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Note

# Distribution of Bacteria with the Arylsulfate Sulfotransferase Activity

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This study is to predict the possible roles of the arylsulfate sulfotransferase (ASST) in the microorganism. At first we studied the spectrum of a distribution of the ASST enzyme through about 1,300 bacteria and the several selected strains were compared with *Klebsiella* K-36 previously reported in the level of DNA homology using the Southern blot method. From this study, we could predict that this enzyme would not exist in specific bacteria and it might not be a critical enzyme for the life of bacteria.

**Key words :** Arylsulfate sulfotransferase, Screening, 4-Methylumbelliferyl sulfate, Southern blotting, pKASST100

## INTRODUCTION

Bacterial arylsulfate sulfotransferase (ASST) catalyzes the transfer of the sulfate group from phenolic sulfate esters to phenolic acceptor substrates (Kim *et al.* 1991, 1994a). The three arylsulfate sulfotransferase-producing bacteria, *Eubacterium* A-44 (Kim *et al.* 1986), *Klebsiella* K-36 (Kim *et al.* 1992b), and *Haemophilus* K-12 (Lee *et al.* 1995) were isolated until now. This enzyme can sulfate exogenous compounds including sodium picosulfate (Kim *et al.* 1992a), quercetin (Koizume *et al.* 1990), and phenolic antibiotics (Kim *et al.* 1992c). It is assumed that intestinal flora harboring the ASST are involved in the metabolism and detoxification of phenolic compounds. The ASST also catalyzed the sulfation of the tyrosine residue of peptides and proteins such as kyotorphin, enkephalin, cholecystokinin-8, trypsin inhibitor, and insulin (Kim *et al.* 1994b, Kobashi and Kim 1986). In addition, the antithrombin activity of recombinant hirudin, which is sulfated by ASST obtained from *Eubacterium* A-44, was increased about 3.4-fold compared with that of the unsulfated hirudin (Muramatsu *et al.* 1994). The ASST may also be used to introduce the sulfate group in organic synthesis, while this is difficult to do using organic synthetic methods. Recently, we cloned an *astA* gene (Baek *et al.* 1996) from *Klebsiella* K-36 and expressed as a fusion protein (Baek *et al.* 1997) with glutathione S-transferase.

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The purpose of this study is to predict why this protein exists, in the microorganism. As a preliminary experiment, we studied in this paper the spectrum of a distribution of the ASST enzyme and the selected strains were compared with *Klebsiella* K-36 in the level of DNA homology using the Southern blot method.

## MATERIALS AND METHODS

### The first and second screening

For the first screening of the ASST enzyme activity, about 1,300 bacteria maintained in our laboratory were streaked with a loop on the agar plate media containing 0.1 mM 4-methylumbelliferyl sulfate (MUS). These plates were incubated at 37°C for 1-2 days and the plates with fluorescence were selected under UV (320 nm). These selected bacteria were judged as ASST-positive strains. For the second screening, selected microorganisms above were inoculated in the LB broth containing 0.1 mM MUS. The cultured media were harvested and then disrupted by a sonicator. The sonicated suspensions were used as enzyme sources and the enzyme assay was conducted according to previously reported method (Kim *et al.* 1992b).

### Southern blotting

Each total DNA from bacterial strains selected was prepared and digested to completion with the appropriate endonucleases. The DNA samples were then separated on an ethidium-bromide-stained 1% (w/v) agarose gel, blotted to nylon membranes. The plasmid

pKASST100 (Baek *et al.* 1996) which contained the *astA* gene from *Klebsiella* K-36 was digested to completion with *Bam*HI and *Eco*RV to release a 560 bp fragment including some part of the *astA*. A 560 bp *Bam*HI-*Eco*RV fragment of pKASST100 was purified from an agarose gel and labelled by random-primer extension (New England Biolabs) using [α-$^{32}$P]dATP and the Klenow fragment of *E. coli* polymerase I. The blot was probed overnight at 42°C with the $^{32}$P-labelled pKASST100 fragment and washing was performed twice with 2×SSC, 0.1% SDS at room temperature, followed by washing twice with 0.1×SSC, 0.1% SDS at 52°C (Sambrook *et al.* 1989). The blot was subsequently exposed for 16 h to X-ray film to visualize hybridized DNA fragments.

## RESULTS AND DISCUSSION

We selected (Table I) 29 bacterial strains with a high ASST activity using the first and the second screening methods. These selected bacteria are all Gram(-) strains. In a previously reported study (Baek *et al.* 1996) about *Klebsiella* K-36, it was predicted that the enzyme has a signal peptide placed at a preceding part of the expressed enzyme and may be located at the periplasmic space. From the previous result, the enzyme was likely to be included mainly in Gram(-) which have a developed periplasmic space. In fact, in this study, the high enzyme activity was detected in only Gram(-) bacteria. However, the enzyme was also found in Gram(+) *Eubacterium* A-44 in a previous report (Kim *et al.* 1986). So we can not say that the enzyme belongs to only G(-).

