PREDICTION OF NEW ENZYME INVOLVED IN PEPTIDOGLYCAN RECYCLING

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Resume

We demonstrate here a bioinformatics approach to prediction of a new enzyme involved in peptidoglycan recycling in bacteria. YjjK encoding this enzyme is frequently co-localized with the genes involved in murein turnover. Moreover, in the genome of E. coli, a common candidate regulatory sequence was found in the upstream region of yjjK as well as two other genes involved in cell wall biogenesis, namely mltE and ampC.

Introduction

Turnover and recycling of the cell wall murein represent a major, though often non-essential, metabolic pathway of most bacteria. Degradation products of the peptidoglycan are formed during the enlargement of the murein sacculus as a consequence of a growth mechanism, which couples the controlled degradation of the cell wall polymer with the insertion of new material. Consequently, the recycling pathway is viewed as a possible signaling vehicle, informing the cell of the condition of the essential structure existing outside the cell itself (Park, 1995; 1996). Alginase production, bacterial encystment in Azotobacter vinelandii and induction of Escherichia coli β-lactamase genes were shown to be greatly influenced by the bacterial ability to recycle their cell wall (Nunez et al., 2000; Tuomanen, 1991; Tolg et al., 1993; Normak, 1995; Park, 1996; Dietz, 1997; Wiedemann et al., 1998).

β-Lactamase (AmpC; Edlund et al., 1979) induction and modulation of the composition of the cell wall share elements of a regulatory circuit that involves AmpD, cytosolic N-acetyl-anhydromuramyl-L-alanine amidase (Jacobs, 1995). Amidases were shown to act as powerful autolytic enzymes in the presence of antibiotics (Heidrich et al., 2001). Cell wall turnover products may relay the signal to AmpR, the ampC transcription activator (Lindberg et al., 1985; Lindquist et al., 1989) or act on AmpR indirectly through the AmpE member of AmpD/AmpE signal system (Park, 2001). There is also a possible connection between septation/division and induction of ampC β-lactamase promoted byftsZ (Ottolenghi, Ayala, 1991).

The released cell-wall peptides are regulated by the Opp system in gram-negative bacteria and highly homologous Spo system in gram-positive (Goodell, Higgins, 1987; Perego et al., 1991). The MppA protein is responsible for tripeptide uptake (Li, Park, 1999) in gram-negative bacteria. In E. coli, the transmembrane protein AmpG (Lindquist et al., 1993) transports not only D-tripeptide but also D-pentapeptide into the cell (Park, 2001). Still, many aspects of this system should be yet discovered and understood.

Knowledge about the machinery performing regulation, turnover, and recycling of cell-wall components in bacteria seems to be of a major importance in designing inhibitors that could prevent the establishment of β-lactam resistance of bacteria possessing inducible β-lactamases. It also can help in developing new classes of antibiotics. We demonstrate here a bioinformatics approach to prediction of a new enzyme involved in the peptidoglycan recycling in bacteria. The yjjK gene, encoding this enzyme, is frequently co-localized with the genes involved in the murein turnover. Moreover, in the genome of E. coli, a common candidate regulatory sequence was found in the upstream region of yjjK and two genes involved in the cell wall biogenesis (Engel et al., 1992), namely mltE and ampC.

Materials and Methods

Genome sequences of analyzed species were extracted from the ERGO Database (http://wit.mcs.anl.gov/WIT2/). Profiles for signal recognition were constructed as described in (Gelfand, 1999). Positional nucleotide weights in these profiles are defined as

\[ W(b,k) = \log \left[ N(b,k) + 0.5 \right] - 0.25 \sum_{a=C,G,T} \log \left[ N(a,k) + 0.5 \right], \]

where \( N(b,k) \) is the count of nucleotide \( b \) at position \( k \). The score of the candidate site is calculated as the sum of the respective positional nucleotide weights:

\[ Z(b_1...b_L) = \sum_{k=1...L} W(b,k), \]

where \( k \) is the length of the site.

