ELSEVIER

Biochemistry, genetics and regulation of bacilysin biosynthesis and its significance more than an antibiotic

Research Paper

Gülay Özcengiz[1] and İsmail Öğülür[2]

[1] Department of Biological Sciences and Molecular Biology and Genetics, Middle East Technical University, 06800 Ankara, Turkey
[2] Marmara University, Research and Training Hospital, Division of Pediatric Allergy and Immunology, 34899 Istanbul, Turkey

Bacillus subtilis **has the capacity to produce more than two dozen bioactive compounds with an amazing variety of chemical structures. Among them, bacilysin is a non-ribosomally synthesized dipeptide antibiotic consisting of L-alanine residue at the N terminus and a non-proteinogenic amino acid, L-anticapsin, at the C terminus. In spite of its simple structure, it is active against a wide range of bacteria and fungi. As a potent antimicrobial agent, we briefly review the biochemistry and genetics as well as the regulation of bacilysin biosynthesis within the frame of peptide pheromones-based control of secondary activities. Biological functions of bacilysin in the producer *B. subtilis* beyond its antimicrobial activity as well as potential biotechnological use of the biosynthetic enzyme L-amino acid ligase (Lal) are also discussed.**

General

The potential of *Bacillus subtilis* to produce more than two dozen bioactive compounds has been recognized for over 50 years with low molecular weight, bioactive peptides constituting the majority. Such peptides have rigid, hydrophobic and/or cyclic components and unusual molecules, like D-amino acids [1]. In principle, there are two distinct pathways leading to the combination of such non-proteinaceous constituents [2]: (i) the non-ribosomal peptides are synthesized by non-ribosomal peptide synthetases (NRPSs), which are large multienzymes catalyzing a series of reactions explained by 'multicarrier thiotemplate model', and (ii) the linear precursor peptides synthesized ribosomally are modified post-translationally and processed proteolytically. The non-ribosomal synthesis of peptide antibiotics is commonly seen in bacteria and fungi [3–5]. The NRPSs with modular catalytic domains do catalyze all steps in peptide biosynthesis involving the selection and serial condensation of amino acid residues.

Bacilysin, the simplest peptide antibiotic known, was discovered in as early as 1946 as an antibiotic synthesized by a strain of *B. subtilis* that causes partial lysis of growing cultures of *Staphylococcus aureus* [6]. In the same year, it was independently reported by Foster and Woodruff with the name bacillin, as an antibiotic formed by *B. subtilis* in synthetic medium with an action antagonized by complex media, blood and crude materials [7]. In late 1940s, bacilysin from *B. subtilis* A14 was shown to be a very heat stable bactericidal agent unextractable with organic solvents [8]. The production of bacilysin and its isolation in low yields from culture filtrates of A14 strain was first reported in 1965 [9]. The molecule was described as $C_{12}H_{18}N_2O_5$ with a molecular weight of 270 Da. Its hydrolysis by means of acid treatment yielded l-alanine and an uncharacterized L-amino acid like a tyrosine derivative [10]. Walker and Abraham isolated bacilysin and its terminal epoxy amino acid in improved yields from the culture fluids and by the application of physicochemical methods, assigned its exact chemical structure as L-alanyl-β-(2,3-epoxycyclohexanonyl)-L-alanine [11,12]. The C-terminal amino acid was identical to anticapsin produced by *Streptomyces griseoplanus* which inhibits the formation of hyaluronic acid capsule of group A streptococci [13,14], and thus named anticapsin (Fig. 1b). On the other hand, the dipeptide antibiotic tetaine produced by *Bacillus pumilus* B-180 [15] was reported as chemically and physically identical to bacilysin [16]. Atsumi et al. found bacillin,

Corresponding author: Özcengiz, G. (ozcengiz@metu.edu.tr)

http://dx.doi.org/10.1016/j.nbt.2015.01.006
1871-6784/

Please cite this article in press as: Özcengiz, G., and Öğülür, I., Biochemistry, genetics and regulation of bacilysin biosynthesis and its significance more than an antibiotic, New Biotechnol. (2015), http://dx.doi.org/10.1016/j.nbt.2015.01.006

