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The role of AmpR in regulation of L1 and L2 β-lactamases in *Stenotrophomonas maltophilia*

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Abstract

Stenotrophomonas maltophilia is known to produce at least two chromosomal-mediated inducible β-lactamases, L1 and L2. Gene *L2*, which encodes a class A β-lactamase, and the adjacent *ampR* gene form an *ampR*-class A β-lactamase module. L1 belongs to the class B β-lactamase and has no neighbor *ampR*-like regulatory gene. In this study, the *ampR-L2* module from *S. maltophilia* KH was compared with *ampR*-β-lactamase modules from several microorganisms with respect to the AmpR and β-lactamase proteins and the intergenic (IG) region. *S. maltophilia* and *Xanthomonas campestris* showed the most closely phylogenetic relationship among the microorganisms considered. The regulatory role of AmpR towards L1 and L2 was further analyzed. In the absence of an inducer, AmpR acted as an activator for *L1* expression and as a repressor for *L2* expression, whereas AmpR was an activator for both genes in an induced state. In addition, inducibility of *L1* and *L2* genes depended on the presence of AmpR. The *ampR* transcript was weakly and constitutively expressed, but was not autoregulated.

Keywords: *Stenotrophomonas maltophilia*; β-lactamase; AmpR

1. Introduction

β-lactamase production is a major mechanism causing bacterial resistance to β-lactam antibiotics [1]. The genomic module of *ampR* and an inducible β-lactamase gene has been commonly seen in many gram-negative bacteria, in which both genes are divergently transcribed and the intergenic region (IG) between them contains the promoters and the regulatory domains [12]. Two different classes of β-lactamases have been identified in such an *ampR*-β-lactamase module, i.e. AmpC and class A β-lactamase.

Chromosomally encoded *ampR-ampC* systems have been found in *Citrobacter freundii* [18], *Enterobacter cloacae* [13], *Yersinia enterocolitica* [40], *Morganella morganii* [33], *Hafnia alvei* [11], *Ochrobactrum anthropi* [25], *Buttiauxella agrestis* [5], *Serratia marcescens* [22] and *Pseudomonas aeruginosa* [21]. The regulation of chromosomal *ampR-ampC* systems is well documented [15,19,29,44]. In *C. freundii*, induction of *ampR-ampC* is intimately linked to peptidoglycan recycling. There are at least three gene products known to be involved in the induction mechanism: AmpG, AmpD and AmpR. AmpG encodes a transmembrane protein that functions as a permease to transport the cell wall degradation products such as anhMurNAc-tripeptide from the periplasm into the cytoplasm. AmpD encodes a cytosolic *N*-acetyl-anhydromuramyl-L-alanine amidase, which hydrolyzes the anhMurNAc-tripeptide for further recycling. The *ampR* gene encodes an LysR-type transcriptional regulator (LTTR), which controls *ampC* gene transcription. The *ampR-ampC* IG region of *C. freundii* has been characterized. The promoters for *ampR* and *ampC* are located in the IG region in opposite orientation and partially overlap. A consensus T-N11-A LysR-motif is conserved and located in the *ampR* promoter region, resulting in the negative autoregulation of *ampR* [20]. Similar IG architecture is observed in many other *ampR-ampC* modules. Furthermore, AmpR acts as a repressor in

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the absence and as an activator in the presence of the β-lactams [15,18,19]. In addition to the chromosomally encoded *ampR-ampC* systems, many plasmid-born AmpC β-lactamases have been discovered in bacteria [4,6,10,26,31,32], including some clinical isolates [3,9,27,35,42].

Another similar regulatory module, *ampR*-class A β-lactamase, has been identified in *Rhodopseudomonas capsulate* sp108 [7], *E. cloacae* NOR-1 [23], *Proteus vulgaris* B317 [8], *Serratia marcescens* S6 [24], *Burkholderia cepacia* 249 [41], *Citrobacter sedlakii* 2596 [37], *Xanthomonas campestris* [43] and *Stenotrophomonas maltophilia* [14]. The *ampR*-class A β-lactamase modules described so far are all chromosomal. Compared to the *ampR-ampC* module, the IG region architectures of *ampR*-class A β-lactamase modules are more diverse. For example, in *S. marcescens* S6, the promoters of the *ampR* and class A β-lactamase genes exhibit a face-to-face architecture [24]. In addition, the regulatory role of AmpR proteins is different in the different *ampR*-class A β-lactamase modules. AmpR acts as an activator regardless of the presence of an inducer in *E. cloacae* NOR-1 [23] and *S. marcescens* S6 [24], but it acts as a repressor in *B. cepacia* 249 [41].

S. maltophilia, a non-fermenting gram-negative bacillus, is known to produce at least two chromosomally encoded inducible β-lactamases, L1 and L2 [2,36,37]. The corresponding genes *L1* and *L2* and their flanking DNA sequences have been separately cloned and analyzed in our previous study [14]. Recently, AmpR was shown to be a key regulator for inducible expression of *L1* and *L2* [30]. This work aims at identifying the regulatory role of AmpR towards basal *L1* and *L2* gene expression and at testing the autoregulation of AmpR expression. Moreover, the *ampR-ampC* and *ampR*-class A β-lactamase systems are compared and discussed in detail.

