

Restriction Endonuclease Ajnl from *Acinetobacter johnsonii* R2, an Isoschizomer of EcoRII, recognizes 5'-CCWGG-3' and cleaves dcm-methylated sites

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A producer of restriction endonuclease Ajnl has been isolated from natural resources and identified as bacterial species Acinetobacter johnsonii R2. The restriction enzyme purification and estimation of its activity is described. It has been shown that Ajnl produces DNA fragments with 5'-CCWGG sticky ends similar to EcoRII, but cleavage is not blocked by dcm-methylation. A new restriction endonuclease Ajnl may be widely used in genetic engineering.

INTRODUCTION

EcoRII restriction - modification system were discovered among the first RM-systems [1, 2]. EcoRII restriction endonuclease produces DNA fragments with 5'-CCWGG sticky ends (where W - A or T) [3]. DNA- methyltransferase M.EcoRII modifies internal cytosine of both strands of recognition sequence protecting bacterial DNA from the cleavage by EcoRII restriction endonuclease [1, 4]. Many laboratory *E. coli* strains have dcm-methyltransferase [5, 6] which modifies DNA similar to M.EcoRII. Plasmid DNAs isolated from these *E.coli* strains are resistant against cleavage by EcoRII. This is an obvious limitation of EcoRII application in genetic engineering. There are some isoschizomers of EcoRII named ApyI [7], MvaI [8, 9], BstNI [10, 11] and others, which are not blocked by dcm-methylation, but cleave the same DNA sequence in another way: 5'-CC[^]WGG-3'. Such isoschizomers may replace the prototype, however, not in all situations. For example, in the experiments on plasmid libraries preparation, it is convenient to use a vector molecule linearized by rare-cutting enzyme and short DNA fragments which may be produced by 4-bp recognition restriction endonuclease. However, both these enzymes should produce the same sticky ends. There is such a couple of enzymes SexAI (recognition sequence 5'-A[^]CCWGGT-3 ') and EcoRII (5'-[^]CCWGG-3 '). Unfortunately, both these enzymes are sensitive to dcm-methylation. Recently we have discovered MabI - isoschizomer of restriction endonuclease SexAI which is not blocked by dcm-methylation, but similar isoschizomers of EcoRII are unknown.

In this work we describe isolation and an investigation of some properties of the new restriction

endonuclease Ajnl from bacterium *Acinetobacter johnsonii* R2 which is the isoschizomer of EcoRII. Activity of Ajnl is not blocked by dcm-methylation.

MATERIALS AND METHODS

A search of restriction endonucleases was carrying out according to [12, 13]. The producing strain *A. johnsonii* R2 (SE collection of microorganisms #5M95) was isolated from natural water supply and identified according to [14, 15]. *A. johnsonii* R2 was grown in a special media containing 10 g/l trypton, 5 g/l yeast extract, and 5 g/l NaCl, 0.5 g/l MgCl₂, 0.01 g/l thiamine at pH 7.5 and 28°C with aeration. Cells were harvested by centrifugation when the culture reached the stationary phase.

