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EXPERIMENTAL ARTICLES

Nuclease Biosynthesis and Growth of *Serratia marcescens* in the Presence of 2-(*p*-Aminobenzenesulfonamide)-thiazole

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Abstract—The biosynthesis of nuclease in *Serratia marcescens* has been studied under the conditions of purine synthesis inhibition with 2-(*p*-aminobenzenesulfonamide)-thiazole. The addition of this sulfonamide to *S. marcescens* at different growth stages is found to inhibit both culture growth and nuclease synthesis.

Key words: nuclease Sma nuc, *Serratia marcescens*, biosynthesis, sulfonamide, 2-(*p*-aminobenzenesulfonamide)-thiazole.

The endonuclease (EC 3.1.30.2) of the gram-negative enterobacterium *Serratia marcescens* (also known as Sma nuc) is one of the extracellular hydrolases synthesized by this bacterium. Its biochemical properties, structure, mechanism of action, and amino acid homology to other pro- and eukaryotic nucleases have been comprehensively studied, although the biosynthesis of this enzyme is as yet poorly understood [1–3]. Sma nuc is synthesized as two isoforms: Sm1 and Sm2 [4]. There are two peaks of nuclease activity in the culture liquid of *S. marcescens* [5]. Biosynthesis of this nuclease depends on the composition of the nutrient medium and the aeration level [6], but not on the presence of nucleic acids or glucose in the medium [7]. Its biosynthesis is enhanced in the presence of mitomycin C, which hinders the separation of complementary DNA strands, and nalidixic acid, which interacts with the A subunit of DNA gyrase [7, 8]. Although the mechanisms of action of these antibiotics differ, the final result of the action of either antibiotic is the inhibition of DNA replication. This finding suggests that the inhibition of purine biosynthesis can also enhance the biosynthesis of the nuclease, as this inhibition hinders DNA replication.

The aim of this work was to investigate the above suggestion. To accomplish this, we selected the most efficient sulfonamide (as sulfonamides are well known as inhibitors of purine biosynthesis in prokaryotes [9]), determined the most susceptible *S. marcescens* strain, and found the bacteriostatic concentration of the most efficient sulfonamide. Then, the special features of *S. marcescens* growth and nuclease synthesis were studied by adding the sulfonamide to bacterial cultures in different growth phases. In order to confirm the identity of Sma nuc and the newly synthesized nuclease, we compared their molecular mass, charge, and stability at high temperatures and different pH values.

MATERIALS AND METHODS

The experiments were carried out with two *Serratia marcescens* strains, 28 and 24, obtained from D.V. Yusupova (Kazan State University) and two strains, Sm6 and W1050, that were kindly provided by M. Benedik (the University of Houston, United States).

In order to select the most active nuclease producer, the strains were plated onto 2% nutrient agar supplemented with 0.2% DNA or RNA and 8% methyl green (ICN, United States). The activity of the extracellular nuclease was evaluated from the diameter of the changed color zones that formed around colonies grown in the course of plate incubation at 30°C for 24 h.

The basal medium for *S. marcescens* cultivation contained (g/l) NaCl, 4.7; NH_4Cl, 1.1; Na_2SO_4, 0.4; $MgCl_2$, 0.95; $K_2HPO_4 \cdot 3H_2O$, 2.8; glucose, 5; casein hydrolysate, 1; and yeast extract, 3; *p*-Aminobenzenesulfonamide, 2-(*p*-aminobenzenesulfonamide)-thiazole (2-pABST), and 2-(*p*-phthalylaminobenzenesulfonamide)-thiazole were dissolved according to the method described by Mashkovskii [10], and the pH of the solutions was adjusted to a neutral value with either 0.1 M NaOH or 0.1 M HCl.

In order to select the most efficient sulfonamide and determine its bacteriostatic concentration, the strains were cultivated at 30°C for 24 h both with and without a sulfonamide. In the former case, the sulfonamide concentration was varied from 0.0001 to 1%. The inoculum consisted of cells washed off from nutrient agar with a fresh liquid medium. The cultivation medium was inoculated to give an optical density of 0.05.

