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Biotechnological advances on Penicillin G acylase: Pharmaceutical implications, unique expression mechanism and production strategies

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ABSTRACT

In light of unrestricted use of first-generation penicillins, these antibiotics are now superseded by their semi-synthetic counterparts for augmented antibiosis. Traditional penicillin chemistry involves the use of hazardous chemicals and harsh reaction conditions for the production of semisynthetic derivatives and, therefore, is being displaced by the biosynthetic platform using enzymatic transformations. Penicillin G acylase (PGA) is one of the most relevant and widely used biocatalysts for the industrial production of β-lactam semisynthetic antibiotics. Accordingly, considerable genetic and biochemical engineering strategies have been devoted towards PGA applications. This article provides a state-of-the-art review in recent biotechnological advances associated with PGA, particularly in the production technologies with an emphasis on using the *Escherichia coli* expression platform.

Contents

Abbreviations: 6-APA, 6-aminopenicillianic acid; 7-ACA, 7-aminocephalosporanic acid; 7-ADCA, 7-amino-desacetoxycephalonsporic acid; AA, amino acid; AfPGA, PGA from *A. faecalis*; βLA, β-lactam antibiotics; BRP, bacteroicin release protein; CRP, cAMP receptor protein; D-(−)-PGA, D-(−)-phenylglycine amide; D-(−)-PGM, D-(−)-phenylglycine methyl ester; D-(−)-HPGA, D-(−)-4-hydroxyphenylglycine amide; D-(−)-HPGM, D-(−)-4-hydroxyphenylglycine methyl ester; DNA, deoxyribonucleic acid; DO, dissolved oxygen; DAO, D-amino acid oxidase; EcPGA, PGA from *E. coli*; GA, glutaryl acylase; HSP, heat-shock protein; IM, inner membrane; IPTG, isopropyl-β-D-thio-galactoside; KcPGA, PGA from *K. cryocrescens*; LL, leaderless; mRNA, messenger-RNA; Ntn, N-terminal nucleophilic; nt, nucleotides; OM, outer membrane; PAA, phenylacetic acid; PEG, polyethylene glycol; PenG, penicillin G; PG, peptidoglycan; PGA, penicillin G acylase; PrPGA, PGA from *P. rettgeri*; PVA, penicillin V acylase; RBS, ribosome binding site; RNA, ribonucleic acid; SSC, semi-synthetic cephalosporins; SSP, semi-synthetic penicillin; (S/H), synthesis/hydrolysis; Tat, Twin-Arg translocation; TCA, tricarboxylic acid; TF, trigger factor; TFF-AMEC, tangential flow filtration anion-exchange membrane chromatography; tRNA, transfer-RNA; TtPGA, PGA from *T. thermophiles*.

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1. Introduction

Penicillin is the first β-lactam antibiotic (βLA) to be discovered and, approximately 80 years later, is still one of the most common antibiotics in terms of annual bulk production (~3×10^7 kg/year), annual sales (~$15 billion), and market share (~65% of the total antibiotic market) (Chandel et al., 2008; Parmar et al., 2000; Peñalva et al., 1998). However, the unfettered use of first-generation penicillins (i.e. penicillin G and penicillin V) in the second half of the 20th century led to the development of many penicillin-resistant pathogens. Consequently, only a small fraction of penicillins produced today are used for therapeutic purposes, whereas the majority are used as raw materials for the production of semisynthetic penicillins (SSPs) (e.g. amoxicillin and ampicillin) to further augment the potency of penicillins and broaden their antimicrobial range (Bush, 2007; Parmar et al., 2000). In comparison to their first-generation counterparts, SSPs are engineered to confer novel properties, such as an improved side effect profile, lower toxicity, and superior pharmacokinetics (Chandel et al., 2008; Parmar et al., 2000; Peñalva et al., 1998). SSPs are commonly produced in a two-step fashion (Fig. 1). First, bulk penicillins are transformed into 6-aminopenicillianic acid (6-APA) either chemically or enzymatically. Next, 6-APA is further processed into SSPs enzymatically by condensation with the amide or ester of D-(−)-4-hydroxyphenylglycine and D-(−)-phenylglycine derivatives, respectively (Bruggink et al., 1998). On the other hand, upon expanding the 5-membered thiazolidine ring (which is fused to the β-lactam ring) to a 6-membered thiazine ring, penicillins can be converted to cephalosporin G and then another intermediate of 7-amino-desacetoxycephalonsporic acid (7-ADCA) for subsequent enzymatic production of semisynthetic cephalosporins (SSCs). Alternatively, SSCs can be produced using the raw material of cephalosporin C via another intermediate of 7-aminocephalosporanic acid (7-ACA) (Bruggink et al., 1998). A schematic overview of chemical and enzymatic reactions for the production of a selection of semisynthetic β-lactam antibiotics is presented in Fig. 1.