2. Materials and methods

2.1. Bacterial strains, plasmids and media

Table 1 shows the bacterial strains and plasmids used in this study. *S. maltophilia* KH is a clinical isolate. The active β-lactamases present in *S. maltophilia* KH are encoded by the *L1* and *L2* genes, as verified by isoelectric focusing electrophoresis after nitrocefin staining (our unpublished data). *Escherichia coli* DH5α and *E. coli* S17-1 were used for general cloning and conjugation experiments, respectively. For the induction experiment, 10 μg/ml of cefuroxime was added.

2.2. Construction of recombinant plasmids pKHR174L2$_{xylE}$, pKHΔRL2, pKHΔL2, pKHΔR, pKHR$_{xylE}$174L2, and pKHΔL1

Fig. 1A shows the restriction enzyme map of the *S. maltophilia* KH *ampR-L2* module (accession No. EU032534). A 1.3-bp *xylE* cassette derived from pTXylE [14] was inserted into the SmaI site in the *ampR* gene and in the SphI site of *L2* in pKHR174L2. The resultant recombinant plasmids, pKHR$_{xylE}$174L2 and pKHR174L2$_{xylE}$, were sequenced to confirm the correct orientation of *xylE* gene. The recombinant plasmids pKHΔRL2 and pKHΔR were derived from pKHR174L2 by deleting a 797-bp StuI and a 468-bp PstI fragment, respectively. The 608-bp SmaI-StuI fragment from pKHR174L2 was ligated into the SmaI-digested pEX18Tc, and the resultant plasmid was further ligated with the 854-bp SphI-HindIII fragment from pKHR174L2, producing pKHΔL2. The correctness of both inserted DNA fragments in pKHΔL2 was checked by DNA sequencing. The intact *L1* gene of *S. maltophilia* KH was amplified by PCR using the paired primers 5′-AAG GAG GCC CAT GCT AGT TT-3′ and 5′-TTC TGA CCG GCA CCC TTC-3′, and cloned into the T-vector (pTKHL1). The recombinant plasmid pKHΔL1 was constructed by a two-step cut-ligation of HindIII-PstI and XbaI-SphI from pTKHL1 to pEX18Tc treated with the same restriction enzymes. Again, DNA sequencing was performed to check the correctness of both insertions.

Table 1
Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or properties
Stenotrophomonas maltophilia	
KH	Wild type, a clinical isolate from Taiwan
KHΔL1	*S. maltophilia* KH isogenic mutant of *L1* gene
KHΔL2	*S. maltophilia* KH isogenic mutant of *L2* gene
KHΔRL2	*S. maltophilia* KH double mutant of *L2* and *ampR* gene; deletion 797 bp StuI-StuI DNA fragment which including partial 5′ terminus of *ampR*, intergenic region of *ampR* and L2, and partial 5′ terminus of *L2*
Escherichia coli	
DH5α	F- Φ80d*lacZ*Δ*M15* Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 hsdR17* (r_k^- m_k^+) *phoA supE44*χ *thi-1 gyrA96 relA1*
S17-1	λ pir + mating strain
Plasmids	
pEX18Tc	*sacB oriT*, Tc^r
pKHR174L2	pEX18Tc vector with a 2.8 kb PCR amplicon containing the partial 3′ terminus of ABC transport protein gene, *ampR* gene, 174-bp IG region, and *L2* gene; Tc^r
pKHR174L2$_{xylE}$	Derived from pKHR174L2, inserting an *xylE* cassette of the same orientation as *L2* gene into SphI site of *ampR* gene; Tc^r
pKHΔL1	pEX18Tc vector with a 298-bp XbaI-SphI DNA fragment of 5′ terminus of *L1* gene and a 336-bp PstI-HindIII DNA fragment of 3′ terminus of *L1* gene, deleting the internal 407-bp DNA fragment of *L1* gene
pKHΔL2	Derived from pKHR174L2, deleting the 107-bp StuI-SphI DNAa fragment; Tc^r
pKHΔR	Derived from pKHR174L2, deleting the 468-bp PstI-PstI DNA fragment; Tc^r
pKHΔRL2	Derived from pKHR174L2, deleting the 797-bp StuI-StuI DNA fragment; Tc^r
pKHR$_{xylE}$174L2	Derived from pKHR174L2, inserting an *xylE* cassette of the same orientation as *ampR* gene into the SmaI site of *ampR* gene; Tc^r

2.3. Construction of isogenic mutants KHΔL2, KHΔRL2, KHΔR, and KHΔL1

Recombinant plasmids pKHΔL2, pKHΔRL2, pKHΔR, and pKHΔL1 were introduced into *S. maltophilia* KH via conjugation to generate isogenic mutants KHΔL2, KHΔRL2,