The characteristics of bacterial growth and nuclease synthesis in the presence of 2-pABST were studied by

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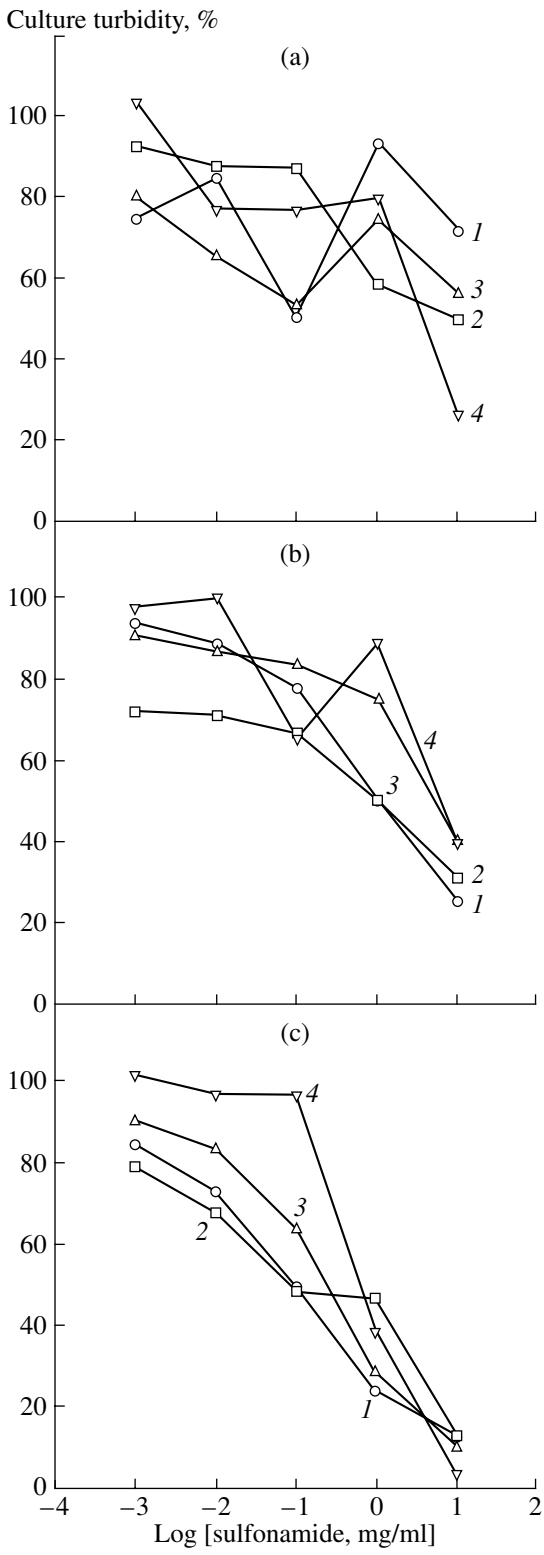

Fig. 1. The effect of various concentrations of (a) *p*-aminobenzenesulfonamide, (b) 2-(*p*-phthalylaminobenzenesulfonamide)-thiazole, and (c) 2-pABST on the culture turbidity of *S. marcescens* strains 24 (curves *1*), 28 (curves *2*), Sm6 (curves *3*), and W1050 (curves *4*). Culture turbidity in the absence of sulfonamides was taken to be 100%.

