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MINI-REVIEW

# Genome-guided exploration of metabolic features of *Streptomyces peucetius* ATCC 27952: past, current, and prospect

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## Abstract

*Streptomyces peucetius* ATCC 27952 produces two major anthracyclines, doxorubicin (DXR) and daunorubicin (DNR), which are potent chemotherapeutic agents for the treatment of several cancers. In order to gain detailed insight on genetics and biochemistry of the strain, the complete genome was determined and analyzed. The result showed that its complete sequence contains 7187 protein coding genes in a total of 8,023,114 bp, whereas 87% of the genome contributed to the protein coding region. The genomic sequence included 18 rRNA, 66 tRNAs, and 3 non-coding RNAs. In silico studies predicted ~ 68 biosynthetic gene clusters (BCGs) encoding diverse classes of secondary metabolites, including non-ribosomal polyketide synthase (NRPS), polyketide synthase (PKS I, II, and III), terpenes, and others. Detailed analysis of the genome sequence revealed versatile biocatalytic enzymes such as cytochrome P450 (CYP), electron transfer systems (ETS) genes, methyltransferase (MT), glycosyltransferase (GT). In addition, numerous functional genes (transporter gene, SOD, etc.) and regulatory genes (*afsR-sp*, *metK-sp*, etc.) involved in the regulation of secondary metabolites were found. This minireview summarizes the genome-based genome mining (GM) of diverse BCGs and genome exploration (GE) of versatile biocatalytic enzymes, and other enzymes involved in maintenance and regulation of metabolism of *S. peucetius*. The detailed analysis of genome sequence provides critically important knowledge useful in the bioengineering of the strain or harboring catalytically efficient enzymes for biotechnological applications.

**Keywords** *Streptomyces peucetius* ATCC 27952 · Genome mining · Genome exploration · Cytochrome P450 · Methyltransferase · Regulatory genes

## Introduction

*Actinobacteria* includes Gram-positive filamentous soil bacteria with high G+C content (Barka et al. 2016; Thuan et al. 2014; Dhakal et al. 2017b). The genus *Streptomyces* under this phylum are capable of producing a multitude and diversity of bioactive secondary metabolites of great interest to medicine and industry (Čihák et al. 2017; Hopwood 2007; Dhakal and Sohng 2015). *Streptomyces* generally undergo a complex process of morphological development, leading to the formation of such bioactive secondary metabolites under depleted nutrient conditions (Čihák et al. 2017; Chaudhary et al. 2013). Actinomycetes, mostly including *Streptomyces*, are prominent source of approximately 80% of the known antibiotics (macrolide, polyketide, non-ribosomal polyketide, aminoglycoside, etc.) (Bérdy 2005; Dhakal et al. 2017a).

Recent advances in the development of DNA sequence and analysis provide access to huge knowledge about the whole or partial genome structure of diverse actinobacteria. This provides deep insight into their metabolism, biochemical properties, and potential for production of secondary metabolites (Ventura et al. 2007; Ziemert et al. 2016). In particular, sequences are interpreted to obtain a large number of cryptic gene clusters that encode putative natural products, and

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provide the base for further experimental characterizations (Ziemert et al. 2016; Dhakal et al. 2017c).

*S. peucetius* 27952 was obtained as a mutant strain derived from *S. peucetius* ATCC 29050, prominently established as producer of anthracycline as daunorubicin (DNR). *S. peucetius* ATCC 27952 has been characterized as significant producer of two of the major medicinally important anthracyclines: daunorubicin (DNR) and doxorubicin (DXR) (Arcamone et al. 1969). Although a number of organisms (including *S. peucetius* ATCC 29050) are known to produce DNR, *S. peucetius* ATCC 27952 has been the only organism reported to produce DXR (Grein 1987; Arcamone et al. 1997). Besides, *S. peucetius* ATCC 27952 (hereafter *S. peucetius*) also contain much more interesting and valuable mysteries, such as NRPS and PKS biosynthetic gene clusters, CYPs and their metabolic roles, and sugar genes. It is also characterized as a biotechnologically important strain used in biotransformation experiments, or as a source of biocatalytic enzymes for the transformation of pharmaceutically important compounds (Dhakal et al. 2018). Recently, the complete genome of *S. peucetius* was determined and analyzed, which provided deep insight on genetic bases of its biochemistry and metabolism. The genome information provides the base for metabolic engineering of strain for the production of DXR and other potentially bioactive molecules. This information also opens up new avenues for utilizing its biocatalytic enzymes, such as CYPs, MTs, GTs, and other cryptic enzymes for industrial applications (Dhakal et al. 2018). In this review, we describe the genome-guided GM approach for determining diverse BCGs and genomic exploration (GE) of enzymes involved in the production of diverse secondary metabolites, or utilized as efficient biocatalysts.

## Features of *Streptomyces peucetius* genome

Due to the immense potential of *S. peucetius* as a source of diverse secondary metabolites or efficient biocatalytic enzymes, the genomic information was obtained by genome sequencing, and deposited in GeneBank (accession number CP022438). *S. peucetius* ATCC 27952 consists of 8,023,114 bp chromosomal DNA with 70.6% G+C content. Around 87% of genome is involved in the protein coding region, which constitutes 7187 protein coding genes, 18 rRNA operons, and 65 tRNAs. Table 1 compares the chromosomal features of *S. peucetius* ATCC 27952 with several sequenced *Streptomyces* strains, and reveals its genomic diversity.

"Genome mining" is a successful approach for mining the genomic data, and connecting the BCGs to the bioactive compounds. This is facilitated by predictive (bioinformatics tool)

**Table 1** Comparison of the general chromosomal features of *S. peucetius* and other *Streptomyces* species

| No. | Names of species | Total size (bp) | G+C content (%) | Open reading frames | Coding density (%) | Average ORF length (bp) | tRNA | Secondary metabolites clusters | Cytochromes |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *S. peucetius* | 8,023,114 bp | 70.6 | 7588 | 87 | – | 64 | 68 | 23 |
| 2 | *S. coelicolor* | 8,667,507 | 72.12 | 7825 | 88.9 | 991 | 63 | 25 | 18 |
| 3 | *S. avermitilis* | 9,025,608 | 70.7 | 7574 | 86.2 | 1034 | 68 | 30 | 33 |
| 4 | *S. griseus* | 8,545,929 | 72.2 | 7138 | – | – | 66 | 34 | 27 |
| 5 | *S. fulvissimus* | 7,905,758 | 71.5 | 5194 | 88.8 | – | 73 | 32 | – |
| 6 | *S. collinus* | 8,272,925 | 72.6 | 7008 | – | – | 72 | – | – |
| 7 | *S. laurentii* | 8,032,664 | 72.3 | 7452 | – | – | 69 | – | – |
| 8 | *S. scabies* 87.22 | 10,148,695 | 71.5 | 8746 | 86.2 | 1005 | 75 | – | – |
| 9 | *S. leeuwenhoekii* | 8,285,171 | 73 | – | – | – | 65 | 34 | – |
| 10 | *S. ambofaciens* ATCC23877 | 8,303,940 | 72.22 | 7668 | – | – | 70 | – | |
| 11 | *S. globisporus* C-1027 | 7,608,611 | 71.6 | 7078 | | – | 66 | – | – |
| 12 | *Streptomyces peucetius strain NRRL WC-3868* | 952,866 | 71.9 | 7890 | – | – | – | – | – |

– not determined

and confirmative (sophisticated analytical tool) techniques (Ziemert et al. 2016; Dhakal et al. 2017c). The complete sequencing and appropriate annotation have allowed for rational mining of *S. peucetius*, and the prediction of many previously uncharacterized BCGs. Prior to the availability of the genome sequence of *S. peucetius*, compounds of diverse nature, such as polyketides, terpenes, and NRPS, were already characterized as shown in Fig. 1. However, Table 2 shows the identification and classification of probable secondary metabolites from this strain, based on in silico analysis of the complete genome of *S. peucetius* ATCC 27952. Approximately 68 BGGs are predicted to exist on the genome of the strain, which includes diverse compound classes such as PKS, NRPS, terpenes, and siderophores (Dhakal et al. 2018). Although the chemical structures of most of the putative secondary metabolites are unknown, the strategically devised enhancement and isolation techniques targeting particular secondary metabolites (predicted by in silico studies) will definitely assist for getting information about their chemical structure and biological activities.