Genomic analyses (protein similarity searches using Smith–Waterman algorithm, analysis of orthology, and identification of candidate signals in the genome sequences) were done using GenomeExplorer (Mironov et al., 2000).
Results

The genome of *Escherichia coli* contains two paralogous genes, *yjjK* and *uup*, with an identity of 34%. Each of them encodes a protein previously identified as a putative ATP-binding component of a transport system. We have analyzed these proteins with PROSITE motif-search tool and TMPRED server. Each protein consists of two homologous parts; each part has a nucleotide-binding domain typical of ABC transporters, and, in the case of *YjjK*, a hydrophobic transmembrane alpha helix (predicted by TMPRED, Fig. 1a). The TMPRED output for *Uup* is less clear and predicts two transmembrane alpha helices in the N-terminal part of the protein (Fig. 1b). However, ABC transporters generally have at least four transmembrane alpha helices; thus, *Uup* and *YjjK* are more likely to be membrane-bound ATP-binding enzymes.

![Fig. 1. TMPRED output for (a) yjjK and (b) uup proteins of *E. coli*.](image)

We have found that *yjjK* and *uup* genes are frequently co-localized with the genes involved in murein turnover (Fig. 2). In particular, in the genomes of *Pasteurella multocida*, *Actinobacillus actinomycetemcomitans*, and *Haemophilus influenzae*, *uup* clusters positionally with the gene encoding a murein hydrolase exporter; in the genomes of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, it is co-localized with a gene encoding soluble lytic murein transglycosylase. *yjjK* is adjacent to a soluble lytic murein transglycosylase gene in the genomes of *E. coli*, *Salmonella typhi*, and *Vibrio cholerae*. Moreover, in the genome of *Rhodopseudomonas palustris yjjK* is localized near a gene encoding a non-orthologous membrane-bound lytic murein transglycosylase.

![Fig. 2. Schematic representation of chromosomal loci containing homologs of yjjK and uup genes in (a) *P. multocida*, (b) *P. aeruginosa*, (c) *E. coli*, and (d) *R. palustris*. yjjK and uup orthologs are shown by filled arrows, genes functionally associated with murein are shown by hatched arrows. Gene identifications are given from ERGO database.](image)

*RVK01701* and *RPA01805* are orthologs of *uup*; *RECO4274* and *RRPA03898* are orthologs of *yjjK*. *RVK01698* encodes an unknown hypothetical protein; *RVK01699* encodes deoxyguanosine triphosphate triphosphohydrolase; *RVK01698* encodes murein hydrolase exporter; *RPA01805* and *RECO4274* encode soluble lytic murein transglycosylase; *RRPA05491* encodes an unknown hypothetical protein; *RRPA04141* encodes trans-aconitate methyltransferase; *RRPA05490* encodes DNA polymerase III, chi subunit; and *RRPA03898* encodes membrane-bound lytic murein transglycosylase. In the genomes of *E. coli* and *S. typhimurium*, a common candidate regulatory sequence was found in the upstream region of *yjjK* as well as two other genes involved in cell wall biogenesis, namely *mltE* and *ampC* (Fig. 3). This sequence is a palindrome of length 20 with conserved 7-bp half-sites and a variable 6-bp spacer. This element is not conserved in other species.

![Fig. 3. Candidate regulatory sequence in the upstream regions of yjjK, ampC, and mltE genes of *E. coli*.](image)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
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<tbody>
<tr>
<td><em>yjjK</em></td>
<td>CTcaTTA - 6 - TAAaCAt</td>
</tr>
<tr>
<td><em>mltE</em></td>
<td>CTgtTTA - 6 - TAAcCGG</td>
</tr>
<tr>
<td><em>ampC</em></td>
<td>CCGTTTc - 6 - aAACCAG</td>
</tr>
</tbody>
</table>
Being conserved among most prokaryotic as well as several eukaryotic species, both \textit{yjk} and \textit{uup} genes are absent in the genomes of intracellular endosymbionts, such as \textit{Mycoplasma genitalium} and \textit{Mycoplasma pneumonia}, that lack the cell wall. Moreover, these genes are conserved in those eukaryotic species that possess the cell wall, e.g. \textit{Arabidopsis} and \textit{Drosophila}, and they are absent in mammals that has none. Due to the co-localization of \textit{yjk} and \textit{uup} genes with genes involved in murein recycling, their candidate co-regulation with cell wall genes, and finally, their absence in the genomes of species lacking the cell wall, but not in the species with the cell wall, we propose that \textit{YjjK} and \textit{Uup} are membrane-anchored ATP-binding proteins involved in the cell wall regeneration.

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\section*{References}