Review

Cell Factory for Phenylnaphthacenoid Polyketide Production

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Abstract: Covering 2009–2022. Phenylnaphthacenoid polyketides have gained significant interest in recent years owing to their potent antibacterial and anticancer activities. Notably, more than 100 members of this class of natural products have been discovered from various *Streptomyces* species by different research groups including ours over the last 13 years. This review summarizes the current knowledge of the discovery, chemical diversity, and bioactivity of phenylnaphthacenoid polyketides. The current review also highlights the cell factory for phenylnaphthacenoid production: (1) native strains, (2) mutant strains, (3) heterologous expression, and (4) biocatalytic halogenations. Furthermore, current challenges and future opportunities are also presented as a guide for researchers to explore them more purposefully.

Keywords: cell factory; polyketides; phenylnaphthacenoids; *Streptomyces*; antibiotics; anticancer

1. Introduction

Phenylnaphthacenoids belong to a class of rare aromatic polyketides containing a core *gem*-dimethyl-naphthacenone scaffold (A–B–C–D ring) connected to a phenyl moiety (D ring) [1,2]. These natural polyketides, which are usually substituted with monochloride or multi-chlorides, show potent activity against Gram-positive bacteria including clinically relevant pathogens MRSA and VRE [3,4]. Some congeners also exhibit excellent activities against Gram-negative bacteria and are cytotoxic against several cancer cell lines [5,6]. Furthermore, they also inhibit Poly(ADP-ribose) polymerase-1 (PARP1) [7] and protein tyrosine phosphatase-1B (PTP-1B) [8].

To date, more than 100 members of phenylnaphthacenoid polyketides (PNPs) have been identified (Figure 1). The first disclosed members of this class of natural products were reported in the patent literature from *Streptomyces* sp. KB-3346-5 in 2009 [9]. Thereafter, several other strains of *Streptomyces* were shown to produce PNPs [2,6,10,11]. With the recent advances in engineering microbial cell factories, many more PNPs with diverse structures were discovered that would have been otherwise inaccessible. Notably, more than three-fourths of the identified PNPs to date were discovered over the last 5 years, including naphthacemycins, fasamycins, formicamycins, accramycins, streptovertimycins, and formicapyridines, among others (Figure 1, Table 1) [1–3,11–13].

Tremendous progress in the field of PNPs has been made by many different research groups including ours [1–3,11,12]. Hence, this review summarizes the current knowledge of the discovery, structural diversity, bioactivity, and methods of promoting the expression of PNPs covering the period spanning 2009 to 2022. Furthermore, the current challenges and future opportunities in the field of PNPs are also presented and discussed, thereby providing a guide for researchers to explore them more purposefully.

A.

	R₁	R₂	R₃	R₄	R₅	R₆	R₇
Accramycin A	Me	Me	H	H	H	H	H
Accramycin B	Me	Me	Me	H	H	H	H
Accramycin C	H	Me	H	Cl	H	H	H
Accramycin D	Me	Me	Me	Cl	H	H	H
Accramycin E	Me	Me	H	Cl	Cl	H	H
Accramycin F	Me	H	Me	Cl	Cl	H	H
Accramycin G	Me	Me	Me	Cl	Cl	H	H
Accramycin H	H	H	Me	Cl	H	Cl	Cl
Accramycin I	H	Me	Me	Cl	Cl	Cl	H
Accramycin J	H	H	Me	Cl	Cl	Cl	Cl
Accramycin K	H	Me	Me	Cl	Cl	Cl	Cl

B.

	R₁	R₂	R₃	R₄	R₅
Naphthacemycin A₁	H	H	Me	H	H
Naphthacemycin A₂	H	H	Me	Cl	H
Naphthacemycin A₃	Me	H	Me	H	H
Naphthacemycin A₄	Me	Cl	Me	H	H
Naphthacemycin A₅	H	H	Me	Cl	Me
Naphthacemycin A₆	Me	H	Me	Cl	H
Naphthacemycin A₇	Me	Cl	Me	H	Me
Naphthacemycin A₈	Me	Cl	Me	H	Me
Naphthacemycin A₉	Me	H	Me	H	Me
Naphthacemycin A₁₀	Me	Cl	Me	Cl	Me
Naphthacemycin A₁₁	Me	H	Me	Cl	Me

C.

	R₁	R₂	R₃	R₄	R₅	R₆	R₇	R₈
Naphthacemycin B₁	H	H	H	H	H	H	H	H
Naphthacemycin B₂	H	H	H	H	Me	H	H	H
Naphthacemycin B₃	H	H	H	H	Me	Cl	H	H
Naphthacemycin B₄	H	Cl	H	H	Me	H	H	H
Naphthacemycin B₅	H	H	H	H	Me	H	H	Cl
Naphthacemycin B₆	H	H	H	Cl	Me	H	H	H
Naphthacemycin B₇	H	Cl	H	H	Me	H	H	Cl
Naphthacemycin B₈	H	Br	H	H	Me	H	H	Cl
Naphthacemycin B₉	H	H	H	Cl	Me	H	H	Cl
Naphthacemycin B₁₀	H	H	Cl	Cl	Me	H	H	Cl
Naphthacemycin B₁₁	H	Cl	H	Cl	Me	H	H	Cl
Naphthacemycin B₁₂	H	Cl	Cl	Cl	Me	H	H	Cl
Naphthacemycin B₁₃	H	Cl	Cl	Cl	Me	Cl	H	Cl

D.

	R₁	R₂	R₃	R₄	R₅
Naphthacemycin C₁	Me	Cl	Me	H	H
Naphthacemycin C₂	Me	H	Me	Cl	Me

E.