## BCGs of different secondary metabolites in *S. peucetius*

DXR is most widely used antitumor drug, with broad-spectrum activity (Arnold et al. 2004). However, it is commercially produced from DNR semi-synthetically, as DNR-overproducing strains are generally unable to convert DNR into DXR (Arcamone et al. 1997). Alternatively, they can be isolated from *S. peucetius*, which can produce DXR in reasonable amount. To date, biosynthetic pathways of DXR/DNR have been extensively studied using radical label, gene mutation, and protein expression. Based on these knowledge, current focus is directed on the development of industrial producer strain or bioengineering and media optimization techniques for production of DXR in elevated titer. The details of the biochemical studies, regulation mechanisms, and biological engineering strategies for elevated production of DXR in *S. peucetius* are already reviewed elsewhere (Malla et al. 2010; Niraula et al. 2010a).

A cryptic type III polyketide synthase gene (accession no ABY71276) was predicted to be 1,3,6,8-tetrahydroxynaphthalene (THN) synthase on the basis of in silico analysis and named Sp-RppA. The gene was introduced to *S. lividans* TK24 for heterologous expression, and flaviolin, an auto-oxidized product of THN, was detected as product. In particular, the gene was heterologously expressed in *Escherichia coli* and purified protein was isolated, which was used for enzyme reaction with malonyl-CoA. This enzymatic reaction generated flaviolin as final product (Ghimire et al. 2008). Thus, the biosynthetic mechanism of flaviolin was proposed based on in vivo and in vitro studies as shown in Fig. 2.

Furthermore, another biosynthetic gene cluster encoding for cryptic NRPS was reported in *S. peucetius* genome by the combination of sequence-based genome mining and mass spectrometry. By using tandem mass spectrometry of the obtained product, a cyclic structure with approximate molecular weight of 644.4 Da was deduced for a siderophore peptide. This peptide consisted of several domains in the order of C-A-T-C-A-T-E-C-A-T-C-A-T-C (Park et al. 2013). Later, the siderophore compound was isolated from *S. peucetius* NBRC 14660 (=ATCC 27952), grown in 2 L of iron-deficient media, and its final structure was confirmed based on NMR analysis. Unlike previously hypothesized structure,

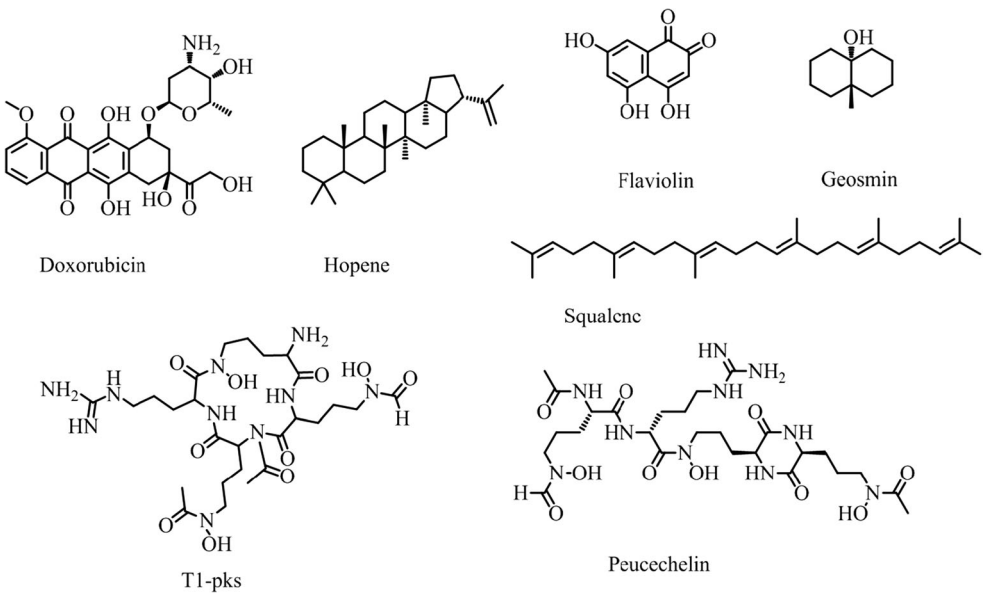


**Fig. 1** Structures of different secondary metabolites isolated and characterized from *S. peucetius* ATCC 27952

**Table 2** Putative BCGs encoding for natural products from *S. peucetius* ATCC 27952

| Cluster | Types | Most similar known biosynthetic gene cluster (% similarity) |
|---|---|---|
| 1 | Terpene | NA |
| 2 | Terpene | NA |
| 3 | Bacteriocin-lantipeptide | Sch47554_/_Sch47555_biosynthetic_gene_cluster (3% of genes show similarity) |
| 4 | Nrps-siderophore | Albachelin_biosynthetic_gene_cluster (80% of genes show similarity) |
| 5 | Otherks | Daptomycin_biosynthetic_gene_cluster (7% of genes show similarity) |
| 6 | Other | Daptomycin_biosynthetic_gene_cluster (4% of genes show similarity) |
| 7 | Cf_putative | NA |
| 8 | Cf_saccharide | NA |
| 9 | Cf_putative | Streptomycin_biosynthetic_gene_cluster (31% of genes show similarity) |
| 10 | Cf_putative | NA |
| 11 | Cf_putative | NA |
| 12 | Cf_putative | NA |
| 13 | Ectoine | Ectoine_biosynthetic_gene_cluster (75% of genes show similarity) |
| 14 | T1pks | Bafilomycin_biosynthetic_gene_cluster (33% of genes show similarity) |
| 15 | Cf_putative | Lomofungin_biosynthetic_gene_cluster (13% of genes show similarity) |
| 16 | Cf_putative | NA |
| 17 | Cf_putative | NA |
| 18 | Cf_putative | NA |
| 19 | Cf_fatty_acid | Carbapenem_MM_4550_biosynthetic_gene_cluster (6% of genes show similarity) |
| 20 | Cf_saccharide | Caprazamycin_biosynthetic_gene_cluster (71% of genes show similarity) |
| 21 | Terpene | NA |
| 22 | Cf_saccharide | NA |
| 23 | Cf_putative | NA |
| 24 | Cf_putative | Siomycin_biosynthetic_gene_cluster (7% of genes show similarity) |
| 25 | Other | Salinosporamide_biosynthetic_gene_cluster (16% of genes show similarity) |
| 26 | Cf_putative | NA |
| 27 | Lantipeptide | NA |
| 28 | Cf_fatty_acid | Auricin_biosynthetic_gene_cluster (6% of genes show similarity) |
| 29 | Cf_putative | NA |
| 30 | Cf_putative | NA |
| 31 | Lassopeptide | NA |
| 32 | Cf_putative | NA |
| 33 | Cf_putative | NA |
| 34 | Linaridin | Zorbamycin_biosynthetic_gene_cluster (8% of genes show similarity) |
| 35 | Cf_putative | NA |
| 36 | Cf_putative | NA |
| 37 | Cf_putative | NA |
| 38 | Cf_putative | NA |
| 39 | Cf_putative | NA |
| 40 | Cf_saccharide | Cypemycin_biosynthetic_gene_cluster (22% of genes show similarity) |
| 41 | Cf_putative | NA |
| 42 | Otherks-T2pks | Cinerubin_B_biosynthetic_gene_cluster (65% of genes show similarity) |
| 43 | Cf_putative | Mithramycin_biosynthetic_gene_cluster (5% of genes show similarity) |
| 44 | Cf_saccharide | NA |
| 45 | Ladderane-arylpolyene-Cf_fatty_acid-Nrps | Skyllamycin_biosynthetic_gene_cluster (51% of genes show similarity) |
| 46 | Siderophore | NA |
| 47 | T1pks | Cremimycin_biosynthetic_gene_cluster (25% of genes show similarity) |
| 48 | T2pks-Cf_saccharide | Spore_pigment_biosynthetic_gene_cluster (83% of genes show similarity) |
| 49 | Cf_putative | T3_toxin_biosynthetic_gene_cluster (5% of genes show similarity) |