20 grams of the frozen cells biomass was suspended in 60 ml of 0.2 M NaCl and 0.3 g/l of lysozyme and incubated 16 hours. Cells were disrupted by sonification and centrifuged. The enzyme isolation was performed at 4°C. All solutions included buffer 1 (10 mM K-phosphate buffer (pH 7.2), 7 mM beta-mercaptoethanol and 0.1 mM EDTA). Supernatant after centrifugation was diluted 3 times by buffer 1 and loaded on 40 ml of P11 phosphocellulose (Whatman, UK). A column was washed by 0.05M NaCl in buffer 1 and than a gradient of 0.05-0.6M NaCl in buffer 1 was used to eluate the enzyme. Enzymatic activity was determined in SE Y-buffer at 55°C with subsequent analysis of the reaction products by electrophoresis in 1% agarose according to [16]. Fractions with enzymatic activity were loaded on 5 ml of hydroxylapatite (BioRad, USA) column, washed by 0.2M NaCl in buffer 1 and than a gradient chromatography was carried out from washing buffer to 0.2M NaCl, 0.25M K-phosphate in buffer 1. Fractions with enzymatic activity dialyzed against buffer 1 and loaded on 4 ml of heparin-sepharose (Sigma, USA). A column was washed by 0.05M NaCl and enzyme was eluated by NaCl gradient 0.05M -0.6M. Fractions with Ajnl dialyzed against buffer 1 with 50% glycerol and stored at -20°C. Optimal buffer for enzyme's activity was determined by a direct comparison of DNA pattern cleavage in 5 SE buffers at 30°C, 40°C, 50°C, 60°C and 70°C. ³²P-labeled oligonucleotide (5'-AAGGTCACCTGGGACGAACGCGTCGACCGGTATACTAA-3') with a corresponding complementary strand was used to determine the phosphodiester bond to be hydrolyzed. The oligonucleotide duplex was synthesized according to [17] and was cleaved by restriction endonucleases Ajnl, Psp6I and Bst2UI. The products of hydrolysis were separated by electrophoresis in 20% polyacrylamide gel in the Tris-borate buffer in the presence of 7 M urea. DNA ladder was prepared from oligonucleotide by a treatment with exoIII. Resulting gel was scanned in Cyclon (Packard,USA).

The DNA preparations, oligonucleotides, and restriction endonucleases used in this work were from SibEnzyme Ltd. (Novosibirsk, Russia).

RESULTS AND DISCUSSION

Bacterial strain - producer of restriction endonuclease Ajnl has the following characteristics. Cells are short rods, not motile, in the size 1,3x2 micron; occur as single, in pairs, and also in chains; do not form spores. Gram-negative and stained cells look as cocki in pairs. The strain is aerobic, catalase-positive and oxydase-negative. The strain grows on the minimal agar with ethanol, acetate, alanine and arginine but doesn't not grow with glucose, lactose, rhamnose, arabinose, sacrose, maltose, galactose, sorbitol, mannitol, dulcitol inositol, glycerol, citrate, glycine, ornithine and histidine. The strain forms the small amount of acid at growth on a nutritious agar with ethanol, but not with carbohydrates or with multinuclear spirits. The starin does not hydrolize gelatin. The strain grows at concentration NaCl from 0

up to 3 %, pH 6.8 - 7.7, temperature from 4°C to 30°C, but not at 37°C. It is sensitive to ampicillin, kanamycin and chloramphenicol, but it is resistant to tetracycline at concentration of an antibiotic in a nutritious agar of 0.05 mg/ml. In nutritious broth Luria without stirring the culture forms homogeneous dregs and a deposit, and forms a superficial film at concentration NaCl of 1.5 and 3 %. On a nutritious agar of a colony grew for 2 days at 22°C and were round, creeping away up to 2 cm in diameter, flat and little bit convex in the center, crude-brilliant, translucent, grayish with a salad shade. The strain was identified according to [15] as a species *Acinetobacter johnsonii* R2, and its restriction endonuclease have been named Ajnl according to [18].

Biomass of *A. johnsonii* R2 was grown at aeration in modified Luria broth. Yield was 4 g cells from 1 litre of a media. Enzyme was isolated from a cellular extract by consecutive chromatographic steps as described in the methods. 3.5 ml of enzyme Ajnl with activity of 5000 units/ml were received from 20 grams of frozen cells. Optimal conditions of restriction enzyme's reaction were determined comparing activity in five SE standard buffers (see methods and the table). Ajnl is most active at 55°C in buffer Y.