	R₁
Naphthacemycin D₁	H
Naphthacemycin D₂	Cl

Figure 1. *Cont.*

F.
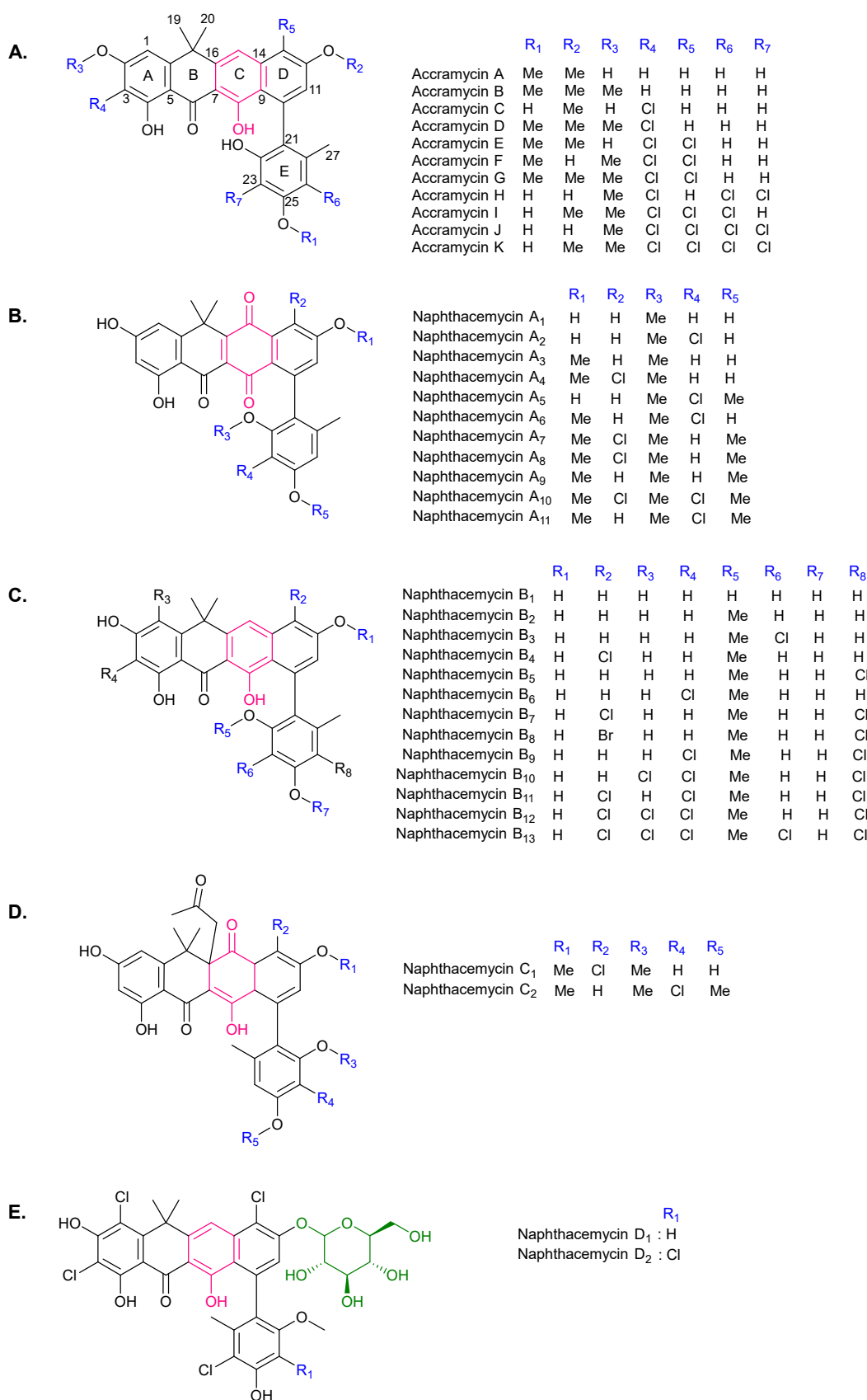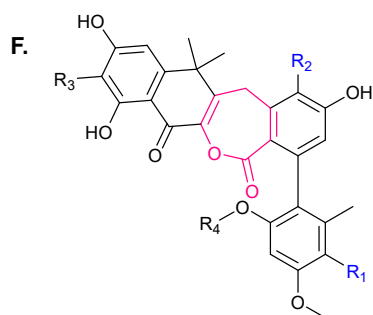
	R_1	R_2	R_3	R_4
Baeyer villiger lactone intermediate A	H	Cl	H	H
Baeyer villiger lactone intermediate B	Cl	Cl	H	H
Baeyer villiger lactone intermediate C	Cl	Cl	H	Me
Baeyer villiger lactone intermediate D	Cl	Cl	Cl	H
Baeyer villiger lactone intermediate E	Cl	Cl	Cl	Me

G.
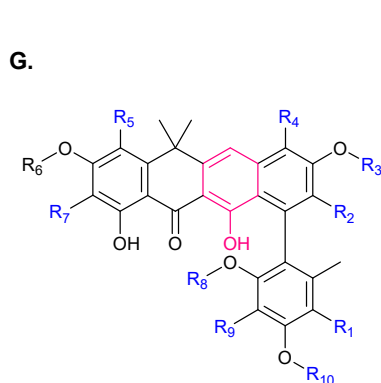
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
Fasamycin A	H	H	H	H	Cl	H	H	H	H	H
Fasamycin B	H	H	H	H	Cl	H	Cl	H	H	H
Fasamycin C	H	H	H	H	H	H	H	H	H	Me
Fasamycin D	H	H	H	Cl	H	H	H	H	H	Me
Fasamycin E	Cl	H	H	Cl	H	H	H	H	H	Me
Fasamycin F	Cl	COOH	H	H	H	H	H	H	H	H
Fasamycin G	H	H	Me	Cl	H	H	H	Me	H	H
Fasamycin H	H	H	Me	H	H	H	H	H	H	H
Fasamycin I	Cl	H	Me	H	H	H	H	H	H	H
Fasamycin J	Cl	H	H	H	H	H	H	H	H	H
Fasamycin K	H	H	H	Cl	H	H	H	Me	H	H
Fasamycin L	Cl	H	H	Cl	H	H	Cl	H	H	H
Fasamycin M	Cl	H	H	Cl	H	H	H	Me	Cl	H
Fasamycin N	Cl	H	H	Cl	Cl	H	H	H	H	Me
Fasamycin O	Cl	H	H	Cl	H	H	Cl	Me	H	Me
Fasamycin P	Cl	H	H	Cl	Cl	H	H	Me	Cl	H
Fasamycin Q	Cl	H	H	Cl	Cl	H	Cl	H	H	Me
Fasamycin R	H	H	H	H	H	Me	H	H	H	H
Fasamycin S	H	H	H	H	H	Me	Br	H	H	H
Fasamycin T	H	H	H	H	H	H	Cl	H	H	H
Fasamycin U	Br	H	H	H	H	H	H	H	H	H
Fasamycin V	Br	H	H	Br	H	H	H	H	H	H
Fasamycin W	Cl	H	H	H	H	Me	H	H	H	H
Fasamycin X	Br	H	H	H	H	Me	H	H	H	H
Fasamcyin Y	Br	H	H	H	H	Me	Cl	H	H	H

