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EXPERIMENTAL ARTICLES

Endophytic Bacilli Producing Type II Restriction Endonucleases

V. V. Lukyanchuk, O. N. Reva, and L. V. Polishchuk[1]

Zabolotnyi Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, ul. Zabolotnogo 154, Kiev, 03143 Ukraine

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Abstract—Two of thirteen bacillar strains isolated from the inner tissues of cotton plants were found to produce type II restriction endonucleases. The investigation of the site specificity of these enzymes showed that they are *Asu*I and *Eco*31I isoschizomers.

Key words: bacilli, restriction endonucleases.

Restriction endonucleases have been found in many microorganisms. Type II restriction endonucleases have been isolated from *Helicobacter pylori* (*Hpy*N1) [1], *Haemophilus aegyptius* (*Hae*II and *Hae*III) [2], *Klebsiella pneumonia* (*Kpn*I) [2], and some other bacteria pathogenic to humans and animals. Restriction endonucleases have also been found in phytopathogenic bacteria of the genera *Pseudomonas*, *Bacillus*, and *Erwinia* [2, 3].

The aim of this study was to investigate the occurrence and the site specificity of restriction endonucleases in the bacilli isolated from the inner tissues of cotton plants.

MATERIALS AND METHODS

Thirteen bacillar strains used in this study (*Bacillus subtilis* UKM B-5017, UKM B-5044, UKM B-5048, and 70D, *Bacillus licheniformis* UKM B-5508, UKM B-5512, UKM B-5913, and UKM B-5559, "*Bacillus endophyticus"* UKM B-5710, UKM B-5912, and UKM B-5915, and *Bacillus pumilus* 7-91D) were obtained from the Ukrainian Collection of Microorganisms (UKM B). The strains were isolated from the inner tissues of cotton plants (Tajikistan)[4].

The DNA of phage lambda was purchased from MBI Fermentas (Lithuania). The DNA of phage T7 was kindly donated by F.I. Tovkach, Institute of Microbiology and Virology. The restriction endonucleases *Cfr*13I (isoschizomer *Asu*I) and *Eco*31I were purchased from MBI Fermentas, lysozyme A was purchased from NPO Biolar (Olaine, Latvia), Triton X-100 was purchased from Serva (Germany), and ethidium bromide and type I agarose were purchased from Sigma (United States).

The strains were grown in nutrient broth or on nutrient agar and tested for the presence of restriction endonucleases using the Belavin method [5] with a minor modification, which lay in the cultivation of bacteria in nutrient broth. The site specificity of endonucleases was analyzed with the aid of the DIGEST software program developed by Jeff Elhai.

RESULTS AND DISCUSSION

Among the 13 endophytic bacilli tested, which were taken randomly from the Ukrainian Collection of Microorganisms, two strains were found to possess endonuclease activity. This corresponds to an occurrence rate of restriction endonuclease producers of 15.4%. This rate is more than two times lower than the mean occurrence rate of such producers among bacilli (36%) [6, 7], which can be explained by the specific habitat of the strains under study (the inner tissues of cotton plants). The microorganisms inhabiting this econiche depend on the metabolism of host plants and constitute a steady community of symbiotic and phytopathogenic organisms.

Figure 1 shows the electropherogram of the fragments of two DNA substrates digested by two new restriction endonucleases, *Bsu*5044BI (is produced by *B. subtilis* UKM B-5044) and *Bli*5508BI (is produced by *B. licheniformis* UKM B-5508). The use of two DNA substrates was dictated by the different occurrence rates of palindromic sites in these substrates [7, 8].

It can be seen that the restriction endonuclease *Bsu*5044BI digests the DNAs of phages lambda and T7 into many fragments, whose size was determined using a calibration curve constructed with the aid of the *Hin*dIII-produced fragments of the phage lambda DNA. Data on the hydrolysis sites in the phage DNAs and on the molecular mass of the DNA fragments produced were analyzed for the site specificity of restriction endonucleases using the DIGEST program. The analysis showed that *Bsu*5044BI is an isoschizomer of the restriction endonuclease *Eco*31I. This fact was confirmed by comparing the fragments of the phage DNAs

