Characterization of two Restriction Endonucleases, SenPT14b1 and SenPT16l, in Standard Phage-Type Strains of Salmonella Enteritidis

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Two restriction endonucleases (ENases) were found by screening 38 standard phage strains of Salmonella (S.) Enteritidis. An isoschizomer of SacII ENase that recognizes the sequence 5'-CCGC/GG-3' was identified in S. Enteritidis PT14b, and an isoschizomer of XmaIII ENase (5'-CGCGCGG-3') was found in S. Enteritidis PT16. It is of special interest that the recognition specificities of all known ENases in Salmonella, including those of the S. Enteritidis ENases, are very similar to each other.

Key words restriction endonuclease; Salmonella Enteritidis; SacII isoschizomer; XmaIII isoschizomer

Food poisoning caused by Salmonella (S.) Enteritidis has been a serious problem in most countries in the world. The majority of food poisoning cases have been caused by the ingestion of eggs or egg products contaminated with S. Enteritidis. A phage-typing method has been devised and applied to the epidemiology of S. Enteritidis. In England, phage type (PT) 4 of S. Enteritidis has been popular, isolated from patients and the environment around poultry farms, while in the U.S.A. PT8, PT13 and PT14b have been frequently isolated from the environment around poultry farms.1,2)

The occurrence of restriction endonuclease (ENase) has been studied in this laboratory3–6) in pathogenic bacteria belonging to Enterobacteriaceae in the hope that novel ENases useful for recombinant DNA technology might be found, as the screening of ENases has rarely been tried in these bacteria. This is the case, and several ENases of pathogen origin have been commercially available after the isolation of safe ENase-positive strains. We have tested the occurrence of ENases in standard PT strains in S. Enteritidis, as the PT is divided on the basis of host range and host controlled variation; the specificity of the host-controlled variation is highly dependent upon the occurrence of specific ENase in the bacterium. The isolation and purification of two ENases in S. Enteritidis are described in this paper together with general characteristics of Salmonella ENases.

MATERIALS AND METHODS

Standard PT strains of S. Enteritidis employed for the screening of ENases were from our collection. A total of 38 standard PT strains of S. Enteritidis were screened for ENases by the lysozyme lysis method described previously,7,8) a safe method for the detection of ENases in pathogenic bacteria, including Salmonella. The medium used for the growth of S. Enteritidis was a nutrient broth. For the purification of ENase from a larger scale culture, the methods of Pirrotta and Bickle9) were employed. After the extraction of ENase, three kinds of chromatography were employed for the further purification as described minutely in RESULTS. λ or T4dC DNA was employed as a substrate of ENases for the primary screening, and λ, Adenovirus-2 (Ad-2), ColE1 and ϕX174 DNAs were used for further analysis of the specificity of the ENases detected.

RESULTS

Screening of ENases in S. Enteritidis Two strains, S. Enteritidis PT14b and PT16, were shown to produce an ENase by the lysozyme lysis method. PT14b is one of the major causes of illness in the U.S.A., as described above. The ENases in S. Enteritidis PT14b and PT16 were designated as SenPT14b1 and SenPT16l, respectively, according to the proposal of Smith and Nathans.10) No conclusive data suggesting the location of the hsdA gene of these two strains have been obtained in spite of several trials.

Characterization of SenPT14b1 and SenPT16l SenPT16l ENase was purified free of detectable non-specific nucleases from the extract of 12.3 g wet weight of S. Enteritidis PT16 grown in a 2.4 l of Luria broth by polyethylenimine precipitation, followed by DEAE cellulose, heparin agarose, and hydroxyapatite column chromatography. SenPT16l was eluted immediately without being retained on DEAE cellulose, at 250 mm NaCl on heparin agarose, and at 50 mm phosphate on a hydroxyapatite column in buffer B.11) The final yield was 40 units/g of wet cells. SenPT14b1 was purified from S. Enteritidis PT14b by essentially the same procedure as described above, except that the hydroxyapatite column chromatography was omitted. SenPT14b1 was eluted at 50 mm NaCl on DEAE cellulose and at 480 mm NaCl on heparin agarose. The yield of SenPT14b1 was higher than that of SenPT16l, i.e., 220 units/g of wet cells.