H.
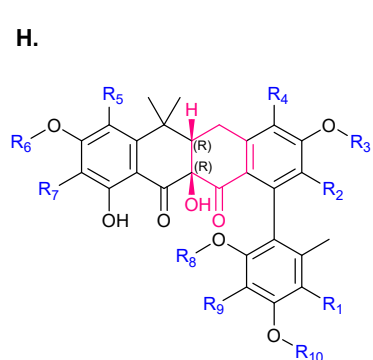
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
Formicamycin A	H	H	Me	Cl	H	H	H	Me	H	Me
Formicamycin B	Cl	H	Me	Cl	H	H	H	H	H	Me
Formicamycin C	H	H	Me	Cl	Cl	H	H	Me	H	Me
Formicamycin D	Cl	H	Me	Cl	Cl	H	H	H	H	Me
Formicamycin E	H	H	Me	H	H	H	H	H	H	Me
Formicamycin F	Cl	H	Me	Cl	H	H	Cl	Me	H	Me
Formicamycin G	H	H	Me	Cl	Cl	H	Cl	Me	H	Me
Formicamycin H	Cl	H	Me	H	Cl	H	Cl	Me	H	Me
Formicamycin I	Cl	H	Me	Cl	Cl	H	Cl	H	H	Me
Formicamycin J	Cl	H	Me	Cl	Cl	H	Cl	Me	H	Me
Formicamycin K	H	H	Me	Cl	Br	H	Cl	Me	H	Me
Formicamycin L	Cl	H	Me	Cl	Br	H	Cl	Me	H	Me
Formicamycin M	H	H	Me	Br	H	H	H	Me	H	Me
Formicamycin N	H	H	Me	Cl	H	H	H	H	H	Me
Formicamycin O	Cl	H	Me	H	H	H	H	H	H	Me
Formicamycin P	H	H	H	Cl	H	H	H	H	H	Me
Formicamycin Q	Cl	H	Me	Cl	H	H	H	Me	H	Me
Formicamycin R	Cl	H	Me	Cl	Cl	H	Cl	Me	Cl	H
Formicamycin S	Cl	H	Me	Cl	Cl	H	Cl	Me	Cl	Me

I.
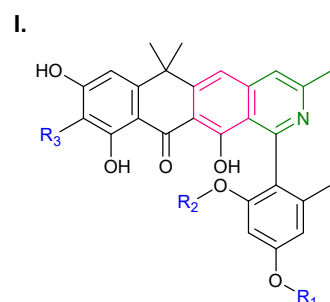
	R_1	R_2	R_3
Formicapyridine A	H	H	H
Formicapyridine B	Me	H	H
Formicapyridine C	Me	Me	H
Formicapyridine D	H	H	Cl
Formicapyridine E	Me	H	Cl
Formicapyridine F	Me	Me	Cl
Formicapyridine G	H	H	Br
Formicapyridine H	Me	H	Br
Formicapyridine I	Me	Me	Br

Figure 1. *Cont.*

J.

	R$_1$	R$_2$	R$_3$	R$_4$	R$_5$	R$_6$	R$_7$	R$_8$
Streptovertimycin A	H	H	H	Me	H	H	Me	H
Streptovertimycin B	Cl	H	H	Me	H	H	Me	H
Streptovertimycin C	H	Cl	H	Me	H	H	Me	H
Streptovertimycin D	H	Cl	H	H	H	H	Me	H
Streptovertimycin E	Cl	Cl	H	H	H	H	Me	H
Streptovertimycin F	H	Cl	Cl	H	H	H	Me	H
Streptovertimycin G	H	Cl	Cl	H	Me	H	Me	H
Streptovertimycin H	Cl	Cl	Cl	H	H	H	Me	H
Streptovertimycin J	H	H	H	Me	H	Me	Me	H
Streptovertimycin K	H	H	H	Me	H	Me	Me	Me
Streptovertimycin L	Cl	H	H	Me	H	Me	Me	Me
Streptovertimycin M	H	OMe	Cl	Me	H	H	Me	H
Streptovertimycin N	H	H	H	Me	H	H	H	Me
Streptovertimycin O	H	H	H	Me	H	H	H	H
Streptovertimycin P	Cl	H	H	Me	H	H	H	H
Streptovertimycin Q	H	Cl	H	Me	H	H	Me	Me
Streptovertimycin R	H	Cl	H	H	H	H	Me	Me
Streptovertimycin S	H	Cl	Cl	Me	H	H	H	H
Streptovertimycin T	Cl	Cl	Cl	Me	H	H	Me	H

K.

Streptovertimycin I

L.

Streptovertidine A: R = H
Streptovertidine B: R = Cl

M.

