Note

Distribution of Highly Specific Arylamidase Activities in Bacteria

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There are numerous papers on the application of proteolytic enzymes of microbial origin not only in the medical field but also in food, leather, detergent, and many other industrial fields. Among these enzymes, highly specific ones have attracted great attention in the basic research field as a useful tool for separation, structure analysis, and modification of proteins and peptides. However, systematic studies on the distribution of highly specific peptidases in bacteria have been scarce. We have tried to obtain such peptidases by screening bacteria which can hydrolyze only one substrate out of various amino acid \( p \)-nitroanilides and amino acid 2-naphthylamides, though arylamidase is not always a peptidase and vice versa.

This paper deals with the distribution of highly specific arylamidase activity in bacteria and the close relationship among productivity of such enzymes, cellular localization of the activity, and taxonomical properties of the producer bacteria.

Seventeen strains of gram positive bacteria in Genera Arthrobacter, Bacillus, Brevibacterium, Corynebacterium, Micrococcus, Nocardia, Mycobacterium, and Staphylococcus, and 26 strains of gram negative bacteria in Genera Achromobacter, Aeromonas, Alcaligenes, Azotobacter, Enterobacter, Erwinia, Escherichia, Proteus, Pseudomonas, Serratia, and Xanthomonas were obtained from the IAM Culture Collection of our institute. One thousand and seventy-two strains of soil bacteria and 531 strains of sea water bacteria were newly isolated from the Kanto District in the spring of 1980, by using a conventional smear method with tryptosoy agar plates (in the case of sea water bacteria, the medium was supplemented with 3\% NaCl). These bacteria were inoculated into a medium (pH 7.0) containing 3\% peptone, 0.5\% yeast extract, and 0.5\% NaCl (in the case of sea water bacteria, 3\%) and cultured at 30°C for 20 hr with shaking. The culture broths were directly used as samples for amidase assays. A drop of culture broth was mixed on a microtiter plate with a drop each of 1 mg/ml solutions of the substrates listed in Fig. 1 in 0.05m phosphate buffer, pH 7.0, and incubated at 30°C for 1 hr. \( p \)-Nitroaniline liberated from amino acid \( p \)-nitroanilides was directly detected with the naked eye and 2-naphthylamine from amino acid 2-naphthylamides was detected in a similar manner after successive addition of a drop of 1 N HCl, a drop of 0.1\% Na nitrite solution, a drop of 0.5\% ammonium sulfamate solution, and a drop of 0.5 mg/ml \( N \)-(1-naphthyl)ethylenediamine diHCl solution to the reaction mixture.

Figure 1 shows various arylamidase activities present in bacterial culture broths. In general, alanine \( p \)-nitroanilide (Ala-pNA), Leu-pNA, alanine 2-naphthylamide (Ala-2-NNap), and Lys-2-NNap were hydrolyzed by most bac-

![Graph showing the hydrolysis of amino acid arylamidases by bacterial cultures.](image-url)
teria examined, whereas Cys-di-2-NNap, Tyr-2-NNap, and Trp-2-NNap were rather resistant to bacterial enzymes. Soil bacteria and sea water bacteria showed a difference between each other in the enzyme specificity for Ala-2-NNap and Asp-2-NNap.

Figure 2 shows the number of substrates hydrolyzed by a bacterium. In most cases, 4 to 5 substrates were hydrolyzed by one bacterium. Under the conditions employed, 11% of the IAM strains and 3~5% of the new isolates from natural sources showed no amidase activity with the substrates examined. Bacteria which hydrolyzed only one substrate, contrary to expectation, were many; about 7% of the IAM and soil bacteria, and 2% of the sea water bacteria.

Among them, 24 strains showed an increase in optical density at 410 nm of more than 0.5 when 0.1 ml of culture broth was mixed with 0.2 mg substrate in 1.0 ml of 0.05 m
phosphate buffer (pH 7.0) and incubated at 30°C for 10 min. As shown in Table I, such bacteria could be obtained only from soil, and the substrates hydrolyzed specifically were only leucine and γ-glutamyl-pNAs.

Taxonomical properties of these bacteria are shown in Table II. All of them were gram positive rods and formed thermostable endospores, indicating that they belong to Genus Bacillus. Cellular localization of the amidase activity was different between them, according to the assay of enzyme activity in the supernatants of culture broth and washed cells obtained by centrifugation at 10,000 × g for 10 min; some were extracellular, some were intracellular, and some were observed both inside and outside the cells. Such enzyme localization was also dependent on taxonomical properties of the producer bacteria such as oxidase, VP reaction, and/or motility.

These supernatants and the cells showing Leu-pNA amidase activity were able to hydrolyze leucylglycine and leucylglycylglycine, but not Ala-pNA or Ala-2-NNap; those showing γ-Glu-pNA amidase activity hydrolyzed glutathione and l-glutamine, but not l-asparagine. Among bacteria, leucine arylamidases were reported to be present in Aeromonas proteolytica,9) Bacillus stearothermophilus,10) B. subtilis,11) and Clostridium histolyticum,12) and γ-glutamyl arylamidases in Pseudomonas aeruginosa13) and some other bacteria.14) The former enzymes, however, also hydrolyzed Ala-pNA and the latter enzymes also hydrolyzed l-asparagin or were produced accompanying other amidases and/or peptidases. For this reason, our amidases and/or their producers are different from the known bacterial amidases and their producers.

The result that the bacteria which produced a single highly specific arylamidase could be obtained from soil rather than from sea water should be due to that few of the sea water bacteria were gram positive (most of them hydrolyzed Ala-pNA, suggesting that they were gram negative15), spore-forming ones. Such an ecologically and taxonomically inclined distribution of highly specific arylamidase producers may suggest the physiological importance of these enzymes in their producer bacteria and make it easier to screen these enzyme-producing bacteria.

REFERENCES