**Table 2** (continued)

| Cluster | Types | Most similar known biosynthetic gene cluster (% similarity) |
|---|---|---|
| 50 | Bacteriocin-T1pks | Enduracidin_biosynthetic_gene_cluster (29% of genes show similarity) |
| 51 | Cf_putative | Fostriecin_biosynthetic_gene_cluster (9% of genes show similarity) |
| 52 | Cf_putative | Kanamycin_biosynthetic_gene_cluster (46% of genes show similarity) |
| 53 | Melanin | Melanin_biosynthetic_gene_cluster (28% of genes show similarity) |
| 54 | Cf_fatty_acid | NA |
| 55 | Lantipeptide-T1pks | SapB_biosynthetic_gene_cluster (100% of genes show similarity) |
| 56 | Cf_saccharide-Cf_fatty_acid | Paromomycin_biosynthetic_gene_cluster (5% of genes show similarity) |
| 57 | Cf_putative | NA |
| 58 | Terpene | Hopene_biosynthetic_gene_cluster (69% of genes show similarity) |
| 59 | Cf_saccharide | Chrysomycin_biosynthetic_gene_cluster (8% of genes show similarity) |
| 60 | Cf_putative | NA |
| 61 | Other | Stenothricin_biosynthetic_gene_cluster (13% of genes show similarity) |
| 62 | Cf_putative | Herboxidiene_biosynthetic_gene_cluster (7% of genes show similarity) |
| 63 | Cf_putative | Frankiamicin_biosynthetic_gene_cluster (21% of genes show similarity) |
| 64 | Cf_putative | NA |
| 65 | Cf_putative | Chalcomycin_biosynthetic_gene_cluster (4% of genes show similarity) |
| 66 | Cf_putative | NA |
| 67 | Cf_putative | Borrelidin_biosynthetic_gene_cluster (4% of genes show similarity) |
| 68 | Cf_putative | Echosides_biosynthetic_gene_cluster (17% of genes show similarity) |

NA not applicable

the siderophore was confirmed to be linear peptide, and named as peucechelin (PCN). The biosynthetic gene cluster was predicted, and biosynthetic mechanism (Fig. 3) was also proposed based on organization of different open reading frames (ORF) (Kodani et al. 2015). Similarly, an ORF (accession no EU492927.1) was predicted to encode for squalene-hopene cyclase (*Spterp25*), responsible for cyclization of squalene to form hopene. The ORF was successfully expressed in *E. coli* BL21(DE3) pLySs, and purified protein was able to cyclilize squalene to hopene (Ghimire et al. 2015) (Table 3).

## Genomic exploration of metabolically crucial genes from *S. peucetius* ATCC 27952

Biocatalysis utilizing specific microbial biotransformation or bioconversion (with multiple catalytic steps) is highly active area of research for preparation of pharmaceutical products (Liu and Yu 2010; Hegazy et al. 2015). The biocatalysis mediated by suitable microbial host tends to be a superior approach than chemical methods. The most crucial aspect is that microbial catalysis can be operated at non-extreme reaction conditions, such as near neutral pH, ambient temperature, and

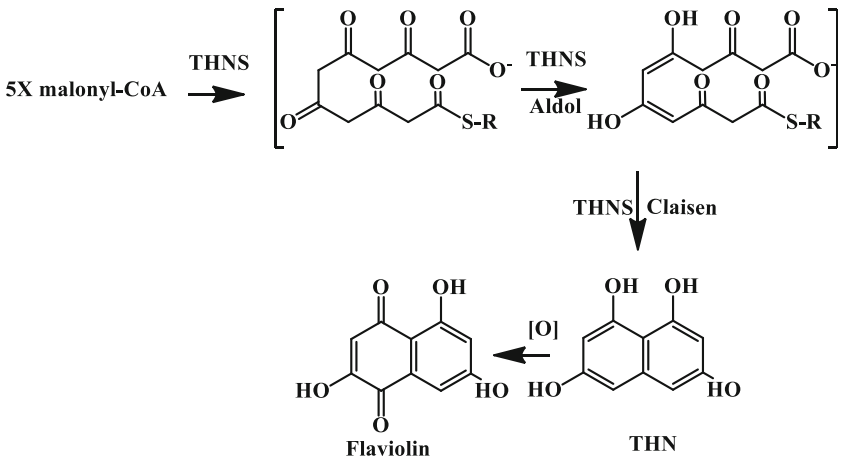


**Fig. 2** Proposed biosynthetic mechanism of flaviolin (2,5,7-trihydroxy-1,4-naphthoquinone). R coenzyme A (CoA), THN 1,3,6,8-tetrahydroxynaphthalene, THNS 1,3,6,8-tetrahydroxynaphthalene synthase

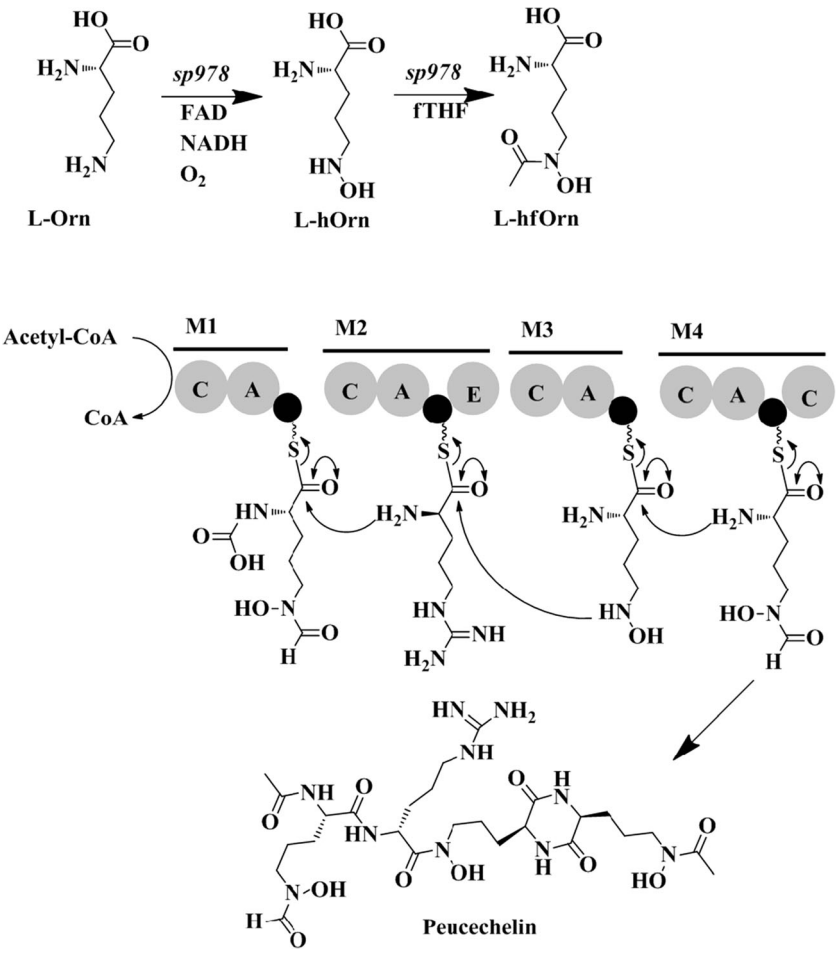


**Fig. 3** Proposed biosynthetic pathway of Peucechelin (PCN) in *S. peucetius* NBRC 14660 (=ATCC 27952). A, C, E represent adenylation, condensation, and epimerization domains, respectively. Black filled circle represents a peptidyl carrier protein domain (PCP). sp970, sp980 lysine/ornithine N-monooxygenase and formyl transferase, respectively, FAD flavin adenine dinucleotide, NADH nicotinamide adenine dinucleotide, L-Orn L-ornitine, L-hOrn δ-N-hydroxy-L-ornithine, L-hfOrn L-δ-N-formyl-δ-N-hydroxyornithine

