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Induction of a secretable beta-lactamase requires a long lag time in *Xanthomonas campestris* pv. campestris str. 17

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Abstract

Xanthomonas campestris pv. campestris (Xcc) constitutively expresses penicillinase activity in the absence of an inducer. An *ampR-bla* module is required for the antibiotic resistance phenotype. In this study, we demonstrate that AmpR negatively autoregulates its own expression in a β-lactam-independent manner. In the absence of inducer, *bla* is expressed at a high basal level. Expression of *bla* is inducible by β-lactam, however, with a period. AmpR protein and the LysR-motif located upstream of *bla* promoter are essential for basal expression and induction of *bla*. Most β-lactamase activity is present in the culture medium, suggesting that Bla protein can be secreted by Xcc into the environment.

Keywords: *Xanthomonas campestris* pv. campestris; β-lactamase; *ampR*; *bla*; LysR-motif

1. Introduction

Penicillin was discovered in 1928 and used to treat bacterial infections during World War II. Since then, numerous natural and synthetic β-lactam antibiotics have been introduced into the pharmacotherapeutic market and have been remarkably successful. Unfortunately, overuse and misuse of antibiotics has resulted in development of antibiotic-resistant bacteria. This is one of the most challenging problems in infection control. One of the major mechanisms of β-lactam resistance results from enzymatic hydrolysis of β-lactam by chromosomally encoded β-lactamase in Gram-negative bacteria. The mode of action and transcriptional regulation of chromosomally encoded β-lactamase have been extensively studied. The expression of the chromosomal β-lactamase gene is generally regulated by a divergently transcribed LysR-type transcriptional regulator gene, *ampR*, forming an *ampR-β-lactamase* module. The *ampR-ampC* of *Pseudomonas aeruginosa* and the *ampR*-L2 of *Stenotrophomonas maltophilia* are representative of class C and class A type β-lactamases, respectively (Hanson and Sanders, 1999; Hu et al., 2008).

Expression of *ampC* of *P. aeruginosa* is linked to peptidoglycan recycling (Hanson and Sanders, 1999; Jacoby, 2009; Jones, 1998; Wiedemann et al., 1998). The normally degraded murein debris, anhydromuropeptides, can be transported into the cytoplasm by AmpG (Huang et al., 2010; Zhang et al., 2010). Anhydromuropetides are further processed either by AmpD (Jacobs et al., 1995; Langaee et al., 1998; Lee et al., 2009) or by NagZ (Asgarali et al., 2009; Lindberg et al., 1987) to produce the UDP-MurNAc-pentapeptide or anhMurNAc-peptide, respectively. In the absence of an inducer, UDP-MurNAc-peptapeptide acts as a co-repressor of AmpR to keep expression of *ampC* at a very low basal level (Asgarali et al., 2009; Jacobs et al., 1994, 1995; Kopp et al., 1993; Lindberg et al., 1987; Uehara and Park, 2002; Votsch and Templin, 2000). Accumulated anhMurNAc-peptide, upon treatment with β-lactam, displaces UDP-MurNAc-

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pentapeptide from AmpR, leading to high-level expression of *ampC* (Langaee et al., 1998; Lindberg and Normark, 1987).

S. maltophilia, an important opportunistic pathogen, expresses two chromosomally mediated β-lactamases, L1 and L2, belonging to class B and A enzymes, respectively. The expression of both enzymes is inducible and positively regulated by AmpR divergently transcribed from a gene upstream of *L2* (Hu et al., 2008; Okazaki and Avison, 2008).

Growing evidence suggests that antibiotic resistance genes in pathogenic organisms have been acquired from environmental antibiotic-resistant strains through horizontal gene transfer (Wright, 2010). Understanding the mechanism of antibiotic resistance in environmental microbes is essential in antimicrobial control. All completely sequenced members of the genus *Xanthomona* harbor an *ampR-β-lactamase* module. *Xanthomonas* spp. are soil bacteria and important plant pathogens. *Xanthomonas campestris* pv. campestris (Xcc) is the causal agent of black rot disease in cruciferous plants. In Xcc, an *ampR1-bla* module is attracting our attention. Like the L2 β-lactamase of *S. maltophilia*, a class A type β-lactamase (Bla) encoded by the *bla* gene contributes to penicillinase activity (Weng et al., 1999) and its production requires the AmpR1 protein, a homolog of the AmpR protein (Weng et al., 2004). The predicted promoters of *bla* and *ampR* are overlapped and oppositely oriented (Weng et al., 2004) (Fig. 1A). Distinct from the known inducible *ampR-β-lactamase* module, the *ampR1-bla* module of Xcc is constitutively expressed (Weng et al., 1999).