Streptovertidione

Figure 1. (**A**) Structures of accramycins A–K isolated from *Streptomyces* sp. MA37; (**B**) naphthacemycins A$_1$–A$_{11}$ from *Streptomyces* sp. KB-3346-5; (**C**) naphthacemycins B$_1$–B$_4$ from *Streptomyces* sp. KB-3346-5 and naphthacemycins B$_5$-B$_{13}$ from *Streptomyces* sp. N12W1565; (**D**) naphthacemycins C$_1$–C$_2$ from *Streptomyces* sp. KB-3346-5; (**E**) naphthacemycins D$_1$–D$_2$ from *Streptomyces* sp. N12W15; (**F**) Baeyer–Villiger lactone intermediates A–E from *Streptomyces formicae* Δ*forY* mutant; (**G**) fasamycins A–F, L–Q from *Streptomyces formicae*, fasamycins G–K from *Streptomyces* sp. KIB-1414, and fasamycins R–Y from *Streptomyces kanamyceticus*; (**H**) formicamycins A–M, R–S from *Streptomyces formicae*, formicamycins N–Q from *Streptomyces* sp. KIB-1414, (**I**) formicapyridines A–I from *Streptomyces* formicae; (**J**) streptovertimycins A–T from *Streptomyces morookaense*; (**K**) streptovertimycin I from *Streptomyces morookaense*; (**L**) streptovertidines A-B from *Streptomyces morookaense*; and (**M**) streptovertidione from *Streptomyces morookaense*.

2. Chemical Diversity of PNPs

The biosynthesis of PNP natural products originates from a conserved type II polyketide synthase (PKS) system to generate the phenylnaphthacenoid scaffold (Figure 2A). In brief, a linear polyketide chain is assembled by Claisen condensation of short-chain acyl-CoA, which is catalyzed by the minimal PKS, consisting of the ketosynthase KS$_\alpha$, a chain-length factor (CLF, or KS$_\beta$), and the acyl carrier protein (ACP) [14]. To date, several biosynthetic pathways encoding the PNPs have been reported, including the accramycin biosynthetic gene cluster (BGC) from *Streptomyces* sp. MA37 [1,2,15], fasamycin BGC from environmental DNA (eDNA)(cosAZ154) [3], fasamycin BGC from *Streptomyces kanamyceticus* [16], formicamycin BGC from *S. formicae* [12,17], and naphthacemycin BGC from *Streptomyces* sp. N12W1565 [10] (Figure 2B). Consistent with the very similar structures of fasamycins, accramycins, and naphthacemycin type-B compounds, their biosynthetic genes showed very high sequence homology [1,2,10,12,15,17]. Post-PKS tailoring enzymes are also involved to introduce various structural modifications to the polyketide core, including *O*-methylation and halogenation, plus oxidative and reductive modifications to generate a plethora of PNPs with great structural diversity [1–3,12,15–17]. On the other hand, the NatY enzyme responsible for the installation of the sugar moiety to the

phenolic hydroxyl group of the naphthacemycin core was identified in vitro and is located outside the *nat* gene cluster [10].

A.

B.
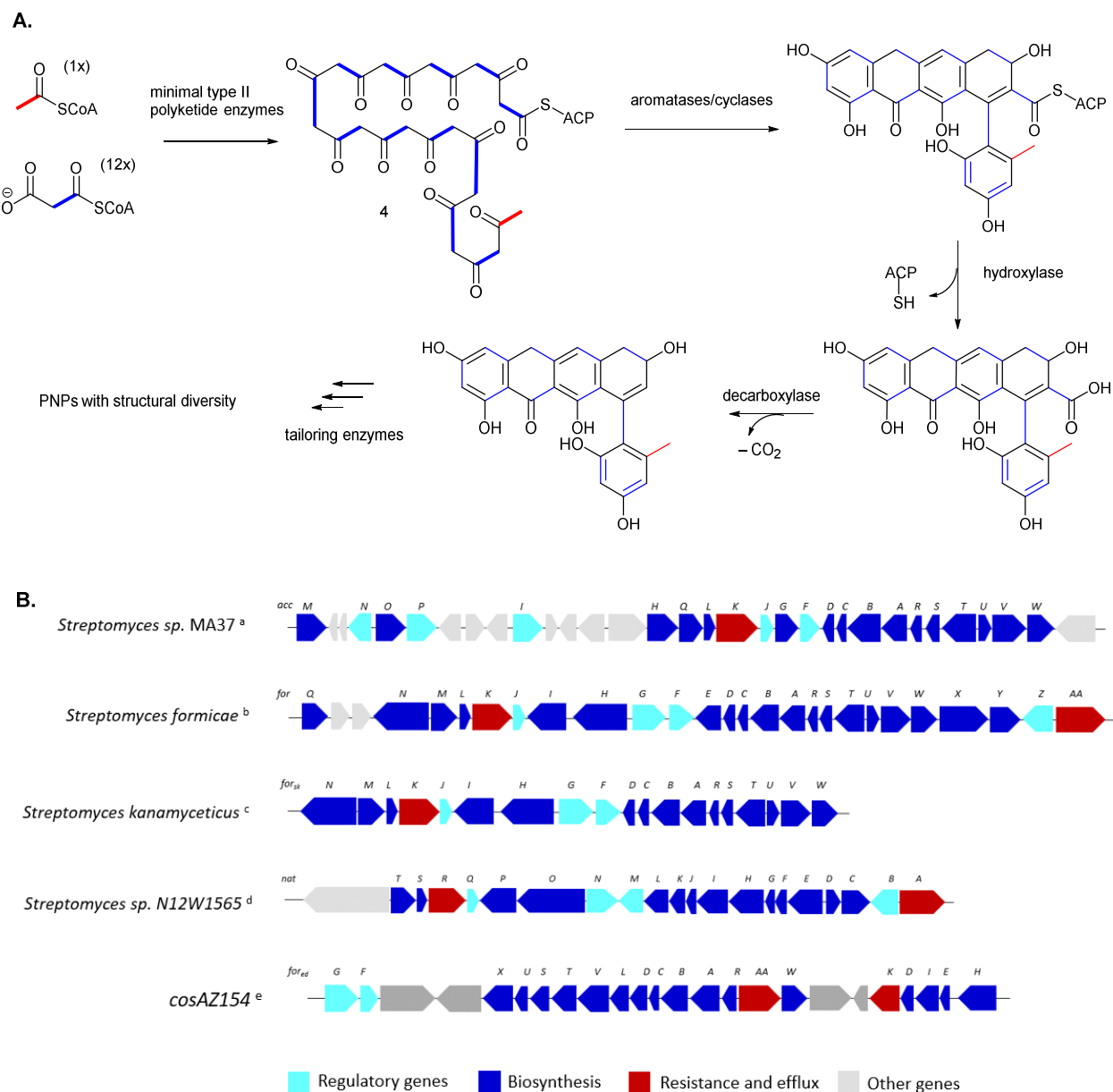
Figure 2. (**A**) The biosynthetic model of type II polyketide backbone formation leading to the ring systems of PNP, and (**B**) comparison of the fasamycin, accramycin, formicamycin, and naphthacemycin biosynthetic gene clusters ([a] from accramycins produced by *Streptomyces* sp. MA37, [b] from formicamycins produced by *Streptomyces formicae*, [c] from fasamycins produced by *Streptomyces kanamyceticus*, [d] naphthacemycin BGC from *Streptomyces* sp. N12W1565, [e] from environmental DNA, containing the fasamycin BGC).