atmospheric pressures. In addition, the reaction products are also generated in predictive manner due to regio-selective and enantio-specific conversions in most cases (Collins and Kennedy 1999). Recently, *S. peucetius* was explored for its biocatalytic properties against diverse compounds. The result indicates that the strain was biocatalytically efficient for generating diverse molecules with pharmaceutical values by whole-cell biotransformation, which included methylation and hydroxylation as major biocatalytic steps (Dhakal et al. 2018). The genome of *S. peucetius* contains ORFs encoding for diverse MTs and CYPs, which may be involved in such bioconversion by methylation and oxidations/hydroxylations, respectively. In addition, some of the ORFs corresponding to respective MTs, CYPs, GTsF, or other biocatalytic enzyme have been studied by heterologous expression in *E. coli*. They have been utilized for in vivo and in vitro reaction studies for determining their suitable substrate and performing optimized enzymatic bioconversions. Particularly, these enzymes are involved in the biosynthesis of primary and secondary metabolites in *S. peucetius*, which in turn help in growth and maintenance. In addition, there are numerous ORFs which are directly or indirectly involved in growth, regulation, and maintenance of the bacteria, as well as primary/secondary metabolite biosynthesis. Genome mining is directly associated with genome to secondary metabolite connection, so "GE" is considered as a suitable term for defining the "association of genomic information with such biocatalytic/regulatory/maintenance enzymes." GE can be used to discover the homologs of functionally characterized enzymes and to find the putative enzymes with novel substrate specificities by tracking the ORF by in silico studies followed by functional characterization by in vivo and in vitro studies. The GE approach was utilized for exploring diverse enzymes encoded in genome of *S. peucetius*, and detailed information is represented in the following sections.

## Cytochrome P450 genes

CYPs are most important biocatalytic enzymes involved in diverse reactions including hydroxylation; alcohol and carbonyl oxidation; epoxidation; dealkylation; heteroatom oxidation; desaturation; ring cleavage, expansion, coupling and formation; dehydration; and even reduction (Rudolf et al. 2017; Jha et al. 2015; Pokhrel et al. 2015; Dhakal et al. 2016). Some of the representative enzymatic reactions facilitated by CYPs

**Table 3** Major enzymes characterized from *S. peucetius* ATCC 27952 that are involved in biosynthesis or modification of secondary metabolites

| Enzyme/Gene names | Gene accession number | Biochemical characterizations | References |
|---|---|---|---|
| *spterp13* | EU334502.1 | 2199 bp; 732 AA; encoding for geosmin synthase. Overexpression of the *spterp13* gene in *S. peucetius* DM07 resulting in 2.4 ± 0.4-fold increased production of geosmin. | Singh et al. (2009) |
| *hopA*, *hopB*, and *hopD* | FJ529811 (hopA), FJ529812 (hopB), and FJ529814 (hopD) | *hopA*, *hopB*, and *hopD* encoding for squalene, phytoene synthases, and diphosphate synthase, respectively; improvement in the production of squalene in *E. coli*. | Ghimire et al. (2009a) |
| *spterp25* | EU492927.1 | 2.025 bp; 674 AA; encoding for squalene-hopene cyclase to form hopene. | Ghimire et al. (2009b) |
| CYP105P2 | CAE53708 | 399 AA; flavone hydroxylase; coexpressed with redox partner CamA (putidaredoxin reductase)/CamB (putidaredoxin) from *Pseudomonas putida* in *E. coli* BL21(DE3) | Niraula et al. (2012) |
| CYP107AJ1 | AJ605547 | 441 AA; hydrogen peroxide ($H_2O_2$)-mediated in vitro dealkylation of 7-ethoxycoumarin | Niraula et al. (2011) |
| CYP105F2 | AJ605543 | 405 AA; in vitro hydroxylation of oleandomycin. | Shrestha et al. (2008a) |
| CYP166B1 | AJ605542 | 405 AA; *O*-dealkylation of 7-hydroxycoumarin | Shrestha et al. (2010) |
| CYP107P3 (CSP4) | AJ605551 (CAE53719) | 406 AA; coexpression along with CamA/CamB in *E. coli* for *O*-dealkylation of 7-ethoxycumarin. | Shrestha et al. (2008b) |
| CYP147F1 | AJ605536 | 413 AA; ω-fatty acid hydroxylase. The order of CYP147F1 affinity to the fatty acids tested was lauric acid > myristic acid > palmitic acid. | Bhattarai et al. (2013) |
| CYP107N3 | AJ605544 | 414 AA; in vitro C9-C10 epoxidation of oleic acid | Bhattarai et al. (2012) |
| CYP157C4 | AJ605537 | 476 AA; 7-ethoxycoumarin hydroxylase | Rimal et al. (2015b) |
| CYP129A2 (DoxA) | AF403708 | 644 AA; involved in multiple steps in biosynthesis of DXR; hydroxylation of the daunorubicin enhanced by two-fold with native redox partners via NADH → FDR2 → FDX1 → DoxA pathway | Rimal et al. (2015a) |
| DnrK | AAA99001 (L40425) | 357 AA; methylation of carminomycin deoxycarminomycin and ε-rhodomycin. Also able to methylate epelmycin D and various flavonoids. | Madduri et al. (1993) Miyamoto et al. (2000) Kim et al. (2007) |
| SpOMT2884 | KF420279 | 224 AA; methylation of diverse flavonoids as 7,8-dihydroflavone, 8-hydroxydaidzein, and 3′ hydroxygenestein | Koirala et al. (2014a) Chiang et al. (2015) Chiang et al. (2017) |
| SpOMT7740 | KU745626 | 343 AA; methylation of various natural products including flavonoids, anthraquinones, anthracyclines and sterols | Parajuli et al. (2017) |
| DnrS/DnrQ | DnrS (L47164) DnrQ (L47164) | DnrS(432 AA)/DnrQ (439 AA); glycosylation of ε-rhodomycin and other anthracyclines | Han et al. (2011) |

in secondary metabolites are as hydroxylation in antibacterial pikromycin (Xue et al. 1998), ether formation in antiparasite, avermectin (Ikeda et al. 1999), and hydroxylation/oxidation to ketone in anticancer compound, doxorubicin (Grimm et al. 1994), etc., which helps in attributing functionality in the final compound.

The genome sequence of *Streptomyces peucetius* was reported to contain 19 CYPs, along with two FDXs and four FDRs (Parajuli et al. 2004). However, Shrestha et al. subsequently re-annotated the CYP system in *S. peucetius* by in silico analysis and comparison with CYP in *S. coelicolor* A3 (2) and *S. avermitilis* (Shrestha et al. 2010). Bacterial CYPs are known to use various types of electron transport systems for catalyzing their specific reactions. Many types of bacterial CYPs accept electron from flavoproteins or FDX proteins, which accept electron from FDR (Paine et al. 2005). Shrestha et al. successfully identified six novel ferredoxin in *S. peucetius*, in which fdx743 was determined as nitrite/sulfate reductase. Furthermore, they also found four types of ferredoxin reductase in *S. peucetius*. Thus, it is evident that among the total 23 CYPs, each of them is supported by any of the six ferredoxins and nine ferredoxin reductases (Shrestha et al. 2010). These are involved in catalysis for various types of reactions, such as oxygenation, hydroxylation, and C-H activation (Table 3).

DoxA, known as daunorubicin 14-hydroxylase, is most important CYP characterized from *S. peucetius*. DoxA was characterized to take part in three major steps in the biosynthesis of DXR by using in vitro reactions (Dickens et al. 1997). But all the reaction were conducted with commercially

available redox partners; however, Rimal et al. successfully developed an efficient native redox system to support DoxA, to convert daunorubicin to doxorubicin under in vitro conditions. Under the optimal pH and temperature (7.6 and 30 °C, respectively), the DoxA activity was increased by 2-fold, via electron transport chain: NADH → FDR2 → FDX1 → DoxA. This observation provides information on most suitable endogenous electron transport system for the modification of DNR to DXR in *S. peucetius* (Rimal et al. 2015a).