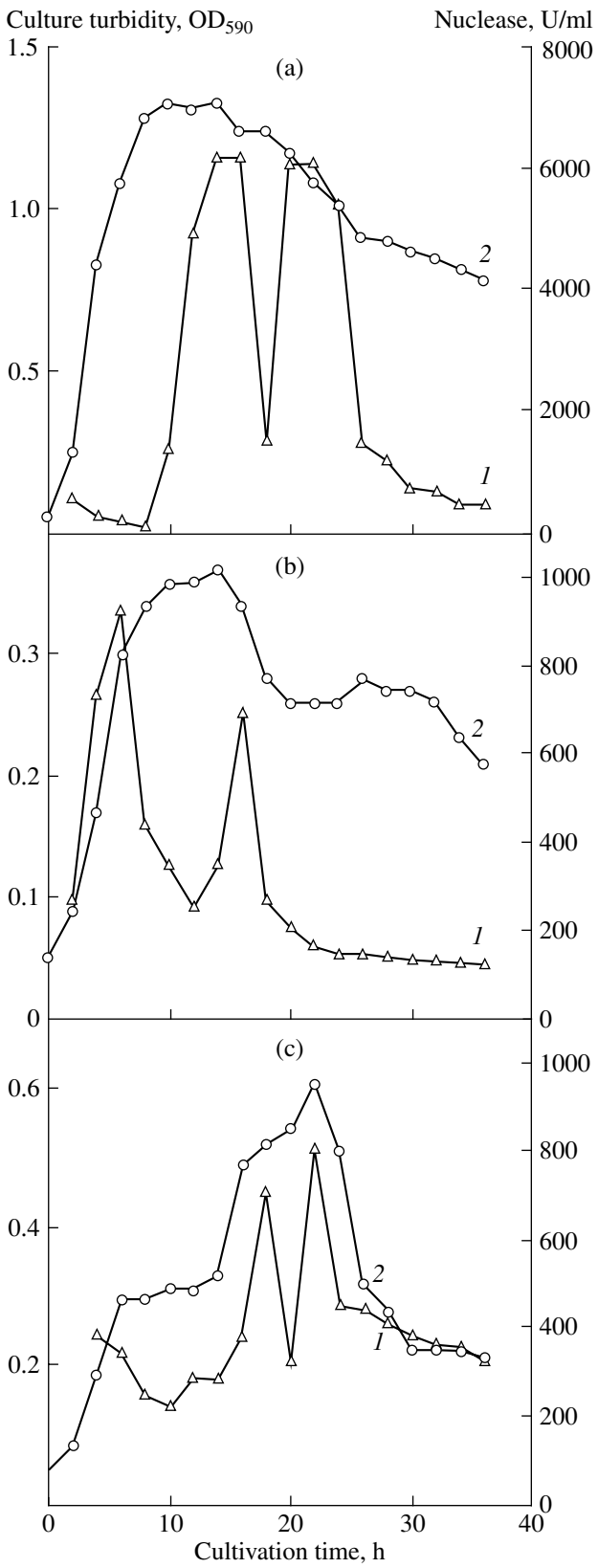

Fig. 2. Dynamics of (*1*) nuclease synthesis and (*2*) growth of *S. marcescens* W1050 without 2-pABST (a) and with 2-pABST added to the medium before inoculation (b) and 2 h after inoculation (c).

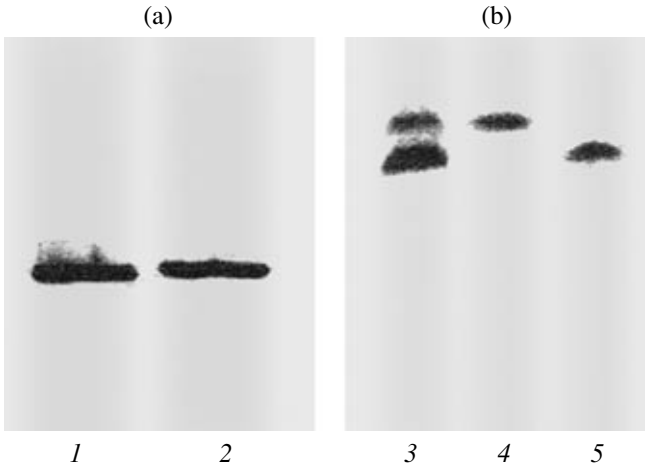

Fig. 3. Electrophoresis of *S. marcescens* nucleases in 15% PAAG under (a) denaturing and (b) nondenaturing conditions. Lanes: (*1, 3*) nuclease synthesized in the presence of 2-pABST, (*2*) Sma nuc, (*4*) Sm1, and (*5*) Sm2.

incubating the strains at 37°C on a shaker (200 rpm) for 36 h. 2-pABST was added immediately before the inoculation or 2 h afterwards. The inoculum was a 12-h culture grown in the liquid medium without 2-pABST.

Bacterial growth was monitored nephelometrically by measuring the optical density of the culture at 590 nm with a KFK-2 photoelectrocolorimeter in a 3.55-mm pathlength cuvette. The nuclease activity in the culture liquid was measured using an acid precipitation method [11]. For this purpose, the cells were removed by centrifugation at 7000 *g* for 15 min. The productivity of the strains was calculated as the ratio of their nuclease activity to the optical density of the culture.

The nuclease was isolated by ion-exchange chromatography [12]. Nondenaturing and denaturing electrophoreses were carried out in 15% PAAG at pH 8.3 [13] using the preparations of Sma nuc, Sm1, and Sm2 [4] as molecular weight markers. The nuclease bands were visualized by contact blotting of the developed PAAG and agarose plates containing nucleic acids [14].