[1]Corresponding author. E-mail: lvpolishchuk@yahoo.com

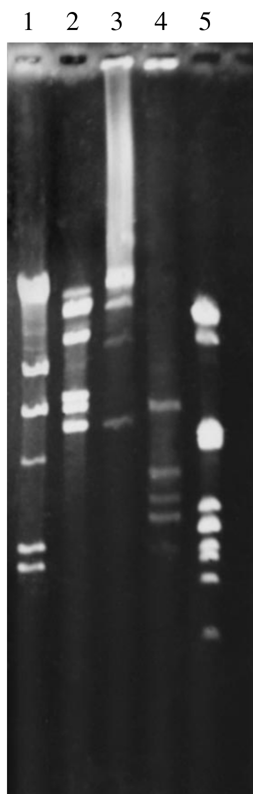

Fig. 1. Electrophoresis of the DNA of phages lambda and T7 digested by *Hin*dIII and lysates of *B. subtilis* UKM B-5044 and *B. licheniformis* UKM B-5508). Lanes: 1, the DNA of phage lambda + *Hin*dIII; 2, the DNA of phage T7 + *B. subtilis* UKM B-5044 lysate; 3, the DNA of phage lambda + *B. subtilis* UKM B-5044 lysate; 4, the DNA of phage T7 + *B. licheniformis* UKM B-5508 lysate; and 5, the DNA of phage lambda + *B. licheniformis* UKM B-5508 lysate.

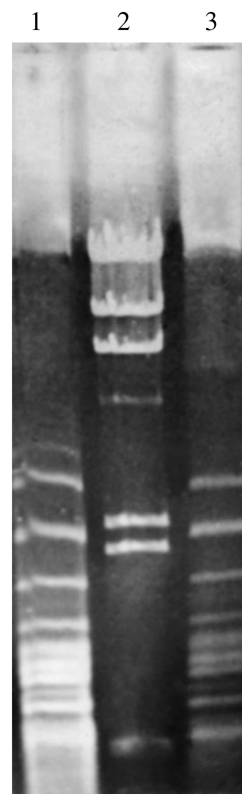

Fig. 2. Electrophoresis of the digested phage DNAs. Lanes: 1, the DNA of phage T7 + *B. subtilis* UKM B-5044 lysate; 2, the DNA of phage lambda + *Hin*dIII; and 3, the DNA of phage T7 + *Cfr*13I.

digested by *Bli*5508BI and the commercial restriction endonuclease *Cfr*13I (the *Asu*I isoschizomer), which showed that these fragments are identical (Fig. 2).

The DNA of the phage lambda contained two restriction sites for *Bli*5508BI, whereas the DNA of the phage T7 contained more than 25 restriction sites. Analysis with the DIGEST program showed that the restriction endonuclease *Bli*5508BI is an isoschizomer of *Eco*31I (the electropherograms of the restriction fragments of the substrate DNA digested by *Eco*31I and *Bli*5508BI are not presented).

Thus, the new restriction endonucleases *Bsu*5044BI and *Bli*5508BI are isoschizomers of the known type II endonucleases *Asu*I and *Eco*31I. The producers of *Bsu*5044BI and *Bli*5508BI, *B. subtilis* UKM B-5044 and *B. licheniformis* UKM B-5508, are easily cultivated and contain the endonucleases in considerable amounts, which facilitates their isolation. In our opinion, these two bacillar strains can be used as industrial producers of *Bsu*5044BI and *Bli*5508BI.

It is known that bacilli produce many prototypes and isoschizomers of restriction endonucleases, which can recognize sequences containing from 4 to 8 and even more nucleotides. Bacillar restriction endonucleases of types II and IIA hydrolyze a nucleotide chain at a recognition site or close to it and form either cohesive or blunt ends. Although about 40% of all of the known bacillar restriction endonucleases recognize four-nucleotide sequences, the prototypes of *Asu*I (*Bsu*5044BI) and *Eco*31I (*Bli*5508BI) (i.e., *Asu*I and E*co*31I) recognize sequences containing five and seven nucleotides, respectively. In this case, *Asu*I recognizes any nucleotide occurring at position 3, whereas *Eco*31I recognizes any nucleotide at position 7 of the recognition site. Both restriction endonucleases hydrolyze nucleotide chains within their recognition sites with the formation of cohesive ends. The recognition sites of *Asu*I and *Eco*31I are rich in G+C, which is typical of the endonuclease producers whose genome is rich in A+T [8].

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