Structure variants with differences in the oxidation state of ring C are commonly encountered in the isolation of PNPs (Figure 1). Accramycins, fasamycins, streptovertimycins, and naphthacemycin B compounds feature a dehyroxyquinol [1,2,4], naphthacemycin A compounds have a quinone motif [6,18], naphthacemycin C molecules consist of a semiquinone-like structure, formicamycins bear a partially oxidized core, and the Baeyer–Villiger intermediates depict a lactone moiety [17]. Streptovertimycin I features a quinone motif in ring E [11]. Naphthacemycins D_1 and D_2 have a glucose moiety linked to ring D

via an *O*-glycosidic bond [10]. Other structural variations observed in ring D include a cleaved ring, such as in streptovertidione, and a rare pyridine moiety in formicapyridines and streptovertidines [6,13]. It is noteworthy that the axial chirality at C10–C21 of all reported streptovertimycin, accramycin, and fasamycin congeners to date was in the *P* (or *S*ₐ) configuration, though they were obtained from different microbial sources [11,12,16]. This stereo configuration is likely a common characteristic of PNP natural products.

3. Cell Factory for Phenylnaphthacenoid Production

Typically, bioactive compounds are generated by the cell factory of the producing microorganism [19]. Several PNPs discovered to date were obtained from the wild-type strains of various *Streptomyces* species (Table 1) [2,6,11,12]. Moreover, the recent advances in microbial cell factory engineering techniques gave rise to a larger number of PNPs with diverse structures and improved product titers [1,17,19]. The next section summarizes the methods for promoting the expression of PNPs (i.e., native strain, mutant strain, heterologous expression, and biocatalytic halogenations) along with their biological activities.

Table 1. Naturally occurring phenylnaphthacenoid polyketide natural products obtained from various *Streptomyces* species and their bioactivity.

	Compound	Producing Organism	Production Method/s	Bioactivity	Ref
1	Accramycin A	*S.* sp. MA37 *S. morookaense*	WT, mutant	G+ MRSA, VRE, MSSA, VSE, A549, HeLa, HepG2, MCF-7, Vero	[1,2,6]
2	Accramycin B	*S.* sp. MA37 *S. kanamyceticus*	mutant heterologous expression	G+	[1,2,16]
3	Accramycin D, E, G, I	*S.* sp. MA37 *S. morookaense*	mutant WT	G+ MRSA, VRE, MSSA, VSE, A549, HeLa, HepG2, MCF-7, Vero	[1,2,6]
4	Accramycin C, F, H, J, K	*S.* sp. MA37	mutant	G+	[1,2]
5	Baeyer–Villiger lactone intermediates	*S. formicae*	mutant	NT	[17]
6	Fasamycin A, B	*S. albus*	heterologous expression	MRSA, VRE	[3,4]
7	Fasamycin C	*S. formicae* *S.* sp. KIB-1414 *S.* sp. MA37 *S. kanamyceticus*	WT mutant heterologous expression	G+ MRSA, VRE	[12] [5] [1] [16]
8	Fasamycin D	*S. formicae*	WT	G+ MRSA, VRE	[12]
9	Fasamycin E	*S. formicae* *S.* sp. KIB-1414	WT	G+ MRSA, VRE	[12] [5]
10	Fasamycin F	*S. formicae*	mutant	NT	[13]
11	Fasamycin G, I, K	*S.* sp. KIB-1414	WT	MRSA	[5]
12	Fasamycin H	*S.* sp. KIB-1414 *S. kanamyceticus*	WT Heterologous expression	G+ MRSA	[5] [16]

Table 1. *Cont.*

	Compound	Producing Organism	Production Method/s	Bioactivity	Ref
13	Fasamycin J	*S.* sp. KIB-1414 *S. kanamyceticus*	WT Enzymatic halogenation	MRSA G+	[5] [16]
14	Fasamycin L–Q	*S. formicae*	mutant	MRSA, MSSA	[20]
15	Fasamycin R–T	*S. kanamyceticus*	Heterologous expression	G+	[16]
16	Fasamycin U–Y	*S. kanamyceticus*	Enzymatic halogenation	G+	[16]
17	Formicamycin A–C, E–G	*S. formicae* *S.* sp. KIB-1414	WT	MRSA, VRE	[12] [5]
18	Formicamycin D, H–M	*S. formicae*	WT	MRSA, VRE	[12]
19	Formicamycin N–Q	*S.* sp. KIB-1414	WT	MRSA	[5]
20	Formicamycin R, S	*S. formicae*	mutant	MRSA, MSSA	[20]
21	Formicapyridine A–I	*S. formicae*	WT mutant	NA	[13]
22	Naphthacemycin $A_1–A_{11}$	*S.* sp. *KB-3346-5*	WT	MRSA, MSSA	[18,21]
23	Naphthacemycin B_1	*S.* sp. *KB-3346-5S.* sp. MA37	WT mutant	MRSA	[18,21,22]
24	Naphthacemycin B_2	*S.* sp. *KB-3346-5S.* sp. N12W1565	WT	MRSA, MSSA	[8,18,21]
25	Naphthacemycin B_3, B_4	*S.* sp. *KB-3346-5*	WT	MRSA, MSSA	[21]
26	Naphthacemycin $B_5–B_{13}$	*S.* sp. N12W1565	WT	PTP1B	[8]
27	Naphthacemycin C_1, C_2	*S.* sp. *KB-3346-5*	WT	MRSA	[21]
28	Naphthacemycin D_1, D_2	*S.* sp. N12W1565	WT	MRSA	[10]
29	Streptovertidine A B	*S. morookaense*	WT	A549, HeLa, HepG2, MCF-7, Vero	[6]
30	Streptovertidione	*S. morookaense*	WT	A549, MCF-7	[6]
31	Streptovertimycin A	*S. morookaense* *S. kanamyceticus*	WT heterologous expression	MRSA, VRE, MSSA, VSE, A549, HeLa, HepG2, MCF-7, Vero	[6,11,16]
32	Streptovertimycin B–T	*S. morookaense*	WT	MRSA, VRE, MSSA, VSE, A549, HeLa, HepG2, MCF-7, Vero	[6,11]