Both SenPT14b1 and SenPT16l were stable during the purification procedures and also when stored at −20°C for more than 6 months. The presence of 10 mM MgCl2 in the reaction buffer is essential for the cleavage of substrate DNA by these two ENases. Neither Mn++ or Zn++ could be substituted for Mg++. Optimal conditions for the cleavage of λDNA with SenPT14b1 and SenPT16l are summarized in Table 1. The cleavage pattern with SenPT14b1 of λ, Adenovirus-2, ColE1, and ϕX174 DNA was very similar to that with SacII, recognizing and cleaving the sequence 5'-CCGC/GG-3'.12) Likewise, the pattern with SenPT16l was identical to that with EagI,

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Table 1. Optimal Condition for Cleavage of λDNA with SenPT14bl and SenPT16l ENases

<table>
<thead>
<tr>
<th>ENase</th>
<th>Optimal condition for cleavage of substrate DNA</th>
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<tr>
<td></td>
<td>Temperature (°C)</td>
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<tr>
<td>SenPT14bl</td>
<td>25</td>
</tr>
<tr>
<td>SenPT16l</td>
<td>37</td>
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</tbody>
</table>

Basal buffer described by Maniatis et al. was employed for cleavage reactions.

Fig. 1. Comparisons of Cleavage Patterns of λDNA after Digestion with ENases SenPT14bl and/or SacII

Lane 1, λDNA + HindIII; lane 2, λDNA + HindIII + SenPT14bl; lane 3, λDNA + HindIII + SenPT14bl + SacII; lane 4, λDNA + HindIII + SacII; lane 5, λDNA + HindIII.

Fig. 2. Comparisons of Cleavage Patterns of λDNA after Digestion with ENases SenPT16l and/or Eagl (XmaIII Isochizomer)

Lane 1, λDNA + HindIII; lane 2, λDNA + Eagl; lane 3, λDNA + EcoRI + SenPT16l; lane 4, λDNA + EcoRI + SenPT16l + Eagl; lane 5, λDNA + EcoRI + Eagl; lane 6, λDNA + HindIII.

DISCUSSION

As described above, two ENases, designated SenPT14bl and SenPT16l, have been purified and characterized. The former is an isochizomer of SacII recognizing the sequence 5'-CCGC/GG-3', and the latter is that of XmaIII recognizing 5'-CCGC/GG-3'. This is the first report on the occurrence of ENases in S. Enteritidis, although several ENases were isolated in other serotypes of Salmonella. Both SenPT14bl and SenPT16l may become commercially available after the introduction of the hsd+ gene into E. coli K-12, since they are stable and since their isochizomers, i.e., SacII, XmaIII and others, are expensive.

In the previous paper, we pointed out that the recognition sequences of all type II Salmonella ENases are similar to each other: these sequences in part overlap with each other; the recognition sequences are GC-rich; the outside nucleotides of the sequences are G and C; and all ENases characterized produce 5'-protruding ends after DNA cleavage. Based on the similarity of recognition sequences, we postulated a divergent evolution from a common ancestor for the gene coding for most ENases in Salmonella, as is the case with ENases in Herpetosiphon giganteus. Our results reported here confirm this hypothesis (Table 2). The recognition sequences of SenPT14bl and SenPT16l are very similar to those of the other Salmonella ENases. These sequences are GC-rich and in part overlap each other. The exception to this rule of the specificities of Salmonella ENases is that SenPT14bl produces 3'-recessive ends in stead of 5'-producing ends after the cleavage of substrate DNA. There are no Salmonella ENases which produce blunt ends (Table 2).

REFERENCES

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