We could think that these selected bacteria may have any similar DNA sequences encoding the ASST enzyme and we conducted the Southern blotting with arbitrarily selected 7 bacterial strains [*Aeromonas hydrophilia* S-853, *Citrobacter freundii* MB4-6446, *Citrobacter freundii* MB4-8242, *Enterobacter amnigenus* AR-37, *E. coli* MB4-6880, *Pseudomonas aeruginosa* 817, *Salmonella typhimurium* ATCC13311]. In the result of Southern blotting (Fig. 1) using a [$^{32}$P] labelled probe from pKASST100, the K-36 *astA* probe hybridized significantly with genomic DNA from *P. aeruginosa* 817 (lane 1), *C. freundii* MB4-6446 (lane 5), *E. coli* MB4-6880 (lane 7), *Enterobacter amnigenus* AR-37 (lane 8), and *C. freundii* MB4-8242 (lane 9). It hybridized less intensely with *A. hydrophilia* S-853 (lane 3), and only

**Table I.** Screening bacteria with the ASST enzyme activity

A) Gram negative bacteria

| Bacteria (No. of bacteria) | the 1st screening[a] | the 2nd screening[a] |
|---|---|---|
| *Acinetobacter* spp. (62) | 15 | 0 |
| *Citrobacter* spp. (25) | 8 | 4 |
| *Enterobacter cloacae* (50) | 5 | 4 |
| *Enterobacter* spp. (56) | 10 | 3 |
| *Escherichia coli* (69) | 14 | 4 |
| *Klebsiella oxytoca* (60) | 10 | 3 |
| *Klebsiella pneumonia* (60) | 15 | 4 |
| *Proteus* spp. (58) | 3 | 0 |
| *Providensia* spp. (38) | 15 | 0 |
| *Pseudomonas aeruginosa* (76) | 10 | 3 |
| Others (216) | 40 | 4 |
| total G(-) (770) | 145 | 29 |

B) Gram positive bacteria

| Bacteria (No. of bacteria) | the 1st screening[a] | the 2nd screening[a] |
|---|---|---|
| Methicillin Resistant *S. aureus* (43) | 0 | 0 |
| *Staphylococcus aureus* (86) | 0 | 0 |
| *Stahpylococcus* spp. (86) | 1 | 0 |
| *Streptococcus* spp. (100) | 4 | 0 |
| Others (200) | 10 | 0 |
| total G (+) (515) | 15 | 0 |

[a]The number of bacterial strains with the ASST activity

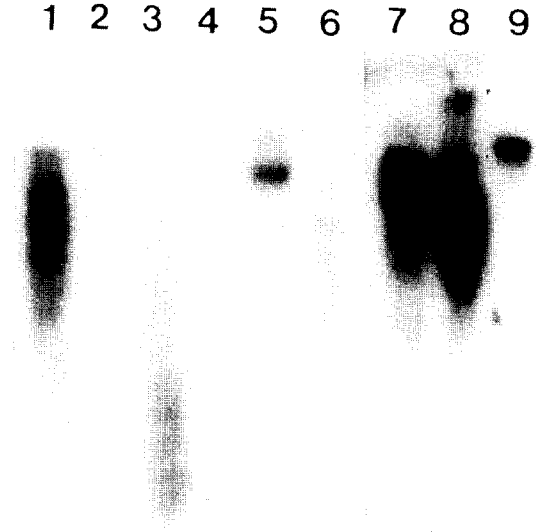


**Fig. 1.** Presence of *astA* homologues in the selected strains detected by Southern blotting. Genomic DNA from the selected strains was digested with a specific restriction enzyme described below respectively, transferred to nylon membrane, and the blot was probed with the $^{32}$P-labelled 560 bp fragment containing part of the *Klebsiella* K-36 *astA* gene. Lanes: 1, *Pseudomonas aeruginosa* 817 (*Hind*III), 2. *Escherichia coli* NM522 (*Bam*HI, Negative control), 3. *Aeromonas hydrophilia* S-853 (*Hind*III), 4. *Eubacterium* A-44 (*Hind*III), 5. *Citobacter freundii* MB4-6446 (*Bam*HI), 6. *Salmonella typhimurium* ATCC13311 (*Hind*III), 7. *Escherichia coli* MB4-6880 (*Hind*III), 8. *Enterobacter amnigenus* AR-37 (*Hind*III), 9. *Citrobacter freundii* MB4-8242 (*Hind*III)

poorly with DNA from *S. typhimurium* ATCC13311 (lane 6). It, however, was not homologous with the DNA from *Eubacterium* A-44 (lane 4). We predicted that the gene encoding an ASST of Gram(-) is significantly different from that of Gram(+). We could guess there may be a conserved region which is shared between these genes from Gram(-). If we find the conserved region it will be helpful for us to locate some active sites. We could not completely exclude the possibility that all bacteria have the *ast* gene in the chromosome but the gene get damaged by unknown mechanism and the enzyme, the product of the gene, may not be expressed. So we also conducted the Southern blotting with the same probe above in several bacteria including the Gram (+) and the Gram (-) which had not shown the enzyme activity. We, however, did not see any positive signals on the X-ray film (data not shown). The result suggests that for the screening of any other microorganism for the detailed study, it is desirable to detect the ASST enzyme activity at first and then to investigate the similarity between the genes from the selected microorganism which will be cloned and sequenced.

From the screening and the Southern blot results, we could understand that the enzyme would not exist in specific bacteria. In other words, there is no specific species or genus having the ASST. At this point, we would like to say that the enzyme is not a critical one for the life of microorganisms. We assumed that the ASST is an ancillary protein which could change toxic materials including phenol into nontoxic materials as a kind of detoxifying enzyme (Jaurin and Grindstrom, 1981) which is located in the periplasmic space. Hereafter, we will study a function of the enzyme through disrupting the gene's structure and observing the changes on the microorganism, for example, the morphology of a colony, the colony size, a certain relationship with other related enzymes, and so on. We will clone the *ast* genes from various bacteria and study the genes in more detail. Especially, we would like to reveal the DNA sequences of the *ast* gene from *Eubacterium* A-44 and compare it with the *ast* genes from Gram(-) bacteria. We would like to clarify the divergence between these bacteria.

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