WT: wild type; G+: Gram positive; NA: no activity against *Bacillus subtilis* 168; PTP1B: protein-tyrosine phosphatase 1B; MRSA: methicillin-resistant *Staphylococcus aureus;* MSSA: methicillin-susceptible *Staphylococcus aureus;* VRE: vancomycin-resistant *Enterococci;* VSE: vancomycin-susceptible *Enterococci.*

3.1. Native Strains for Phenylnaphthacenoid Polyketide Production

A myriad array of PNPs have been isolated from the wild-type (WT) strains of various *Streptomyces* bacteria and were given alternative names, such as naphthacemycins, fasamycins, formicamycins, streptovertimycins, and accramycins (Figure 1, Table 1). The earliest identified PNPs were the KB-3346-5 substances reported in the patent literature by Omura et al. in 2009 [9] and then renamed as naphthacemycins in 2017 [18,21]. A total of seventeen naphthacemycins ($A_1–A_{11}$, $B_1–B_4$, and $C_1–C_2$) were isolated from the

culture broth of *Streptomyces* sp. KB-3346-5, all of which showed activity against clinically isolated MRSA (MIC = 8–64 μg·mL^{-1}). Notably, naphthacemycins A$_8$ and A$_9$ displayed comparable anti-MRSA activities to vancomycin antibiotics, and they also inhibited the growth of linezolid-resistant MRSA. Combining naphthacemycins with an imipenem antibiotic enhanced antibacterial activity by 100–500$\times$ against β-lactam-resistant MRSA [18,21]. The addition of naphthacemycins reduced the MIC of imipenem from 32 μg·mL^{-1} to 0.06–0.25 μg·mL^{-1}, breathing new life into old antibiotics that were weakened by the growing antibiotic-resistant strains. Such drug combinations demonstrate an immense potential to treat multidrug-resistant bacterial infections [23]. In 2020, nine new congeners, naphthacemycins B$_5$-B$_{13}$, were isolated from the culture of *Streptomyces* sp. N12W1565. All of the compounds inhibited protein-tyrosine phosphatase 1B (PTP1B) with IC$_{50}$ values of less than 10 μM, indicating that naphthacemycin type-B compounds are promising anti-PTP1B inhibitors [8]. It is noteworthy that the pentachlorinated naphthacemycin B$_{13}$ showed 3.2$\times$ more potent activity (IC$_{50}$ = 0.34 $\pm$ 0.0.06 μM) than the positive control, Na$_3$VO$_4$ (IC$_{50}$ = 1.10 $\pm$ 0.14 μM).

Fasamycins C–E and formicamycins A–M, some of which contain up to four halogen substituents, were identified from *Streptomyces formicae*, isolated from the tree-associated *Tetraponera penzigi* ants [12]. The brominated congeners, formicamycins K–M, were produced by supplementing the culture medium (MS agar) with NaBr elicitor. The compounds effectively inhibited the growth of MRSA, VRE, and *B. subtilis,* with no observed propensity towards resistance in vitro. The potency of the compounds increased with an increasing number of chlorine atoms in the PNP scaffold. Formicamycin congeners with a nonaromatic C ring also appear to be more active than the structurally related fasamycins. Notably, the bromination of formicamycins substantially improved their intrinsic biological activity. The biological effect of halogen atoms on the increased potency of PNPs is probably due to increased lipophilicity resulting in an enhanced ability to cross the bacterial cell membrane [24].

Fasamycins G–K and formicamycins N–Q were discovered from the soil-derived *Streptomyces* sp. KIB-1414. All compounds were active against MRSA, *S. aureus*, *B. subtilis*, and *E. coli* strains (MIC = 0.20–50.00 μg·mL^{-1}) [5]. Streptovertimycins A–H were identified from the wheat-grown cultures of *Streptomyces morookaense* (formerly *Streptoverticillium morookaense*) SC1169 strain [11]. When *S. morookaense* was cultivated on rice, a diverse array of PNP congeners was produced, including streptovertidines A and B, streptovertidione, and streptovertimycins I–T, some of which showed excellent activity against human lung carcinoma (A549), human cervical carcinoma (HeLa), human hepatocellular carcinoma (HepG2), and human breast carcinoma (MCF-7) cell lines [6].

The naphthacemycin repertoire has recently been expanded with two glycosylated molecules, naphthacemycins D$_1$ and D$_2$ isolated from *Streptomyces* sp. N12W1565 [10]. Both compounds showed moderate activity against MRSA and *B. subtilis* (MIC = 16.8–24.4 μg·mL^{-1}) but enhanced aqueous solubility up to 20$\times$ higher than non-glycosylated PNPs. Glycosylation has been a useful approach to enhance the solubility and bioavailability of poorly water-soluble drugs [25].

Formicapyridines are pyridine-containing trace-level metabolites identified from *S. formicae* via a manual curation and bespoke dereplication approach [13]. Their poor productivity in the WT strain indicates that they are shunt products caused by an aberrant derailment in the cyclization step of the fasamycin/formicamycin machinery. Compared to other PNP congeners, formicapyridines are inactive against *B. subtilis*, suggesting that the pyridine moiety diminished its activity.