Since β-lactam antibiotics are seldom used as antibacterial drugs to treat plant infections, intrinsic constitutively expressed Bla must have particular significance other than hydrolyzing the β-lactam treated. In this report, we used Xcc strain Xc17 as a model to study the role of the LysR box and the long-term induction effect of β-lactam in transcription of *bla*, as well as the extracellular secretion phenotype of β-lactamase. We find that intrinsic β−lactamase expression of *Xanthomonas* spp. appears to adapt bacteria to the natural environmental habitat during the process of evolution, which is very different from the clinical setting of clinical isolates.

2. Materials and methods

2.1. Bacterial strains, plasmids, media and growth conditions

Bacterial strains and plasmids used in this study are listed in Table 1. Unless otherwise specified, Luria-Bertani (LB) broth and LB agar plates were employed as media for growth of Xcc and *Escherichia coli* at 28 °C and 37 °C, respectively. Antibiotics added for selection were ampicillin (100 μg/ml), kanamycin (50 μg/ml), gentamicin (50 μg/ml) and tetracycline (10 μg/ml).

2.2. Recombinant DNA techniques

Sequences of primers used in this study are listed in Table 2. Standard methods for DNA manipulation are those described in (Sambrook et al., 1989). Pfu DNA polymerase was purchased from Promega (Madison, WI, USA). Restriction enzymes and T4 DNA ligase were purchased from Takara (Kyoto, Japan). Antibiotics, chemicals and reagents were purchased from Sigma (St. Louis, MO) and E. Merck (Darmstadt, Germany). The insert of all recombinant plasmids used in this work was checked by DNA sequencing.

2.3. Construction of ampR and bla mutants

DNA fragments containing the entire coding sequence of *ampR* (876 bp) and *bla* (1155 bp) of Xc17 were amplified by PCR with primers ampRw-F and ampRw-R, or ampRp-R and blaw-R, and then cloned into pBB (Yang et al., 2009) to obtain pBBampRw and pBBbla, respectively. A gentamicin resistance cassette, excised from pUCGM (Schweizer, 1993), was inserted into the *Sph*I and *Pst*I sites of pBBampRw and pBBbla to obtain suicide plasmids pBBampR::Gm and

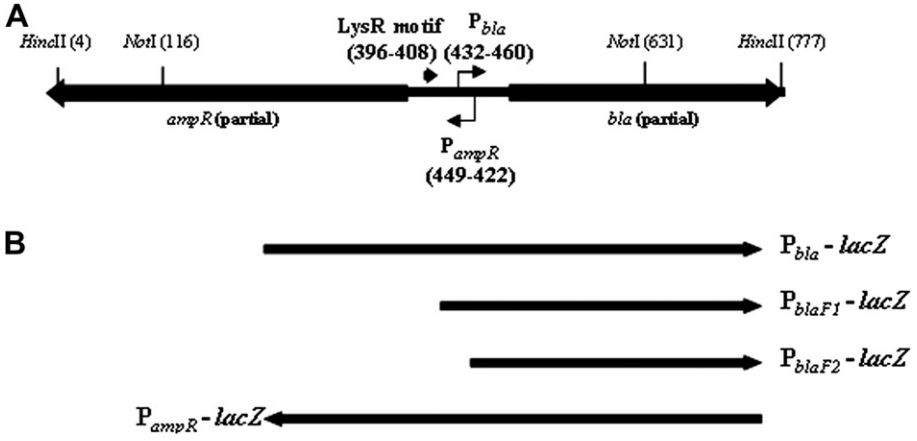

Fig. 1. (A) Genomic organization of the promoters and LysR motif in the intergenic region of the *ampR-bla* module in Xcc. The promoter sequences P_{bla} and P_{ampR} are overlapped and oppositely oriented. (B) Cloning strategy for promoter-*lacZ* transcriptional fusions. The lysR-motif was deleted in P_{blaF1}-*lacZ* fusion and both the LysR motif and the −35 motif of *bla* promoter were deleted in P_{blaF2}-*lacZ* fusion.