VIROLOGY

Study of Antiviral Activity of Metabolites of a New *Serratia species* K-57 Strain

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The results of studies of a newly isolated *Serratia species* K-57 strain are presented. The strain is characterized by antiviral activity towards human influenza A/Aichi/2/68/H3N2, vaccinia, mouse smallpox, and herpes simplex-2 viruses. The detected characteristics of the strain, including the data on activities on nucleolytic enzymes, recommend it for the development of therapeutic and preventive antiviral drugs.

Key Words: *Serratia* genus nucleases; human influenza A/H3N2 virus; mouse smallpox; vaccinia; herpes simplex-2 viruses

Nucleases exhibit antiviral activity *in vitro*. Ribonucleases were found to exhibit antiviral activity towards RNA-containing viruses (influenza, poliomyelitis, and tick-borne encephalitis viruses), while deoxyribonucleases suppress reproduction of DNA-containing viruses [5,7,9].

Pharmaceutical nuclease preparations are used in clinical practice for the treatment of viral diseases: ribonuclease is used in tick-borne encephalitis, deoxyribonuclease — in herpetic keratitis, acute catarrhal inflammation of the upper airways of adenoviral nature and in herpes-1 and -2 [3,5]. Studies aimed at isolation and characterization of secreted RNases of *Pseudomonas* genus bacteria are in progress [9]. Extracellular *B. cereus* ribonuclease is active towards tobacco mosaic virus [10]. Injection of *B. intermedius* RNase to animals infected with street rabies virus has protected 40% guinea pigs and 50-70% rabbits [1]. Endogluquine, a preparation based on *S. marcescens* strain endonuclease, is used for prevention and therapy

of viral infections of bees [3]. The mechanism of antiviral activity of nucleases is related to hydrolysis of nucleic acids by the endonucleolytic mechanism to mono-, di-, tri-, tetra-, and oligonucleotides [4].

We studied the characteristics of a new strain of *Serratia sp.* K-57, including its nuclease activities, and evaluated the possibility of using its metabolites in the antiviral drug biotechnology.

MATERIALS AND METHODS

Serratia sp. K-57 strain is isolated as a result of screening of natural bacterial isolates for nucleolytic enzymes by means of selection of colonies in selective medium with DNA and Toluidine Blue (Difco) [2]. The strain is deposited at Collection of Bacteria, Bacteriophages, and Fungi, Vector State Research Center of Virology and Biotechnology.

The taxonomic group of the studied bacterium was determined by analyzing its phenotypic characteristics by standard methods [2,8], and genomic identification was performed by studying nucleotide sequences of PCR products corresponding to 16S rRNA gene at the Interinstitutional DNA Sequenc-

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Serratia sp. K-57 strain was cultured in liquid or solid LB medium (pH 7.0-7.2; Difco) at 37°C for 18-24 h. *Serratia sp.* K-57 subcultures were maintained by regular reinoculations in agarized LB medium.

Nonspecific nucleases were detected in the strain as described previously [6]. Phages 1 and T7 DNA (SibEnzyme) served as substrates. Nuclease activities were measured by accumulation of 4% HClO₄ soluble product catalyzing the formation of a unit of acid-soluble products at $\lambda=260$ nm over 1 h at 37°C, was taken for a unit of enzyme activity.

The preparations containing the secreted nucleases were obtained by culturing *Serratia sp.* K-57 strain in liquid LB medium on a KT-104 thermostat shaker at 150rpm and 37°C during 18 h. The resultant culture fluid (CF) was centrifuged at 10,000 rpm for 30 min using a JA-21 centrifuge (Beckman). The supernatant was sterilized by ultrafiltration through 0.45- and 0.2- μ Whatman filters and used for testing as the antiviral drug.

Intracellular nucleases were studied as follows: 1 g wet biomass was resuspended in 4 ml sterile distilled water, processed on an MSE ultrasonic disintegrator (4×30 sec, with 30 sec intervals), and centrifuged at 10,000 rpm in an Eppendorf microcentrifuge. The cell extract free from cell debris was sterilized by ultrafiltration. The resultant preparations were stored at -20°C until use.

For evaluation of the toxicity and antiviral activity of the resultant preparations towards RNA-containing viruses, human A/Aichi/2/68 (H3N2) influenza virus (titer 10^{7.5} lg TCD₅₀/ml) and continuous MDCK culture were used. For evaluation of the toxicity and antiviral activity of cell metabolites towards DNA-containing viruses, we used continuous Vero culture and the following viruses: mouse smallpox (MSP, strain K-1), titer 10^{4.6} lg PFU/ml; vaccinia (VV, strain L-IVP), 10^{4.5} lg PFU/ml; herpes simplex type 2 (HSV-2, strain MS), titer 10^{4.5} lg TCD₅₀/ml (all viruses from Collection of Microorganisms, Vector State Research Center of Virology and Biotechnology). Antiviral activity of the experimental samples was studied according to the prophylactic protocol of analysis.

The data were processed by Student's *t* test and expressed as the arithmetic means (*M*) and standard deviations (*SD*) and 95% confidence intervals (95%CI). The differences were significant at *p*≤0.05.