CYP105P2 is characterized to perform hydroxylation of flavone in coexpression with electron transfer system, *camA* and *camB* from *Pseudomonas putida* (Niraula et al. 2012) (Fig. 4). Recently, the crystal structure of CYP105P2 was deduced at 2.1 Å resolution. The comparative analysis with crystal structures of previously determined substrate-bound CYP predicts that binding of the ligand produces large and distinctive conformational changes in α2–α3, α7–α9, and the C-terminal loop regions. This structural flexibility provides strong corroboration to previous observation that CYP105P2 can accommodate a broad range of ligands (Lee et al. 2016). Hence, the detailed structural studies by crystallography assist significantly for determining substrate preference, flexibility, and reaction mechanism. Similarly, CYP107AJ1 is able to catalyze dealkylation reaction, using 7-ethoxycoumarin as substrate (Niraula et al. 2011) (Fig. 4). In addition, a whole-cell bioconversion system was designed to prove the role of enzyme, CYP107P3 in dealkylation reaction (Shrestha et al. 2008b). The specific information about CYPs and their biochemical properties are presented in Table 3.

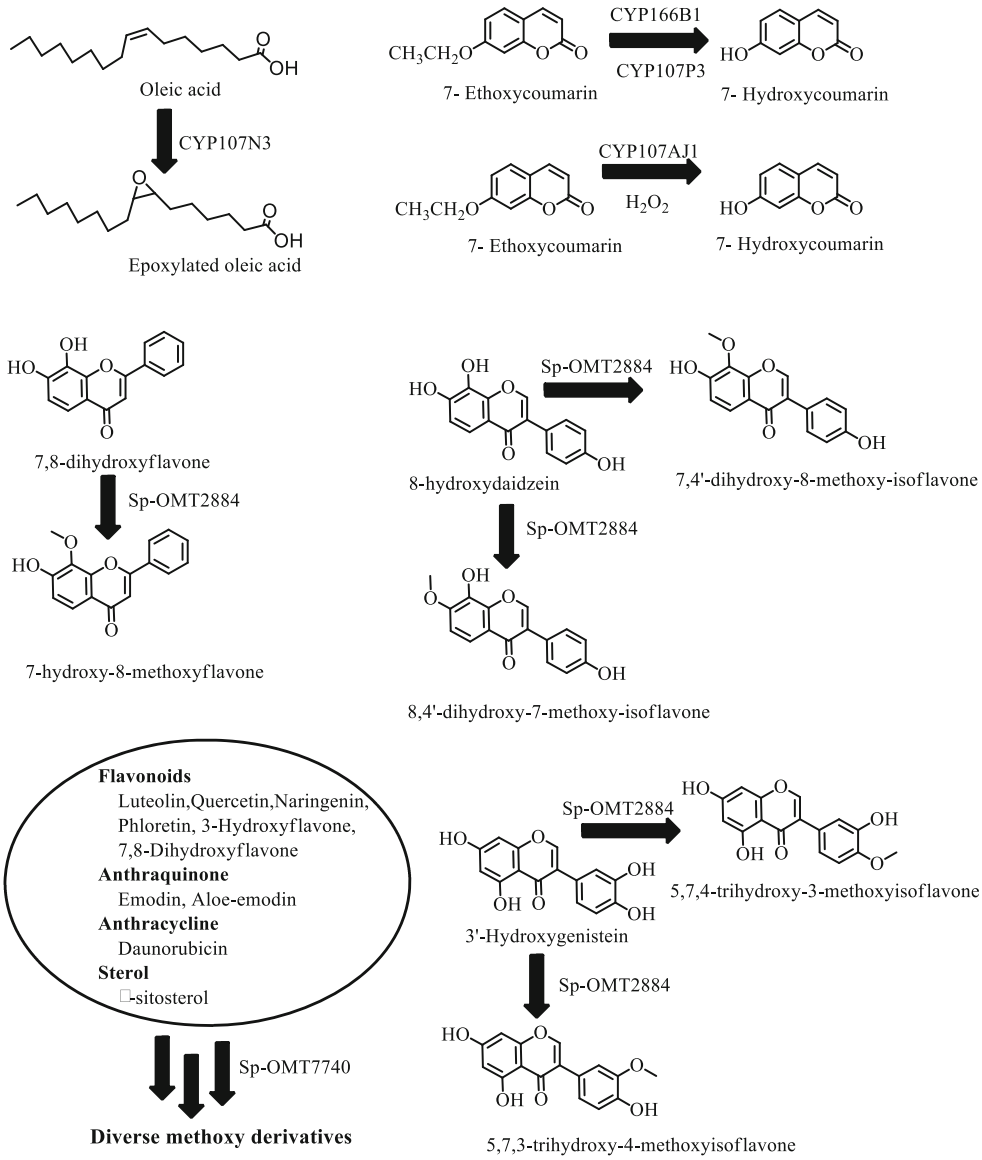


**Fig. 4** Enzymatic catalysis by different CYPs and MTs from *S. peucetius* ATCC 27952. CYPs mediate epoxidations/hydroxylation, whereas MT results methylation in different substrates checked, respectively. $H_2O_2$ hydrogen peroxide

## Methyltransferase genes

Methylation, either N–, C–, or O–, is another important phenomenon critical for maintaining the physiological and biochemical processes in all organisms (Zubieta et al. 2001). It has been observed that *O*-methylation of natural products alters the chemical reactivity of the hydroxyl groups and enhances lipophilicity, increasing their absorption and distribution when used as pharmaceutical components (Darsandhari et al. 2018; Koirala et al. 2014a). Hence, MTs stand as another crucial biocatalytic enzymes involved in growth and maintenance of organism, as well as developing pharmaceutically important compounds. DnrK is first MT studied from biosynthetic pathway of DXR, initially characterized as 4-*O*-methyltransferase of carminomycin (Madduri et al. 1993). Subsequent assessment of enzyme promiscuity established its flexibility for diverse anthracycline intermediates in biosynthetic pathway of DXR (Zubieta et al. 2001), or production of a new hybrid anthracycline 4-*O*-methylpelmycin by heterologous expression in epelmycin producing *Streptomyces violaceus* (Miyamoto et al. 2000). The molecular docking studies and in vitro studies established its substrate flexibility toward diverse flavonoids at 7-hydroxyl group or 4″ hydroxyl group (Kim et al. 2007).

SpOMT2884, another MT present in *S. peucetius* genome, was characterized by heterologous expression in *E. coli* BL21 (DE3). This gene functions as methylation of different flavone (7,8-dihydroxyflavone, luteolin), flavonols (quercetin, rutin), flavanone (naringenin), and isoflavonoids (daidzein, formononetin). However, 7,8-dihydroxyflavone (7,8-DHF) is the most favored substrate (Fig. 4, Table 3). The yield of 7-hydroxy-8-methoxyflavone as product amounted to 52.57 mg/L (Koirala et al. 2014b). Later, Chiang et al. reported biotransformation of 8-hydroxydaidzein to obtain two novel derivatives, consisting of 7,4′-dihydroxy-8-methoxy-isoflavone and 8,4′-dihydroxy-7-methoxy-isoflavone (Fig. 4, Table 3) using SpOMT2884. The test of biological activity showed that it is a highly potent inhibitor of melanogenesis (Chiang et al. 2015). Similarly, genistin was 3′-hydroxylated using recombinant *E. coli* expressing recombinant tyrosinase from *Bacillus megaterium*, and subsequently methylated using another recombinant *E. coli* expressing SpOMT2884 from *S. peucetius*, to generate 5,7,4′-trihydroxy-3′-methoxyisoflavone and 5,7,3′-trihydroxy-4′-methoxyisoflavone (Fig. 4, Table 3). The methoxy derivatives exhibited potent antiproliferative activity toward mouse B16 melanoma cells, with their potential application as another antimelanoma agent (Chiang et al. 2017). Recently, another MT, SpOMT7740, was characterized by in vitro and in vivo biotransformation experiments, after subsequent heterologous expression in *E. coli*. This MT exhibit pronounced substrate flexibility by accepting a diverse nature of chemical scaffolds (Parajuli et al. 2017) such as flavonoids, anthraquinones, anthracyclines, and sterol, as presented in Fig. 4 and Table 3.