Accramycin A was isolated from the Ghanaian soil bacterium *Streptomyces* sp. MA37 [2]. Two other accramycin analogs were detected in the extract by MS, UV, and molecular network analyses but could not be isolated due to very low levels of production. Moreover, the single PNP accramycin A isolated from the native MA37 strain was only produced at 0.2 mg·L^{-1} titer.

3.2. Mutant Strains for Phenylnaphthacenoid Polyketides Production

Streptomyces is the largest antibiotic-producing genus to date [26]. However, native strains usually produce these antibiotics in minute quantities, and thus further chemical investigations remain challenging and are a bottleneck to the molecules being advanced for clinical trials. Their production is controlled by several factors, such as nutrition (carbon and nitrogen sources), biosynthetic efficiency, and the complex regulatory mechanisms in producing strains [27].

Microbial cell factories have been engineered to identify new metabolic pathways and design efficient strains to produce pharmaceutically valuable PNPs. This has been achieved in three ways: (1) by manipulating regulatory genes, (2) by engineering PKS biosynthetic enzymes, and (3) by combining mutations in regulatory and biosynthetic genes.

Four regulatory genes are present in the accramycin cluster, including LuxR (AccF), MarR (AccJ), LysR (AccI), and MerR (AccP) transcriptional regulators [1]. These genes were hypothesized to control the productivity of accramycins in the native microbial cultures. Hence, the roles of the four regulatory genes were investigated by inactivating these genes in MA37 via a double crossover homologous recombination approach. One variant (Δ*accJ*) resulted in the overproduction of accramycin A up to 330-fold higher than WT levels, indicating that the *accJ* gene is a negative regulator in accramycin biosynthesis. Furthermore, the Δ*accJ* engineered strain produced new accramycin congeners (B–K) and other small polyketide molecules that were identified for the first time from bacteria [15]. MarR (multiple antibiotic resistance)-family regulators, such as *accJ*, repress the transcription of their target genes by binding specifically to DNA sequences within promoter regions [28]. All accramycin congeners displayed potent activity against Gram-positive bacteria, including *Enterococcus faecium* (K59-68 and K60-39) and *Staphylococcus haemolyticus* clinical isolates (MIC = 3.1–12.5 μg·mL^{-1}). ForJ, a homolog of AccJ, in formicamycin biosynthesis was also shown to repress the production of formicamycins [20]. Deletion of *forJ* switched biosynthesis from solid agar to liquid cultures, increased formicamycin titers, and induced the production of new congeners (formicamycins R and S, each bearing five chlorine atoms) in engineered *S. formicae* strains. Both molecules exhibited potent antibacterial activity against MSSA and MRSA (MIC = 2 μg·mL^{-1}).

PKS tailoring enzymes have been engineered to understand metabolic pathways and expand the chemical space of PNPs. Formicamycin BGC has an additional set of four grouped unique genes (*forX*, *forY*, *for Z,* and *forAA*) compared to other PNP-producing gene clusters (Figure 2B) [12]. Deletion of the flavin-dependent monooxygenase ForX abrogated formicamycin production and lead to the accumulation of fasamycin E [17]. Deletion of the flavin-dependent reductase ForY also abolished formicamycin biosynthesis and led to the accumulation of new Baeyer–Villiger (BV)-derived lactone intermediates (Figure 1). Therefore, fasamycin molecules are biosynthetic precursors of the formicamycins, and ForX and ForY enzymes are required for this two-step conversion which proceeds via ring expansion to form the BV intermediates followed by a unique reductive ring contraction Favorskii-type reaction.

The production of the shunt metabolites, formicapyridines, increased approximately 25-fold relative to the WT by deletion of the antibiotic biosynthesis monooxygenase (ABM) ForS enzyme [13]. Meanwhile, the production titer of formicamycins in the Δ*forS* mutant was reduced to about one-third that of the WT strain. The Δ*forS* mutant also produced a minor congener, Fasamycin F, which harbors a carboxylic acid group at C-11, indicating that the deletion of ForS in *S. formicae* did not abolish polyketide production (Figure 1). This congener was not observed in any WT cultures. Hence, ForS is not involved in ring-D (pyridine) cyclization as initially perceived nor in the biosynthesis of the *for* BGC polyketide backbone but rather in the maintenance of pathway productivity and fidelity. ForS acts as a chaperone or fidelity factor that ensures that the biosynthetic mechanism proceeds efficiently.

Combining mutations in genes encoding the biosynthetic machinery and regulation resulted in the biosynthesis of additional PNP precursors. Deletion of the halogenase *forV* gene in Δ*forJ* mutant in *S. formicae* (Δ*forJ*Δ*forV*) leads to the accumulation of nonhalogenated fasamycin congeners. Particularly, fasamycin C production is elevated 90-fold higher than the total fasamycins produced by the WT strain [20]. On the other hand, deletion of the *forX* gene involved in the ring expansion during the transformation of fasamycins to formicamycins in Δ*forJ* mutant (Δ*forJ*Δ*forX*) abolished formicamycin production but accumulated chlorinated fasamycins including six new congeners (fasamycins L-Q) (Figure 1). All these congeners showed potent antibacterial activity against MSSA and MRSA (MIC = 2–4 $\mu g \cdot mL^{-1}$). Furthermore, the product titers on solid cultures were increased to approximately 120 times higher than the WT titers.

Two-component systems (TCSs), consisting of sensor kinase and response regulator proteins, are one of the major ways in which bacteria respond and adapt to ever-changing environmental conditions [29]. The large genomes of *Streptomyces* harbor a high number of TCSs compared to other bacterial genera, and the majority have been shown to control secondary metabolism and morphological development [29,30]. The deletion of the TCS ForGF in *S. formicae* diminished fasamycin and formicamycin production, indicating that ForGF is required for the transcription of the *for* BGC [20]. On the other hand, overexpression of ForGF in a Δ*forJ* mutant improved formicamycin yield on solid cultures 10-fold compared to the WT strain. Therefore, cluster-situated TCSs, such as the ForGF, represent a promising target for overexpression to activate silent or cryptic BGCs and obtain higher efficiencies in secondary metabolite production.