Recognition sequence and dcm-sensitivity of Ajnl was determined by comparison of pBR322 dcm+, lambda dcm- and lambda dcm+ DNA cleavage by three enzymes (Fig.1). We used Ajnl, Psp6I (which is blocked by dcm-methylation), and Bst2UI (which is not dependent on dcm-methylation). The experimental data show that Ajnl, Bst2UI and Psp6I identically cut lambda dcm- DNA (Fig.1a, lanes 1-3). Thus, Ajnl recognizes nucleotide sequence 5'-CCWGG-3'. Fig.1b shows the cleavage of pBR322 dcm+ by all these enzymes. Bst2UI and Ajnl cleave this DNA whereas Psp6I doesn't cleave pBR322 dcm+. So, Ajnl recognizes 5'-CCWGG-3' and isn't sensitive to dcm-methylation.

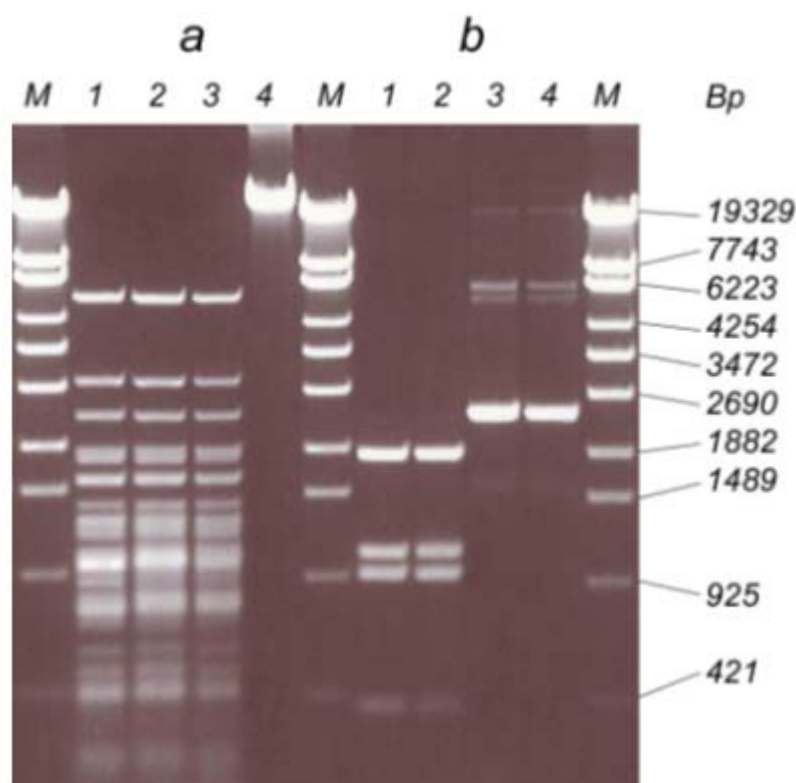


Fig. 1. Determination of recognition sequence and dcm-sensitivity of Ajnl. a) lambda dcm- DNA; b) pBR322 dcm + DNA ; 1 - Bst2UI; 2 - Ajnl; 3 - Psp6I; 4 - native DNA; M - DNA ladder, lambda DNA/BssT1I.

Determination of the cleavage position of Ajnl was carried out as indicated in "Materials and Methods" .
Oligonucleotide duplex as indicated below

5'-AAGGTCA	CCTGG	GACGAACGCGTCGACCGGTATACTAA-3'
3'-TTCCAGT	GGACC	CTGCTTGCGCAGCTGGCCATATGATT-5'

was cleaved by Ajnl, Bst2UI (recognition sequence 5'-CC[^]WGG-3') and Psp6I (recognition sequence 5'-CCWGG-3'). Data on Fig.2 show that Ajnl cleaves DNA similar to Psp6I. Thus, recognition sequence of Ajnl is similar to Psp6I, however, Ajnl isn't sensitive to dcm-methylation. In a [database](#) there is no similar isoschizomers of EcoRII. We believe that enzyme Ajnl will find a wide application in molecular biology investigations.