Given that 6-APA is the key intermediate for the production of SSPs, its production technology and availability directly impact the stability of the world's antibiotic markets. Accordingly, to meet the demand of bulk SSPs, continual effort has been made to improve the scalability, economics, and efficacy of the 6-APA production platform. Traditionally, 6-APA was produced through a laborious yet effective chemical process, in which penicillins were hydrolyzed through the use of hazardous chemicals and solvents, such as trimethylchlorosilane, phosphorous pentachloride, and dichloromethane, at unusually low temperatures (Bruggink et al., 1998). Presently, nearly all bulk penicillins are enzymatically transformed into 6-APA using penicillin acylase (E.C. 3.5.1.11, also known as either penicillin amidase or penicillin amidohydrolase). While the feasibility of this enzymatic approach for the production of 6-APA has been known since the 1950s, it was not economically favorable, primarily due to low conversion yields and high costs of biocatalysts, until its full-scale implementation in the late 1980s. Apart from being an environmentally amicable production process, enzymatic conversions are regio- and stereo-specific, energetically benign, and devoid of undesirable byproducts (Bruggink et al., 1998; Rajendhran and Gunasekaran, 2004). As aforementioned, penicillin acylases may also be used to synthesize a number of SSPs and SSCs by catalyzing the fusion of novel acyl groups with a proper intermediate (i.e. 6-APA, 7-ACA, or 7-ADCA) (Table 1 and Fig. 1).

Broadly, penicillin acylases can be grouped into two classes according to substrate specificity. Type I penicillin acylases hydrolyze penicillin V [thus referred as penicillin V acylase (PVA)], while Type II penicillin acylases hydrolyze penicillin G [thus referred as penicillin G acylase (PGA)]. Moreover, PGA can be further sub-classified into Type IIa specific to an aromatic phenylacetyl moiety and Type IIb specific to an aliphatic moiety (Schmidt, 2010; Sudhakaran et al., 1992). Type IIa PGAs (specifically referred as PGA herein) are the most industrially relevant enzymes and it is estimated that ~85% of enzymatically produced 6-APA (~7650 tons) originates from penicillin G, with the rest from penicillin V (Rajendran et al., 2011; Sudhakaran et al., 1992). While PGA activity has been detected in approximately 40 different microorganisms (including yeast, filamentous fungi, and bacteria), cell factories employed for large-scale production of PGA are limited to a few bacterial platforms with *Escherichia coli* as the major one (Rajendran et al., 2011; Sudhakaran et al., 1992). Being a relatively mature industrial biotechnology, microbial production of PGA, either in native or recombinant hosts, remains riddled with numerous technological issues and limitations. Earlier studies concentrated on bioprocessing and commercial aspects, whereas recent efforts in genetic and protein engineering have been aimed at constructing novel recombinant host/vector systems for PGA overproduction. Herein, we review various novel cellular, molecular, and bioprocessing approaches undertaken to enhance microbial production of PGA.

2. Molecular aspects of PGA

2.1. Gene expression and regulation

Bacterial genes encoding PGAs and their gene expression and regulation mechanisms have been extensively examined, particularly for PGA from *E. coli*. In the native PGA-producing *E. coli* strain of ATCC 11105, phenylacetic acid (PAA) induces the expression of its PGA-encoding gene (*pac*) whereas glucose represses it and the associated regulatory elements have been identified (Radoja et al., 1999). The physiological and metabolic functions of these gene regulations might be associated with the catabolism of carbonaceous aromatic compounds during the organism's 'free-living mode' (Duggleby et al., 1995; Rajendhran and Gunasekaran, 2004). However, regulation of the *pac* gene varies from one organism to another. To exemplify, in *Alcaligenes faecalis*, the expression of *pac* is induced by PAA but not repressed by any carbons (Spence and Ramsden, 2007). On the other hand, the expression of *pac* is also induced by PAA, but repressed by tricarboxylic acid (TCA) cycle intermediates (i.e. succinate, fumarate and malate) in *Providencia rettgeri* (Spence and Ramsden, 2007) and by glucose in *Bacillus megaterium* cultivated with complex media (Pinotti et al., 2000). Apart from PAA induction and catabolite repression, *in vivo* PGA synthesis can be temperature-dependent as well (Deshpande et al., 1994). Based on organisms examined so far, this thermo-regulation appears to be universal in all fungi,