The nuclease productivity of *S. marcescens* W1050 without 2-pABST (column 1) and with 2-pABST added to the medium before inoculation (column 2) and 2 h after inoculation (column 3)

Cultivation time, h	Productivity, arb. units		
	1	2	3
2	2364.83	3026.768	–
4	334.03	4333.33	2029.209
6	185.323	3082.22	1161.346
8	75.5	1281.3*	825.16
10	1043.97	970	697.8741
12	3806.708	702	921.506
12	3912.74	704.763	842.499
14	4623.01*	902.545	763.265
16	4949.61	2046.5	1346.242*
18	1210.14	966.25*	588.3445
20	5194.58*	794.79	1314.536*
22	5544.33	641	877.923
24	5333.99	568.8	1392.63
26	1592.3	517.65	1689.553
28	1314.07	513.42	1663.579
30	850.38	487.809	1606.059
32	806.758	492.4	1577.234
34	581.8984	544.29	1577.234
36	599.9974	576.19	1555

Note: The asterisks * mark the extracellular nuclease activity peaks.

RESULTS AND DISCUSSION

At a concentration of 1%, all three of the sulfonamides studied, *p*-aminobenzenesulfonamide, 2-pABST, and 2-(*p*-phthalylaminobenzenesulfonamide)-thiazole, reduced the growth of *S. marcescens* strains 28, 24, Sm6, and W1050 by approximately two times (Fig. 1). At concentrations of 0.0001–0.001%, the sulfonamides affected bacterial growth similarly. Moreover, the effect of *p*-aminobenzenesulfonamide on bacterial growth did not depend on its concentration, which was probably due to the poor solubility of this sulfonamide in the cultivation medium. The remaining experiments were carried out with *S. marcescens* strain W1050 and 2-pABST at concentrations of 0.1–1%. 2-pABST was chosen because it is the strongest inhibitor of bacterial growth, and strain W1050 was chosen due to the fact that it has the highest nuclease activity, which was indicated with a special indicator medium (data not presented).

The dynamics of the nuclease activity during the growth of strain W1050 in the absence and presence of 0.1% 2-pABST is shown in Fig. 2. If 2-pABST was added before the medium had been inoculated (Fig. 2b), it did not influence the duration of the growth phases but diminished culture turbidity (i.e., the accumulated biomass). However, if 2-pABST was added at the early exponential growth phase (after the second hour of cultivation), it extended the trophophase by about 2.5 times (Fig. 2c). In both cases, the optical density of the culture decreased, and the growth dynamics was stepwise, like that of a synchronous culture.

The curves describing the extracellular nuclease activity had two peaks irrespective of whether 2-pABST was added or not, although its presence diminished the nuclease activity and shifted at least one of the nuclease peaks relative to the growth curve. If 2-pABST was added before the inoculation, both nuclease peaks shifted from the late stationary phase to the phase of growth retardation. If 2-pABST was added 2 h after the inoculation, the first nuclease peak shifted in a similar way, whereas the second peak shifted insignificantly.

The effect of 2-pABST on the nuclease productivity of strain W1050 is shown in the table. In general, 2-pABST decreased the strain productivity at any growth point, the decrease being the greatest (severalfold) in the peaks indicating extracellular nuclease activity.

The nuclease synthesized in the presence of 2-pABST was isolated and purified to an apparent homogeneity (Fig. 3a). This enzyme was found to have an identical molecular mass to Sma nuc (Fig. 3a, lanes *1* and *2*). The nondenaturing electrophoresis of the newly synthesized nuclease, combined with a zymogram analysis (data not presented), showed, in its identity, isoforms Sm1 and Sm2 in relation to the charge of the protein components [4]. The nuclease synthesized in the presence of the sulfonamide contained both isoforms.

The thermal inactivation of the nuclease synthesized in the presence of 2-pABST was similar to that of Sma nuc [13], suggesting that these two nucleases are identical in their biochemical properties. The thermal stability of the nuclease hardly changed in the presence of a 1000-fold excess of magnesium ions. This phenomenon will be described in detail in a forthcoming paper.

Thus, Sma nuc and the nuclease synthesized in the presence of 2-pABST are probably identical in relation to their molecular mass, charge, isoforms, and thermal inactivation. The presence of 2-pABST in the cultivation medium inhibits bacterial growth, suppresses nuclease biosynthesis, and decreases the productivity of a nuclease-producing strain.

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