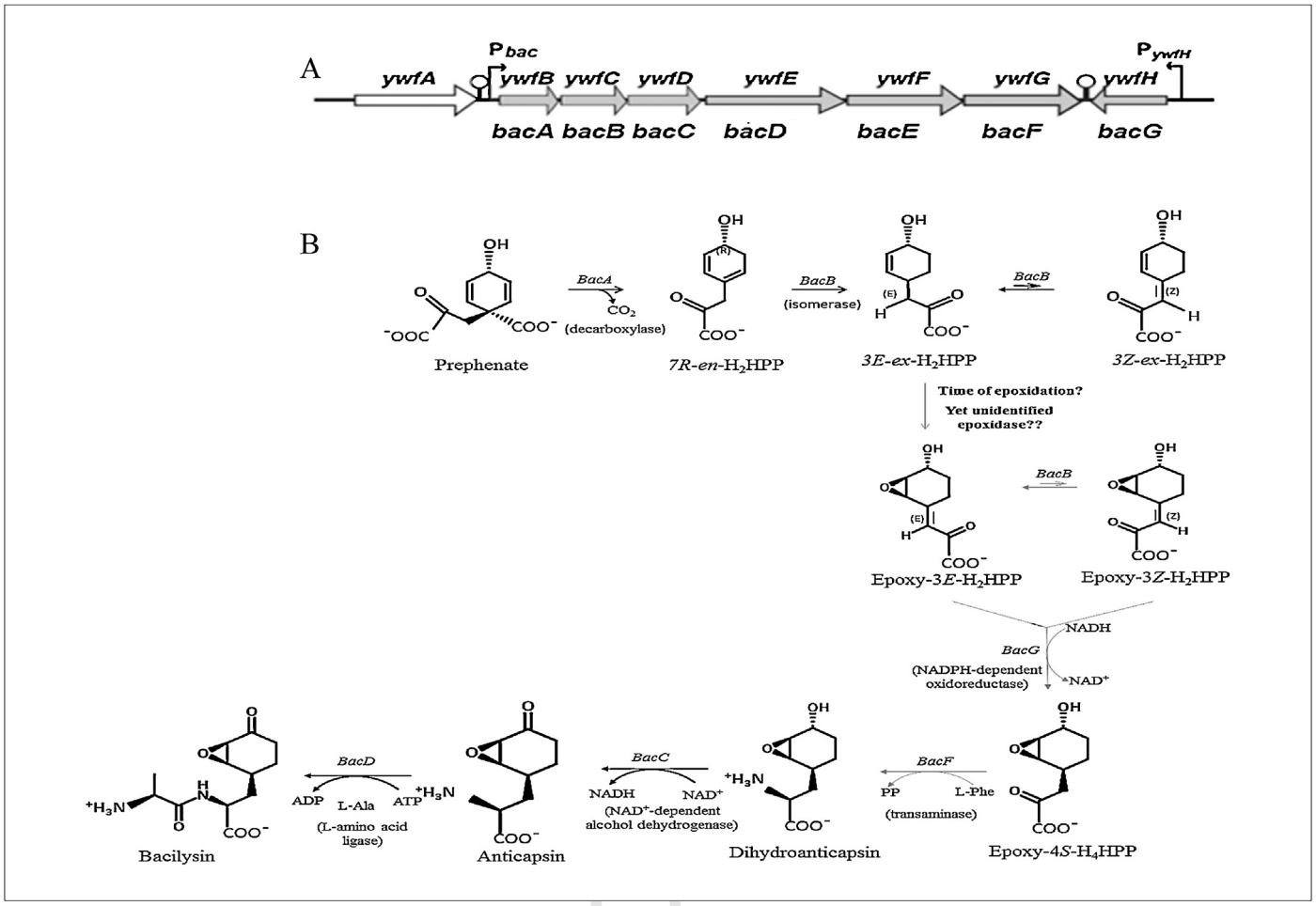

FIGURE 1

(a) Organization of the bacilysin biosynthetic gene cluster *ywfABCDEFG* renamed as *bacABCDE* in *Bacillus subtilis* 168. **(b)** Latest proposed scheme of the biosynthetic pathway from prephenate to bacilysin
Adapted from Parker and Walsh [44].

bacilysin and tetaine, all synonymous [17]. By monitoring the appearance of antibiotic activity with the same analytical techniques in the culture fluids of *B. subtilis* A14 [18] and Marburg [19], the researchers verified that both strains produce bacilysin after the stage of exponential growth.

Bacilysin is active against a broad range of bacteria and some fungi like *Candida albicans* [20]. Its antimicrobial action was reversed by glucosamine and N-acetyl glucosamine [20,21], demonstrating that it interferes with the synthesis of glucosamine and consequently with cell wall formation. Antibacterial action of bacilysin mainly relies on its transport into host cells, its hydrolysis by intracellular peptidases to anticapsin and the inhibition by the latter of glucosamine 6-phospate (GlcN6P) synthase [22]. In *S. aureus*, several di- and tripeptides compete with each other and with bacilysin for uptake [23] since there is only a single peptide transport system for all these peptides in this bacterium [24]. This finding explained the former observations that *S. aureus* mutants resistant to bacilysin arise readily, while this is not the case in *Escherichia coli* having both di- and oligopeptide transport systems for bacilysin uptake. Anticapsin is a more powerful inhibitor of GlcN6P synthase in *S. aureus* cell extracts as compared to bacilysin [25,26]. It was clarified by kinetic studies that anticapsin inhibits GlcN6P synthase activity non-competitively with respect to fructose 6-phosphate, but partly competitively with respect to L-glutamine. When the enzyme GlcN6P synthase was incubated with anticapsin, this resulted in a time-dependent and irreversible inhibition. It was then demonstrated that anticapsin is an analogue of glutamine and covalently binds to the enzyme via cross-linking of the epoxy group of anticapsin and a thiol group of the enzyme [27].

Biochemistry and genetics of bacilysin biosynthesis

Like other peptide antibiotics of the genus *Bacillus*, bacilysin had long been thought to be synthesized by a multifunctional non-ribosomal peptide synthetase (NRPS). The first cell-free synthesis of bacilysin was obtained when L-alanine, L-anticapsin, ATP and Mg^{2+} were provided in reaction mixture [28]. L-Alanyl-L alanine dipeptide was also synthesized in the same reaction mixture. Our group reported partial purification of a biosynthetic enzyme fraction (ca. 125 kDa) by fast flow gel permeation followed by anion exchange FPLC [29]. Enzyme characterization studies, overall, showed that bacilysin biosynthesis does not fit to the general mechanisms of NRPSs. The ATP-PPi exchange reaction of amino acid activation was found to occur with L-alanine, but not with anticapsin. The latter was not activated as an amino acid phosphate, either. In another study, *in vitro* reaction mixtures were

Please cite this article in press as: Özcengiz, G., and Öğülür, I., Biochemistry, genetics and regulation of bacilysin biosynthesis and its significance more than an antibiotic, New Biotechnol. (2015), http://dx.doi.org/10.1016/j.nbt.2015.01.006