotics at MIC. F: filaments; R: round cells; B: cells and filaments with bulges.
Cephalexin, with increased affinity for PBP3, and mecillinam, which binds specifically to PBP2 over a wide concentration range, induced filament and ovoid cell formation, respectively, at their corresponding MICs in all strains tested (Table 1). Competition labeling assays with mecillinam and cephalexin confirmed that, at MICs, their major killing targets are PBP2 and PBP3 (data not shown).

O157:H7, which showed a reduced expression and/or reduced affinity for [3H]benzylpenicillin of PBP2 and PBP3, had a reproducibly higher sensitivity to mecillinam and cephalexin when compared to the other strains including E. coli C600 (Table 1).

The previously described phenotypic and genotypic variability among EPEC strains of distinct serotypes [14–16] led us to determine the PBP profiles of strains belonging to seven different serotypes, in addition to the O127:H6 (E2348/69) strain. As shown in Fig. 2, EPEC strains belonging to serotypes O111:ab:H2, O127:H40, O86:H34, O55:H6, O142:H34, O142:H6 and O119:H2 share a similar set of PBPs. The altered migration patterns of PBPs 1, 2 and 3 observed in some EPEC strains could be attributed, at least in part, to residual amounts of LPS not efficiently removed from the cell envelopes during treatment with sodium metaperiodate. Like the other E. coli strains, all EPEC strains showed the same pattern of morphological changes, and corresponding putative lethal targets, induced by mecillinam, cephalexin or penicillin G (data not shown).

4. Discussion

The PBPs of E. coli K12 were initially reported almost 25 years ago and, since their discovery, several groups have dedicated considerable attention to their enzymatic activities, structural properties, physiological roles and interactions with β-lactam antibiotics [6,8,9]. Nonetheless, in contrast to other bacterial species [7,12,13], the PBP contents of pathogenic E. coli strains have not been studied. In this work we described the PBP profiles of 11 pathogenic E. coli strains representing the major diarrheagenic groups for humans. All strains possessed a similar set of PBPs, based on number and molecular masses. Moreover, preliminary experiments have shown that the physiological roles of at least PBP2 and 3 are similar to those found in E. coli K12.

As observed in E. coli K12 strains, the high molecular mass PBPs of pathogenic E. coli strains represent the main killing targets of β-lactam antibiotics. The MICs for penicillin G, mecillinam and cephalexin corresponded to concentrations able to partially or totally inhibit the binding of radioactive penicillin to their target PBPs. These results indicated also that the permeability barrier mediated by the outer membrane or production of basal levels of type I β-lactamases have little effect upon susceptibility in these strains. Nonetheless, some strains such as E2348/69 (O127:H6), which showed reproducibly lower β-lactam susceptibility levels, and the O157:H7 strain, which showed lower MICs for mecillinam and cephalexin, might have essential PBPs with altered expression and/or affinity to β-lactams. The reduced labeling of PBP2 and 3 of the O157:H7 strain, the most outstanding quantitative difference observed among the PBP profiles of the strains analyzed, probably reflects the reduced concentration of these proteins in the cell envelope since this strain was reproducibly more sensitive to PBP2- and PBP3-specific antibiotics but not to penicillin G or other β-lactam antibiotics which bind preferentially to PBP1 (Table 1 and unpublished observations).

PBP2 and 3 of pathogenic E. coli strains probably perform the same physiological functions described for their corresponding counterparts in E. coli K12 [5,9]. Mecillinam caused formation of osmotically stable round cells and showed high affinity to PBP2. Cephalexin inhibited cell division and bound
preferentially to PBP3. In spite of the reduced amounts of PBP2 and 3 in the cell envelope of the O157:H7, these proteins were responsible for these essential functions in cell wall metabolism based on the induced morphological changes.

During the last few years an increasing amount of evidence has indicated that pathogenic E. coli strains, in contrast to their K12 relatives, can occupy niches and survive the host immune responses due to their flexible genomes and expression of diverse virulence-associated traits such as cell envelope proteins and LPS composition. The present work has shown that the PBP content and, probably, the cell wall metabolism are rather conserved among the laboratory and different diarrheagenic E. coli strains. However, such observations do not rule out that, under specific growth conditions, regulation of the cell wall metabolism, mediated by control of the enzymatic activity and/or stability of PBPs, in pathogenic E. coli strains might present features not shared by laboratory strains.

References