RESULTS

Bacterial isolate K-57 formed significant zones of nuclease activity in selective medium, and hence, this strain was chosen for further research. Molecular ge-

TABLE 1. Phenotypical Signs of *Serratia* Genus Bacteria and Bacterium Strain K-57

Sign	<i>Serratia</i> Genus	Strain K-57
Gelatin hydrolysis	+	+
Pigment	+/-	—
Catalase	+	+
Amylase	—	—
Oxidase	—	—
Simmons Citrate	+	+
MR	>70%	—
VR	+	+
Indole	—	—
Hydrogen sulfide	—	—
Acid:		
glucose	+	+
maltose	+	+
mannitose	+	+
sucrose	+	+
lactose	—	—
Nitrate reduction	+	+
DNase	+	+
Urease	>70%	—
Phenylalanine deaminase	—	—
Lysine decarboxylase	+	+

Note. "+" positive reaction; "—" negative reaction. MR: methyl red reaction; VR: Voges—Proskauer reaction.

netic analysis of the nucleotide sequence of rRNA 16S showed the appurtenance of bacterium K-57 to *Serratia* genus (99% similarity by fragments of identified sequences). The morphophysiology and biochemistry of bacterium K-57 (Table 1) confirmed its appurtenance to *Serratia* genus. By the sum of phenotypical and genomic characteristics, bacterium K-57 was identified as *Serratia sp.* and called *Serratia sp.* K-57.

Testing of *Serratia sp.* K-57 strain in selective media containing nucleic acids demonstrated its RNase and DNase activities. The results of phages 1 and T7 DNA hydrolysis by *Serratia sp.* K-57 CF-based preparations and cell extract were presented in Figure 1.

Measurement of nuclease activity of *Serratia sp.* K-57 strain showed that the RNase activity of CF was 280.5 U/ml and the activity of cell extract was 270.8 U/ml, or 3070 U/g wet biomass.

The data suggest that *Serratia sp.* K-57 strain exhibits DNase activity and hydrolyzes salmon milt DNA and DNA of phage T7 and phage λ . In addition, preparations of *Serratia sp.* K-57 strain CF, diluted 10- and 100-fold, effectively hydrolyzed substrate DNA.

Electrophoregram of phages λ and T7 DNA hydrolysis by CF-based samples of *Serratia* sp. K-57 clones. a) Hydrolysis of phage T7 DNA by CF preparations from clones of *Serratia* sp. K-57 strain (1-7); 11) phage T7 DNA control. b) Hydrolysis of phage λ DNA by CF preparations from the same clones (12-18); 22) CF preparation from control *S. marcescens* B-10 M-1 strain. c) Hydrolysis of phage 1 DNA by CF preparations from *Serratia* sp. K-57 clones 1-4. *Serratia* sp. K-57 CF preparations were used in dilution 1:10 (1, 3, 5, 7) and 1:100 (2, 4, 6, 8); 13, 14) *S. marcescens* strain B10 M1 CF control; 15) phage 1 DNA control.

The antiviral activity of preparations based on the *Serratia* sp. K-57 CF and cell extract was studied using 10-fold dilutions, exhibiting no toxicity towards the cell cultures used. Studies of antiviral activity towards RNA viruses showed that *Serratia* sp. K-57 CF and cell extract inhibited significantly the multiplication of influenza A/Aichi/2/68 (H3N2) virus in MDCK culture. The indexes of this virus reproduction inhibition (difference between virus titer lg in control (without sample) and experiment) under the effects of experimental samples of CF and cell extract were 2.7 and 2.6 lg, with RNase activities in specimens of

CF and cell extract 41.7 and 380.0 U/ml, respectively (Table 2).

The sample based on *Serratia* sp. K-57 CF reduced significantly the efficiency of multiplication in Vero cells of all DNA genome viruses used in the study (Table 2). The indexes of virus multiplication inhibition in Vero cells under the effect of this sample were 2.4, 2.0, and 2.0 lg for mouse smallpox, vaccinia, and HSV-2 viruses, respectively.

The results recommend strain *Serratia* sp. K-57 as a prospective prophylactic antiviral drug with complex activities.

TABLE 2. Antiviral Activities of *Serratia* sp. K-57 CF and Cell Extract towards RNA and DNA Viruses in Continuous Cell Cultures

Sample	RNase activity, U/ml	Virus titer in MDCK, lg TCD ₅₀ /ml ($M \pm SD$)	Virus titer in Vero cells:		
			lg PFU/ml ($M \pm 95\%CI$)		lg TCD ₅₀ /ml ($M \pm SD$)
			MSV ($k=6$)	VV ($k=6$)	HSV-2 ($n=3$)
CF	414.7	2.4 $\pm$ 0.1*	2.2 $\pm$ 0.2*	2.5 $\pm$ 0.1*	3.3 $\pm$ 0.1*
Cellular extract	380.0	2.5 $\pm$ 0.2*	N.s.	N.s.	N.s.
Virus control	—	5.1 $\pm$ 0.1	4.6 $\pm$ 0.1	4.5 $\pm$ 0.2	5.3 $\pm$ 0.1

Note. N.s.: not studied; k : number of wells with monolayer of cells infected with virus in various dilutions; n : number of experiment repeats; MSV: mouse smallpox virus; VV: vaccinia virus; HSV-2: type 2 herpes simplex virus. * $p < 0.05$ in comparison with respective control.

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