## Glycosyltransferase and sugar biosynthetic genes

DXR/DNR contains an unusual sugar thymidine diphosphate (TDP)-L-daunosamine, which is crucial for bioactivity. Due to the biological importance of the sugar component in DXR/DNR, there was interest in characterization of the biosynthesis of TDP-L-daunosamine. The biosynthetic mechanism of TDP-L-daunosamine and enhancement of doxorubicin by overexpression of sugar pathways are already reported (Niraula et al. 2010a). In silico analysis of *S. peucetius* genome reveals the presence of TDP-L-rhamnose gene cluster (RmbA, RmbB, RmbC, and RmbD), and TDP-L-daunosamine gene cluster as well. Singh et al. studied the genetic relationship and biochemical characteristics of *rmbB* and *dnmM* (Singh et al. 2013). The results showed that they share high similarity and encode for TDP-D-glucose 4,6-dehydratase to catalyze the formation of dTDP-4-keto-6-deoxy-D-glucose. In particular, *dnmM* contains a frame shift in its sequence to cause premature termination of translation. Experimental data suggested that inactivation of *rmbB* blocked DXR production in *S. peucetius* and subsequently restored production of DXR upon of complementation of this gene. Thereby, it strongly supports involvement of RmbB as biocatalyst to provide TDP-4-keto-6-deoxy-D-glucose as a precursor for DXR production (Singh et al. 2013).

In another study, Singh et al. found that *dnmU* and *rmbC*, encoding for deoxy-thymidine-diphosphosphate (dTDP)-4-keto-6-deoxyglucose 3,5-epimerase in *S. peucetius*, share high similarity in protein sequence (64%). They catalyze the conversion of dTDP-4-keto-6-deoxy-D-glucose to dTDP-4-keto-L-rhamnose under in vitro conditions. However, it was proved via gene inactivation experiments that the two genes work independently and do not affect each other. *rmbC* inactivation also abolished DXR production. Those effects proved that *rmbC* can work as a key physiological gene that can regulate biosynthesis of secondary metabolites (Singh et al. 2010). Singh et al. also performed the biochemical characterization of two genes encoding for glucose-1-phosphate (G-1-P) thymidylyltransferases: *dnmL* and *rmbA*. They found that DnmL and RmbA only differ in C-terminal amino acid sequence. It was observed that solubility of DnmL in heterologous expression in *E. coli* was determined by *C*-terminal amino acid sequence (Singh et al. 2012).

The biosynthetic pathway of DXN/DXR includes paired protein viz. GT (*dnrS*) and auxiliary protein (*dnrQ*), which are responsible for glycosylation of aglycone, ε-rhodomycinone (RHO) (Han et al. 2011). The detailed understanding of the mechanism of GT and biosynthetic steps of sugar component of DXR/DNR provided clue for developing a combinatorial biosynthetic system for the production of

DXR and its biosynthetic intermediates, using *Streptomyces venezuelae* as a platform heterologous host. This system significantly improved epirubicin production from ε-rhodomycinone, by selecting a substrate of flexible glycosyltransferase, AknS, and efficient TDP-4-ketohexose reductase, AvrE. Meanwhile, seven novel glycosylated derivatives of rhodomycin D were generated (Han et al. 2011). Further, the flexibility and efficiency of AknS toward diverse sugars was utilized for developing a cocultivation-based approach for producing novel anthracyclines related to DXR. In this system, *Streptomyces venezuelae* mutants producing the anthracycline aglycones (aklavinone or ε-rhodomycinone) were cocultivated with *S. venezuelae* mutants coexpressing AknS, along with the production of eight different nucleotides to generate 16 glycosylated anthracyclines containing diverse deoxysugar moieties, 7 of which were entirely new (Kim et al. 2017). This facile cocultivation method for inter-mixing the biosynthetic pathways at strain level provides a new prospect of combining diverse aglycones and deoxysugars to generate structurally diverse polyketides, with potential application to drug discovery and development.

## Regulatory genes and their mechanisms

*S. peucetius* has various types of regulatory genes controlling the production of DNR/DXR, including transcription factors, transcriptional repressors, transcriptional control by a coherent feed forward loop, self-resistance mechanism, and feedback regulation (Vasanthakumar et al. 2013), as listed in Table 4. Previously, inhibition of DNA-DnrO complex formation by DNR and its effect on *dnrO* and *dnrN* expression were studied. DnrO is a transcriptional factor that works as an activator/self-repressor in the DNR biosynthesis in *S. peucetius*. It activates DnrN, and then, DnrN binds to a sequence close to *dnrI* promoter and activates this gene. Finally, *dnrI* stimulates the biosynthesis genes. Ajithkumar et al. analyzed the formation of DNA-DnrO by DNR, and its effect on the expression of *dnrO* and *dnrN*. The authors proposed a model to describe the modulation of *dnrN* and *dnrO* expression by intracellular concentration of the DNR and DnrO protein. This regulatory mechanism may support the intracellular DNR concentration at controlled level and prevent *S. peucetius* from cytotoxicity (Ajithkumar and Prasad 2010a b). Recently, based on computational studies, it was predicted that DnrI is bound as a monomer to a slightly modified heptameric DNA motif, 5′-ACACGCA in *drrA* and 5′-ACAACCT in *drrC*. This was later confirmed by electrophoretic mobility shift assay. This computational and experimental evidence confirms that DnrI binds to the specific resistance genes (*drrA* and *drrC*) at specific sites, which is activated only when an increased load of intracellular DNR is sensed (Prija et al. 2017).

In silico analysis in the complete genome sequence of *S. peucetius* ATCC 27952 led to the presence of AfsR homolog, and named AfsR-p, an antibiotic regulatory protein (SARP) pleiotropic activator. Its protein shares 60% in identity with AfsR from *S. coelicolor* A3(2). This gene triggered the increased production of DXR in *S. peucetius* by 4-fold. The presence of AfsR-p suggested that *Streptomyces* may possess the tyrosine kinase and a serine/threonine-dependent regulatory system (Parajuli et al. 2005). In similar manner, *metK1-sp* (sp1190) and *metK2-sp* (sp1566) were identified, and introduced into *S. peucetius*, which led to the increased production of DXR (Oh et al. 2010). Chaudhary et al. revealed that *doxR* (IclR family) regulates the transcription of NRPS gene as a signal-dependent expression type, and this gene suppress growth, as well as DXR production, in recombinant strain, compared to the control harboring vector only (Chaudhary et al. 2014).

TTA is the rarest codon in genome of *Streptomyces*, which are rich GC content contained. However, TTA codon is reported in several regulatory genes that control sets of antibiotic production genes (Chater 2006). It is also present in *adpA* gene (so-called *bldH*), whereas AdpA acts on several genes involved in the biosynthesis of secondary metabolites and sporulation. The prominent regulatory target of *bldH* (*adpA*) is an extracellular protease inhibitor protein that delays the digestion of the primary biomass until the colony is ready for aerial growth (Chater 2006; Chater and Chandra 2008). Hence, the absence of Leu-tRNAUUA (*bldA*-tRNA) in *bldA* mutant leads to bald phenomenon, due to the inefficient expression of *adpA*. Indeed, the dependence of secondary metabolite profile on *bldA*-tRNA was shown in several cases, including actinorhodin, undecylprodigiosin, and methylenomycin biosynthesis in *S. coelicolor* (Chater and Chandra 2008) and expression of a landomycin regulatory gene in *S. globisporus* (Rebets et al. 2006). Interestingly, TTA codon is found in *dnrO* sequence, whereas *S. peucetius* genome lacks corresponding *bldA*-tRNA. Hence, the effect of heterologous expression of *bldA*-tRNA (accession no. Y00209.1) from *S. coelicolor* A3 on pathway specific regulation of DNR in *S. peucetius* was studied. Particularly, the role of *bldA* on *dnrO*, and overall effect of *dnrO* over other regulatory genes, and in production of DNR, was focused. Experimental results showed that *S. peucetius* containing *bldA* had increased overexpression of pathway transcriptional factors *dnrO* and *dnrI*, along with improved production of DNR (45.7% higher than the control). The comprehensive analysis of transcriptional level, cell growth, and DNR production profiles showed that *bldA*-tRNA facilitates the expression and multiple regulation mechanism of *dnrO* on *S. peucetius* (Pokhrel et al. 2016).