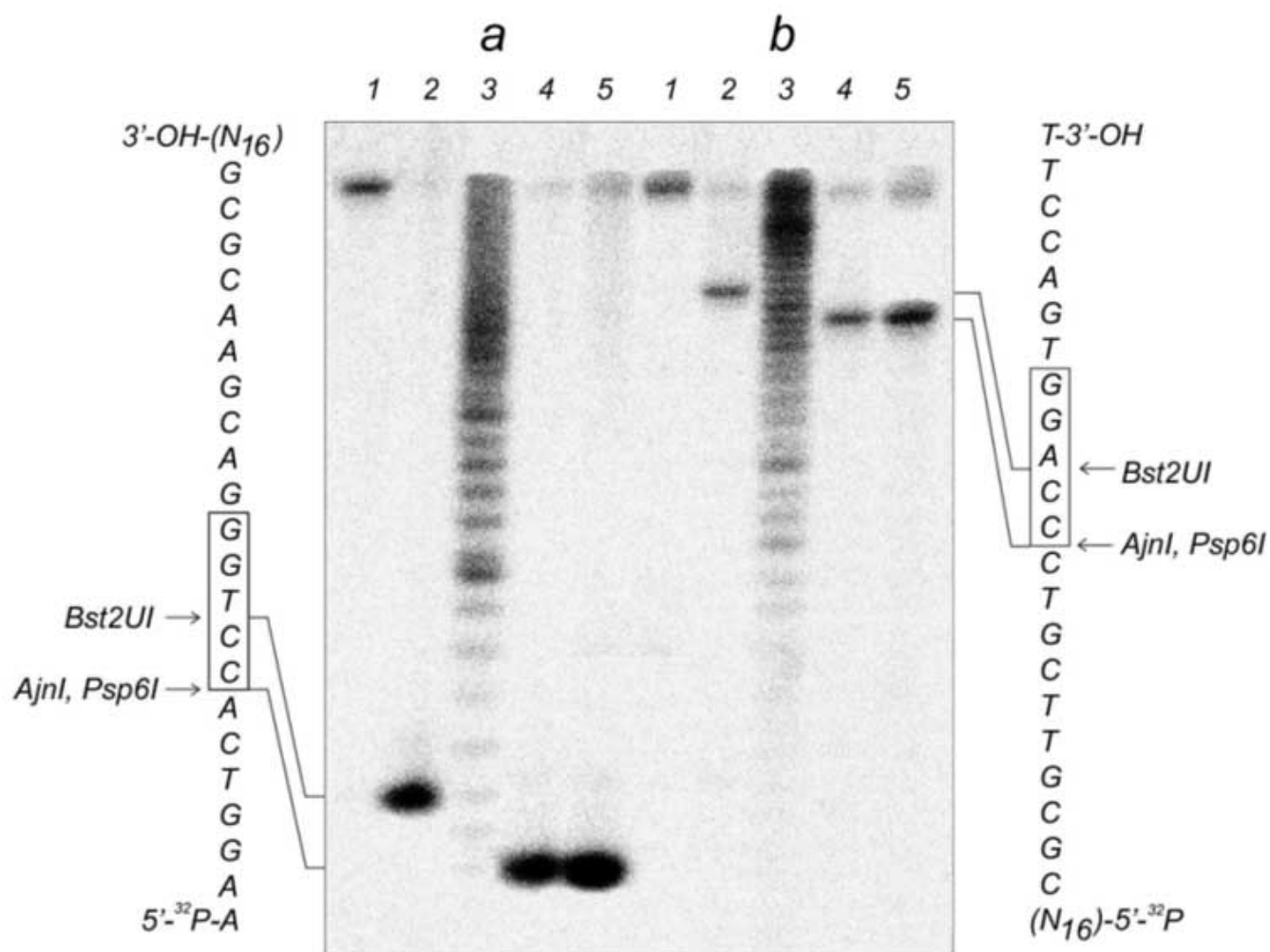


Fig. 2. Determination of cleavage position of Ajl: a - the top strand is labeled by ^{32}P ; b - the bottom strand is labeled by ^{32}P ; 1 - original duplex; 2 - Bst2UI 3 - exoIII, 4 - Ajl, 5 - Psp6I

