Isolation and Characterization of Restriction Endonuclease in
Plesiomonas shigelloides and Aeromonas Species

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Five restriction endonucleases (ENases) and one ENase were found in a screen of 196 strains of Plesiomonas shigelloides and 147 strains of Aeromonas species. Plesiomonas and Aeromonas species are classified as Vibrioaceae, identified as food-poisoning bacteria, are closely genetically related to each other, and their ENases producing abilities have not been reported. ENases were detected at relatively low frequencies in these species as compared to those in other species, such as Salmonella species and Vibrio paraahemolyticus. All ENases were shown to be isoschizomers of already known ENases. One of the Plesiomonas ENases, designated PsbBI, recognizing the sequence 5’-AT/TAAT-3’ should be useful, since PsbBI ENase is produced at a high yield of 7000 units/g of wet cells. The specificities of other ENases are also described in this paper.

Key words restriction endonuclease; Plesiomonas shigelloides; Aeromonas hydrophila; restriction cleavage analysis; food-poisoning bacteria

Restriction endonucleases (ENases) have been widely used as important tools for molecular biology and genetic engineering. To date, more than 209 different specificities of ENases have been identified.1,2 Pathogenic bacteria, including food-poisoning bacteria, have rarely been screened for their ENase activities because of their pathogenicities. Plesiomonas (P.) shigelloides and Aeromonas species are classified as Vibrioaceae, identified as food-poisoning bacteria, and are closely genetically related to each other.3 For useful new ENases, we screened the ENase activities of various strains of P. shigelloides and Aeromonas species, since a new ENase with novel specificity was recently isolated3 from P. shigelloides.

MATERIALS AND METHODS

Among 196 P. shigelloides strains screened for ENases, 65 strains were reference strains for E. coli;4,5 18 strains were from collections of Aldova,5 and another 113 strains were isolated from human origin. The following 147 strains of Aeromonas species were also screened for ENases: 91 O antigen reference strains, 39 strains from the collection of Guinee6 and Gross,7 and another 17 strains, classified as A. salmonicida, A. media, A. shubertii and A. veronii, respectively. The occurrence of ENases in these strains was tested by the modified lysozyme lysis method8 repeated three times. The method consists of gentle lysis of the bacteria to be tested with 200 µg/ml lysozyme and tolueene in the presence of 20% sucrose in the buffer (10 mM Tris-HCl pH 7.5, 30 mM NaCl and 6 mM EDTA). ENases were purified free of detectable non-specific nucleases from stationary phase cells grown in a 400 ml culture of LB (Luria-Bertani) broth by ultrasonication, followed by polyethylene imine precipitation and some column chromatographies, as reported previously.9 To determine the identity of the cleavage site between isoschizomers, ligation and recutting experiments were carried out between substrate DNA fragments produced by the isoschizomers.

RESULTS AND DISCUSSION

Of 196 strains of P. shigelloides tested, ENase activities were detected in 5 strains. On the other hand, only 1 ENase was detected in 147 strains of Aeromonas species. The designation and specificity of these ENases is summarized in Table 1. The cleavage pattern of λ phage DNA by the ENases is shown in Fig. 1. The rate of detection of ENases in each species was relatively low (approximately 3% and 1%, respectively) as compared to that in other species: In Yersinia enterocolitica.10 Salmonella Infantis, Salmonella Thompson, Salmonella Blockley,11 Vibrio paraahemolyticus12 and Vibrio cholerae non O1,13 ENases were detected at a frequency of 22, 91, 34, 30, 17 and 12%, respectively. Figure 2 shows that the PsbBI cleavage site of pBR322 DNA could ligate to the site of VspI, indicating that the cleavage site of PsbBI was the same as that of VspI. Details were shown in the legend to Fig. 2. Of these enzymes shown in Table 1, an isoschizomer of VspI designated PsbBI seemed to be most

Table 1. Characteristics of ENases in Plesiomonas shigelloides and Aeromonas hydrophila

<table>
<thead>
<tr>
<th>ENase</th>
<th>Bacterial strain</th>
<th>Recognition sequence (isoschizomer)</th>
</tr>
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<tbody>
<tr>
<td>PsbBI</td>
<td>Plesiomonas shigelloides TPS970</td>
<td>AT/TAAT (VspI)</td>
</tr>
<tr>
<td>PsbCI</td>
<td>Plesiomonas shigelloides PL50</td>
<td>CAGCTG (PmcCI)</td>
</tr>
<tr>
<td>PsbDI</td>
<td>Plesiomonas shigelloides PL59</td>
<td>CAGCTG (PmcCI)</td>
</tr>
<tr>
<td>PsbEI</td>
<td>Plesiomonas shigelloides TPS044</td>
<td>CTGCAG (PsI)</td>
</tr>
<tr>
<td>PsbAI</td>
<td>Plesiomonas shigelloides 319-73</td>
<td>GACNN/NGNTC (VspI)</td>
</tr>
<tr>
<td>AerAI</td>
<td>Aeromonas hydrophila AH63</td>
<td>CTGCAG (XhoI)</td>
</tr>
</tbody>
</table>

a) Reported previously.21

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useful for genetic engineering because of the high yield; from 1 g of wet cell weight of P. shigelloides TPS970, 7000 units of PshBI were purified. As described previously, the yields of purified SryD41 and YenI10 were 120 and 600 units/g of wet cells. PshBI was stable during the purification procedure and during storage at $-20^\circ$C for 1 year. PshBI showed the highest activity at 37$^\circ$C in universal buffer (10 mm Tris–HCl pH 7.5, 10 mm MgCl$_2$, and 1 mm dithiothreitol) supplemented with 25 mm NaCl. To date, five isoschizomers of PshBI recognizing the sequence 5'-ATTAT-3' have been reported in the database for ENases, REBASE.1) As PshBI is easily purified, stable and reactive under general conditions, PshBI and the producer strain P. shigelloides TPS970 should be useful for bio-engineering reagents.

In our laboratory, several food-poisoning bacteria, such as Yersinia,10 Salmonella,11 Vibrio species (Vibrio parahaemolyticus12) and Vibrio cholerae non O113 were screened for their activities of ENase. Although Plesiomonas or Aeromonas strains have not caused a severe outbreak of food-poisoning, both strains have been detected often from the polluted seafood and patients. It might be important to know these biological characteristics. No one has reported before about their ENase producing abilities.

REFERENCES AND NOTES