**Table 4** Major regulatory and resistance genes characterized in *S. peucetius* ATCC 27952

| Enzyme/Gene name | Gene accession no. | Molecular and biochemical properties | References |
|---|---|---|---|
| *doxR* (IclR) | JQ814785 | Overexpression of doxR in native *S. peucetius* strongly inhibits growth and production of DXR compared to the control. | Chaudhary et al. (2014) |
| *afsR*-p | AJ786384 | *afsR*-p has two conserved ATP binding sites (A-type: 321 GIGGVGKT328 and B-type: 399 MVLLD403) and a catalytic domain (399MVLLD403). *afsR*-p induces increased production of DOX by about 4-fold in *S. peucetius.* | Parajuli et al. (2005) |
| SAM synthase (MetK) | *metK1*-sp (sp1190) and *metK2*-sp (sp1566) | Two genes encoding for S-adenosyl-l-methionine synthetase (MetK). They contain the motifs for ATP binding (RKIIIDTYGG), and two putative metal-bound regions (HGGGAFSGKD) for $Mg^{2+}$ and (VXEGHPDKI) for $K^{+}$. Introduction of *metK1* and *metK2* into *S. peucetius* leading to increased production of DOX by about 2.1-fold and 1.4-fold, whereas inactivation of *metK1* in *S. peucetius* caused significant decrease in production of DOX, compared compared to the control. | Oh et al. (2010) |
| *dnrO*, *dnrN*, and *dnrI* | *dnrO* (L37338)<br>*dnrN* (L37338)<br>*dnrI* (M80237) | DnrN is a DNA-binding protein, essential for transcription of *dnrI*. DnrO activates the transcription of *dnrN*, which in turn activates the transcription of *dnrI*. DnrI is pathway specific regulator which controls the transcription of most of the biosynthetic and resistance genes in DXR biosynthesis. Hence, both DnrN and DnrO play role for the increased activity of *dnrI*. | Ajithkumar and Prasad (2010a, b), Jiang and Hutchinson (2006) |
| *drrA*, *drrB*, *drrC*, and *drrD* | *drrA* (M73758)<br>*drrB* (M73758)<br>*drrC* (L76359)<br>*drrC* (AD00354) | DrrA belongs to ABC transporter family, whereas DrrB is a membrane integral protein. Together, these proteins are believed to construct an ATP-driven pump for the efflux of anthracycline antibiotics out of the cell. DrrC naturally binds at sites where DNR is intercalated. DrrC dislodges DNR along with its release, thereby allowing unhindered DNA replication and transcription. DrrD belongs to family of proteins that does oxidoreductase and transferase activity, and involved in self-resistance mechanism. This combination of DrrABCD provides self-resistance against anthracyclines. | Gandlur et al. (2004), Prija and Prasad (2017), Karuppasamy et al. (2015) |

## Genes involved in self-resistance in *S. peucetius*

Initially, *drrAB* was considered as an operon conferring self-resistance against DXR/DNR in *S. peucetius*. DrrA is a type of ATP-binding protein and belongs to the ABC family of transporter. DrrB is a membrane integral protein that effluxes DNR/DXR out of the cell. Together, these proteins are believed to work to construct an ATP-driven pump to efflux anthracycline antibiotics out of the cell. *drrAB* locus was cloned and expressed in *E. coli*. Membrane topology analysis of DrrB showed that it contains eight membrane-spanning domains in the cytoplasm (Gandlur et al. 2004). DrrA and DrrB combine to ensure their full function and stability. The interaction between them was investigated by generation of single cysteine in DrrB, and a cysteine to amine chemical cross-linker that specifically cross-links a free sulfhydryl group in one protein (DrrB) to an amine in another (DrrA). It is proposed that the motif present in DrrB and other exporters is actually a modified version of the EAA motif, which was originally believed to be present only in the importers of the ABC family (Kaur et al. 2005).

Functionally, DrrAB was conferred as a single-drug transporter. However, it has been recently demonstrated that DrrAB transports not onlyDNR/DXR but also two types of MDR drugs, Hoechst 33342 and ethidium bromide, under in vitro and in vivo conditions. This is the first in-depth study of a drug resistance system from a producer organism, and it shows that a dedicated efflux system like DrrAB contains specificity for multiple drugs. The significance of these findings in the evolution of polyspecificity in drug resistance systems is discussed (Li et al. 2014). In another study, mutational studies were performed in DrrB, and the molecular basis of multispecificity in a prototype efflux system was studied. It was observed that multispecificity in multidrug exporters may be generally determined by the number and location of

aromatic residues, whereas strategically placed negatively charged residues are usually critical for the binding of cationic lipophilic drugs. The targeted mutation was conducted in and/or near the predicted drug-binding pocket of DrrB. Among all the most tested residues, DrrB drastically inhibited doxorubicin efflux, leading to loss of resistance to doxorubicin. However, several mutants simultaneously lost resistance to doxorubicin and verapamil, but retained resistance to Hoechst 33342 and/or ethidium bromide. This suggested the presence of overlapping, as well as independent drug-binding sites, in a common drug-binding pocket of DrrB. Thus, this study provided the comprehensive analysis of residues involved in drug binding by DrrB, a bacterial multidrug resistance protein of the ABC superfamily. A strong similarity was observed in the molecular mechanism of polyspecific drug recognition between *S. peucetius* (DrrAB), and the best characterized multidrug resistance proteins as mammalian ABC (ATP-binding cassette) protein P-glycoprotein (Pgp). These observations provided strong foundation that aromatic residue-based multidrug specificity is conserved across domains, and over long evolutionary periods (Brown et al. 2017).

Recently, few studies were conducted to determine roles of *drrC* and *drrD* in biosynthesis of DXR/DNR, which established that they may be also involved in self-resistance mechanism. DrrC, the DNA binding protein for self-resistance against DNR/DXR, was heterologously expressed in *E. coli,* and its mechanism of action was studied. It was observed that DrrC has the ability to naturally bind at sites where DNR is intercalated. It has been predicted that DrrC possibly scans the DNA for intercalated DNR, and when it encounters DNR, DrrC dislodges DNR, along with its release, thereby allowing unhindered DNA replication and transcription. This novel self-resistance mechanism provided DNA repair-associated self-resistance by DrrC (Prija and Prasad 2017). The *drrD* gene is downstream of *drrA* and *drrB* and hence may be cotranscribed. The in silico analysis predicted that DrrD has N-terminal FAD binding domain, which was proved by in vitro reaction with purified protein. It was allocated to a family of proteins that does oxidoreductase and transferase activity. Previously, *drr*D was categorized as transcriptional repressor, but exact role in biosynthesis of DXR was not determined (Vasanthakumar et al. 2013). Karuppasamy et al. studied the role of drrD by in vivo and in vitro studies. The gene inactivation experiment of *drrD* in *S. peucetius* showed loss of self-resistance partially to DNR toxicity (Karuppasamy et al. 2015). These observations infer that DrrD has plausible role in self-resistance mechanism. Thus based on all studies, it was established that combination of *drrABCD* is entirely involved in self-resistance against anthracyclines in *S. peucetius*.

## Other cryptic genes involved in the maintenance of metabolism and physiology

Glucose is a very important carbon source effecting the differentiation of *S. peucetius* mycelia and antibiotic production. Guzman et al. found that this type of monosaccharide negatively affected the anthracycline biosynthesis. Glucose also prevented the repressive effect of galactose, by suppressing its incorporation. This suggests the participation of an integral regulatory system, which is initiated by an increase in incorporation of repressive sugars and their metabolism, as a prerequisite for establishing the phenomenon of CCR in *S. peucetius* (Guzmán et al. 2005). Recently, a polyphosphate-dependent Glk (Pp-Glk) was found from *S. peucetius* growing in glucose-containing media. A putative Pp-Glk gene is 726 bp, and a deduced amino acid sequence showed polyphosphate-binding motifs, such as GXDIGGXXIK, TXGTGIGSA, and KEX(4)SWXXWA. Glucose was proved as major carbon source for inducing this enzyme. Its maximum activity was measured in stationary growth phase by supplying 100 mM glucose (Ruiz-Villafán et al. 2014). Romero et al. investigated the glucose transportation by GlcP, a membrane protein encoded by *glcp*. This gene was cloned, and a functional analysis of *sp7066* promoter from *S. peucetius* was performed. Immunoblot analysis revealed that D-glucose and its analog 2-deoxyglucose were able to induce expression from *sp7066* promoter. They concluded that D-glucose is a preferred carbon source in *S. peucetius*. Further, it was observed that the expression product from *sp7066*, a putative non-PTS glucose permease, is likely a $H^+$/symporter, which is localized to the membrane and shows a strong specificity for D-glucose for inducing expression (Romero et al. 2015).

