Purification, Properties, and Sequence Specificity of SsI, a New Type II Restriction Endonuclease from Streptococcus salivarius subsp. thermophilus

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SsI, a type II restriction endonuclease, was purified from Streptococcus salivarius subsp. thermophilus strain BSN 45. SsI is an isoschizomer of BsrNI. SsI activity was maximum at pH 8.8, 0 to 50 mM NaCl, 2 to 8 mM Mg2+, and 42°C. Activity against phage DNA in vitro was demonstrated.

Bacteriophage attack is a significant problem in commercial strains of lactic acid bacteria. For lactococci used in cheese production a number of phage insensitivity mechanisms have been reported (6, 10).

Restriction-modification, a mechanism first described for Escherichia coli (1), also exists in lactococci (3). A type II restriction endonuclease has indeed been purified from one Lactococcus sp. (8), and its activity on phage DNA has been established in vitro (5). In the case of Streptococcus salivarius subsp. thermophilus there is biological evidence for the presence of restriction-modification (2, 12). A type II restriction endonuclease has been shown to exist in a strain recalcitrant to electrotransformation with plasmid DNA (18).

We have screened 20 strains of S. salivarius subsp. thermophilus for restriction activity by using λ DNA as substrate. These strains and their lytic phages (2) belong to the collection of the Centre International de Recherche Daniel Carasso and are named by using BSN and d., respectively, followed by a number. All strains were grown in M17 broth (19) supplemented with lactose to a final concentration of 5 g/liter. Specific endonuclease activity was detected among four strains and one of them, BSN 45, was used for further purification of an enzyme named SsI (recommendation of R. J. Roberts, Cold Spring Harbor Laboratory) since the suggested nomenclature (17) could not be followed without confusion with that for a previously described restriction-modification system (15).

Purification of SsI from strain BSN 45. Phosphocellulose was used for ion-exchange chromatography of BSN 45 crude extract by using a salt gradient in phosphate buffer (10 mM NaH2PO4 [pH 7.4], 0.1 mM EDTA, 10 mM β-mercaptoethanol). A single peak of restriction endonuclease activity eluted around 0.65 M NaCl and corresponded to a purification of 750× SsI (Table 1). This preparation was free of nonspecific nuclease activity and was stable for at least 3 months at 4°C. Successive chromatographies on Cibacron Blue Sepharose CL6B, phosphocellulose, and Fractogel TSK-Heparin 650 yielded a more purified enzyme preparation but one with a low enzyme and protein yield. SsI activity seemed associated with a protein with a molecular weight of 41,000. The N-terminal amino acid sequence of this protein band, determined on the first nine amino acids by electroblotting onto polyvinylidene difluoride membranes (11), indicated no obvious resemblance to already known protein sequences.

Recognition and cleavage specificity of SsI. SsI is an isoschizomer of BsrNI. Indeed, identical patterns were obtained with double-stranded pBR322 and φX174 DNA after single digestion with SsI or BsrNI and double digestions with SsI and BsrNI. Thus, SsI recognizes the following sequence.

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5' - C - C - W - G - G - 3' \\
3' - G - G - W - C - C - 5' (W = A or T)
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The cleavage site was determined by primer extension (4) and is indicated by the arrows. Another restriction enzyme which has been characterized from S. salivarius subsp. thermophilus, Ssrh34I, recognizes the sequence 5’-C-C-G-G-3’. The cleavage site of this enzyme remains unknown (18).

Influence of various physical parameters on the activity of SsI. The activity of purified SsI was measured with φX174 DNA linearized with PstI by using classical analytical agarose-ethidium bromide electrophoresis (16). SsI activity requires Mg2+ and is optimum between 2 and 8 mM. Mg2+ cannot be replaced by Mn2+ or Ca2+. The presence of S-adenosylmethionine or ATP was not required for activity, nor did they stimulate activity, indicating that SsI is a classical type II restriction enzyme. SsI was inhibited by an ionic strength higher than 100 mM NaCl. The pH dependence of SsI was determined by using a buffer system whose components are invariant and whose ionic strength remains essentially constant throughout the pH range (6 to 9.5) (7). SsI was active at pHs between 8.3 and 9.1, with an optimum at pH 8.8, and at temperatures between 37 and 47°C, with an optimum at 42°C.

Activity of purified SsI on isolated phage DNA. As restriction enzymes are considered to have a role in the phage insensitivity of the producing strains (14), we determined SsI activity on the DNA of phage that grow lytically on different strains of S. salivarius subsp. thermophilus but not on strain BSN 45. The DNAs of phages φ29, φ57, and φ11 (2) were all digested, producing from 5 to >12 fragments (Fig. 1). We also examined the activity of SsI on the DNA from phage φ15, which lyzes strain BSN 45, and found that φ15 DNA is resistant to SsI cleavage (Fig. 1). This absence...
of cleavage by SslI is due to a modification carried by φ15 DNA since two isoschizomers of SslI which recognize different modification specificities, BstNI and MvaI (13), cleave φ15 DNA into six fragments (data not shown). This suggests that a modification activity which alters the specific site recognized by SslI and makes it resistant to cleavage exists in strain BSN 45. This modification system could be encoded either by the bacterial host, or, as shown recently (9), by phage φ15 itself. The above results suggest a potential biological role for SslI, and studies to determine whether this enzyme is responsible for the in vivo restriction ability of S. salivarius subsp. thermophilus BSN 45 are currently under way.

Cloning the SslI restriction-modification system in a strain other than BSN 45 could introduce a new restriction ability. This should permit the construction of dairy starters with improved phage resistance. In addition, SslI could be a potentially useful type II restriction endonuclease, because it functions between 37 and 47°C with an optimum at 42°C (60°C for BstNI), has a relatively alkaline pH range between 8.3 and 9.1, and may have a different sensitivity to site-specific modification than BstNI, its isoschizomer.

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