REFERENCES

1. Yoshimori, R.N. / In Ph.D. Thesis, University of California, San Francisco, USA. - 1971. - P. 1-73.
2. Roberts R.J., Vincze T., Posfai J., Macelis D. // Nucleic Acids Res. - 2002. - V. 31. - P. 418-420.
3. Bigger, C.H., Murray, K., Murray, N.E. // Nature New Biol. - 1973. - V. 244. - P. 7-10.
4. Buryanov Y.I., Bogdarina I.G., Bayev A.A.// FEBS Lett. - 1978. - V. 88. - P. 251-254.
5. Marinus M.G., Morris N.R. // J. Bacteriol. - 1973. - V. 114. - P. 1143-1150.
6. May M.S., Hattman S. // J. Bacteriol. - 1975. - V. 123. - P. 768-770.
7. Razin A., Urieli S., Pollack Y., et al. // Nucleic Acids Res. - 1980. - V. 8. - P. 1783-1792.
8. Janulajtis A., Vaitkevichus D.P. // Biotechnology. - 1985. - No. 1. - P. 39-51. (Rus.)

9. Butkus, V., Klimasauskas, S., Kersulyte, et al. // Nucleic Acids Res. - 1985. - V. 13. - P. 5727-5746.
10. Baryshev M.M., Buryanov Ya.I., Kosykh V.G., Baev A.A. // Biochemistry. - 1989. - Vol. 54. - P. 1894-1903. (Rus.)
11. Buryanov, Ya.I., Baryshev, M.M., Kosykh, V.G., Baev, A.A. // J. Cell Biochem. Suppl. - 1989. - V. 13D. - P. 211.
12. Dedkov V.S., Pogrebnjak V.B. Definition of restriction endonucleases in lysates from separate colonies of bacteria and measurement of activity BamHI, PstI and MspI in the bacteria which have been brought up on a nutritious agar and in a broth. Thesis in: 6-th working meeting under program "Plasmide". - Pushchino -na - Oke. - M., 1981. - P. 50 - 51. (Rus.)
13. Dedkov V.S., Degtyarev S.Kh. // Applied. biochem. - 1991. - Vol. 28. - P. 309 - 313. (Rus.)
14. A management to practical employment on microbiology. / Ed. Egorov N.S. - M., 1995.
15. Bergey's manual of determinative bacteriology. / Ed. Holt J.G., etc.: 9 edition in 2 volumes: Rus. transl. by ed. akad. Russian Academy of Science G.A. Zavarzin, - M., 1997.
16. Maniatis T., Fritsch E.F., Sambrook J., Methods of genetic engineering: Molecular Cloning. - Rus. transl. - Ì., 1984.
17. Gorbunov Ju.A., Zinovev V.V., Ovechkina L.G., etc. // Bioorgan. Chemistry. - 1987. - Vol. 13. - P. 1629 - 1637. (Rus.)
18. Smith H.O., Nathans D.// J. Mol. Biol. 1973. V. 81. P. 419 423.
19. Sanger F., Coulson A.R., Hong G.F. et al.// J. Mol. Biol. 1982. V. 162. P. 729 773.
20. Sutcliffe J.G. // Proc. Natl. Acad. Sci. USA. 1978. V. 75. P. 3737-3741.
21. Sambrook J., Fritsch E.F., Maniatis T. Molecular Cloning. A Laboratory Manual: Second Edition, CSH, USA. - 1989. - V. 3. - Appendix A.9-A.13.
22. Morgan, R., Xiao, J.-P., Xu, S.-Y. // Appl. Environ. Microbiol. - 1998. - V. 64. - P. 3669-3673.