Pantothenate kinase (PanK) functions as a catalyst for the primary step in the biosynthesis of coenzyme A in many organisms. *S. peucetius* ATCC 27952-derived PanK consists of 332 amino acid, showing high similarity with PanK of *S. avermitilis* and *S. coelicolor* A3(2). Heterologous expression of this gene in *E. coli*, and use for in vitro coupling enzyme assay, showed that the PanK activity was phosphorylation via the decrease in NADH concentration. This result provided clue for using PanK for the increased production of DXR in *S. peucetius* by gene overexpression approach (Mandakh et al. 2010). Furthermore, a proteomic approach was used as an efficient tool to evaluate the cell physiology for antibiotic production. It was observed that *panK*-integrated *S. peucetius* (BG200) accumulated a high amount of RHO, but decreases the production of DXR. To reduce RHO accumulation by synthesizing daunorubicin (DNR) from RHO more efficiently, GT pair (*dnrQS*) was overexpressed (pIBR25::*dnrQS* in *panK*-integrated strain, BG201). 2D gel electrophoresis was used in the *panK*-integrated strain to

understand the results in detail, by investigating the proteome changes. Experimental data showed that the introduction of *drrAB* operon into this strain led to highly increased production of DXR (9.4-fold), compared to the control (Song et al. 2011).

The overexpression of dephosphocoenzyme A (CoaE), an enzyme involved in the final step in biosynthesis of coenzyme A, enhanced the production of DXR by approximately 2.1-fold. The increased production of DXR can be due to increased titer of CoA, which is involved in the formation of diverse biosynthetic precursors, i.e., acyl-CoAs (Lee et al. 2014). Superoxide dismutase (SOD) is significant enzymes with role in the oxidative defense system, in which it catalyzes conversion of superoxide anion ($O_2^-$) to hydrogen peroxide ($H_2O_2$). Two SOD, named *sp-sod1* and *sp-sod2*, were identified and isolated from *S. peucetius* genome. SOD is believed to protect the organism from oxidative damage and elevate the life span of *Streptomyces*. Thereby, increased production of CA in *S. clavuligerus* NRRL 3585, actinorhodin (ACT), and undecylprodigiosin (Red) in *S. lividans* TK24 was recorded by heterologous expression of genes corresponding to SOD (Kanth et al. 2011). Similarly, *paaE* encoding for oxidoreductase has been identified from *S. peucetius* genome. Experimental data showed that PaaE acts as biocatalyst for the degradation of added phenylacetic acid in culture broth (Fig. 5). This study showed the biological role of *S. peucetius* in environment, as an indicator for the bioremediation of environmental pollution (Niraula et al. 2010b).

## Conclusions and future perspectives

Analysis of genome sequence of *S. peucetius* analysis reveals the presence of diverse biosynthetic gene clusters, including potent anticancer compound DNR/DXR. Particularly, the biochemical and biosynthetic aspects of production of DNR/DXR were characterized, using overexpression, heterologous expression, and mutagenesis. The existence of diverse additional BGCs in *S. peucetius* exhibits its potential to produce putative bioactive molecules (Dhakal et al. 2018). This opens up new avenues for obtaining novel bioactive molecules that may be potential drug in the future. But, the major constraint remains the optimal expression of such BGCs, and understanding their biosynthetic and regulatory mechanisms, which requires much effort. However, there is significant advancement on development of versatile DNA assembly systems with higher efficiency, and heterologous expression systems with higher productivity and yield (Luo et al. 2016). There have been several approaches for high-throughput screening of novel bioactive chemicals from *Streptomyces* strains as compound-guided or bioactivity-guided bioassays. Having baseline knowledge of the secondary metabolite BCG can accelerate the production of cryptic molecules, discovery of novel compounds, and enhancement of production titer of known molecules (Smanski et al. 2016). The integrated approaches of strain engineering, biosynthetic pathway engineering, synthetic biology, systems biology, and media optimization technology have immense potential for the production of such cryptic/known biomolecules in significant titers (Dhakal and Sohng 2017; Hwang et al. 2014; Phelan et al. 2017).

There is the presence of several biocatalytic enzymes such as CYPs, MTs, GTs, ferredoxin, and ferredoxin reductase genes, which are involved in biosynthesis of discrete range of secondary metabolites in *S. peucetius* (Dhakal et al. 2018). In addition, it contains diverse protein/enzyme involved in various metabolic pathways, and plentiful types of regulatory genes, that are involved in the development of mycelia, spore formation, production, and regulation (Niraula et al. 2010a; Pokhrel et al. 2016; Karuppasamy et al. 2015; Prija and Prasad 2017). The precise information of these enzymes provides rational approach for holistic approach of metabolic engineering of host strain by multiplex genome engineering (Cobb et al. 2014), rewiring of the regulatory machinery (Park and Wang 2018), or complete reorganization of metabolic pathway by generating artificial pathway or metabolic tweaking by efficient enzymes (Zhang et al. 2016). The use of the suitable genes involved in the regulation of biosynthesis, or growth and maintenance, can equipoise the metabolite constraints observed in most of the pathway engineering approaches. Thus, these precise enzyme-specific engineering provides new prospect for directing both pathway-level engineering and strain-level engineering to new regime (Erb et al. 2017; Pröschel et al. 2015). In addition, the biocatalytic efficiency of enzymes sourced from *S. peucetius* leads to promising result in whole-cell-based in vivo microbial bioconversions or tube-based in vitro enzymatic reactions. Further, the structural elucidation of enzymes by X-ray

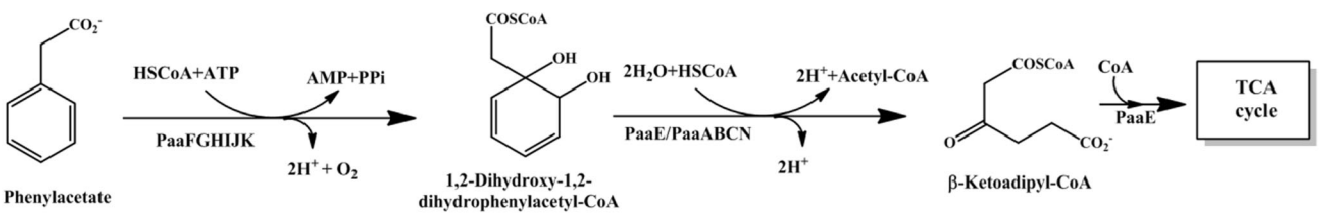


**Fig. 5** Pathway for biodegradation of phenylacetic acid in *S. peucetius* ATCC 27952. HSCoA coenzyme A, TCA cycle tricarboxylic acid

crystallography, and enzyme engineering by mutation, directed that evolution or domain swapping can enhance the overall productivity. The enzymes can also be designed to alter their sterio-/regio-selectivity and expand substrate flexibility, which extends their applicability in broader range (Chen and Zeng 2016; Erb et al. 2017). Hence, the genome-guided exploration of metabolic features of *S. peucetius* in context to secondary metabolite biosynthesis, physiological maintenance of growth and development, and versatile biocatalytic enzymes provides tremendous knowledge. These knowledge can open paradigm shift for the development of designer's producer strain for particular bioactive molecules or magnificently efficient microbial biocalyst for use in industrial application.

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## Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

**Conflict of interest** The authors declare that they have no conflict of interest.

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