3.3. Heterologous Expression for Phenylnaphthacenoid Polyketide Production

Nature holds an enormous amount of microbial diversity with remarkable biosynthetic potential; however, the majority remains recalcitrant to conventional culturing methods. With the recent advances in culture-independent approaches and metagenomics, a large number of unculturable or poorly culturable microbes hitherto became accessible for functional analysis [31]. Functional metagenomics has been used to isolate and characterize small molecules and proteins from soil environmental DNA (e-DNA) metagenomes. Among the rare skeletons isolated using this strategy were fasamycins A and B obtained by the heterologous expression of clones from soil-derived PKS e-DNA cosmid (cosAZ154) in *Streptomyces albus* (Figure 1, Table 1) [3].

The structure of fasamycin B differs from A in the presence of an additional chlorine substituent at carbon-3. Fasamycins A and B showed activity against MRSA and VRE by inhibiting FabF during type II fatty acid elongation [3,4]. The mono-chlorinated fasamycin A (MIC = 0.8 $\mu g \cdot mL^{-1}$) is almost eight times more active than the *di*chlorinated fasamycin B (MIC = 6.25 $\mu g \cdot mL^{-1}$) against VRE, indicating that the specific halogenation pattern is critical for potent antimicrobial efficacy. Both compounds, however, were inactive against yeast or Gram-negative bacteria at the highest concentration tested (50 $\mu g \cdot mL^{-1}$) [3].
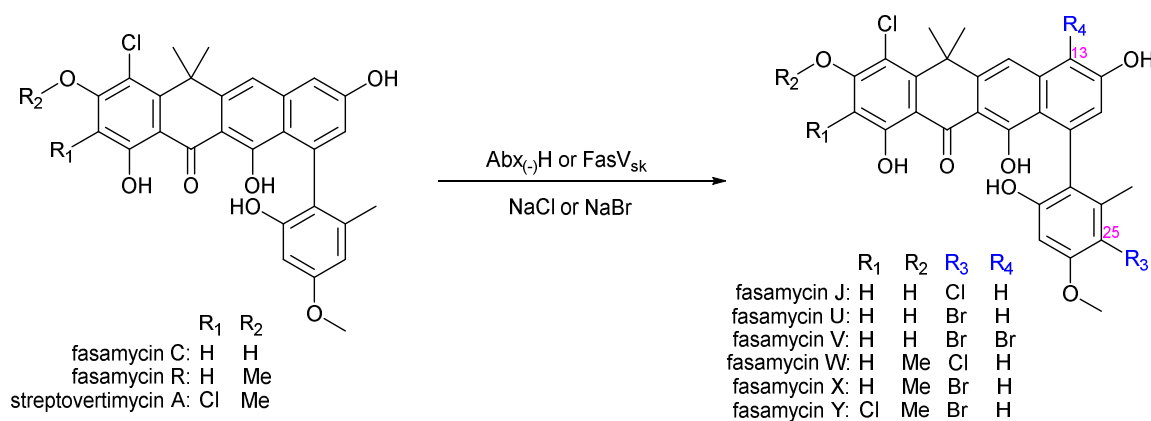
In another study, heterologous expression was used to awaken silent or cryptic biosynthetic pathways to produce medically useful compounds. Three new fasamycin congeners (R–T) along with four known fasamycin-type analogs were obtained via the overexpression of two phosphopantetheinyl transferases (PPtases) in *S. kanamyceticus* [16]. PPtases are responsible for the installation of a 4′-phosphopantetheine arm from Coenzyme A to various carrier proteins (CPs), an essential step in the biosynthesis of PKS, NRPS, and fatty acids (FAs) [32]. All congeners showed potent activity against *B. subtilis* and *S. aureus* (MIC = 1.6–12.5 $\mu g \cdot mL^{-1}$) [16].

3.4. Biocatalytic Halogenations of the Phenylnaphthacenoid Scaffold

Biocatalytic halogenations have been important and valuable reactions in organic synthesis owing to their regio- and stereoselectivity, and they are carried out by enzymes called halogenases or haloperoxidases [33,34]. Halogenases enable the selective installation of halogen atoms in chemical scaffolds of varying complexity under mild reaction conditions.

The introduction of a halogen atom into a molecule can effectively alter its properties (e.g., bioactivity and pharmacokinetic profile), yielding bioactive compounds of industrial and pharmacological significance [34,35].

The fasamycin BGC in *S. kanamyceticus* encodes a single halogenase $FasV_{sk}$, which shows high homology to $FasV_{ed}$ from the fasamycin BGC of eDNA (59.8%), AccV of the accramycin BGC (67.5%), and ForV of the formicamycin BGC (92.8%). The $FasV_{sk}$ was expressed in *Escherichia coli* and its catalytic efficiency was tested in vitro using fasamycin C, fasamycin R, and streptovertimycin R as substrates (Figure 3). Similarly, the halogenase $Abx_{(-)}H29$ in anthrabenzoxocinone (ABX) biosynthesis, which has been shown to introduce halogen modifications into the ABX scaffolds was also investigated in vitro [36]. Both halogenases $FasV_{sk}$ and $Abx_{(-)}H29$ specifically installed the halogen substituent into the C-13 and/or C-25 positions of fasamycins, different from the specific halogenation patterns observed in formicamycins or accramycins [16]. The enzymatic halogenation reactions yielded six new fasamycin congeners (fasamycins J and U-Y), further expanding the bioactive repertoire of PNP scaffolds. An antimicrobial assay of the fasamycins showed that the halogenation profoundly enhanced their antibiotic activity, among which the *di*brominated fasamycin V is four times more potent (MIC = 1.6 $\mu g \cdot mL^{-1}$) than the non-halogenated fasamycin C (MIC = 6.3 $\mu g \cdot mL^{-1}$) against *B. subtilis* and *S. aureus*.

	R_1	R_2	R_3	R_4
fasamycin J:	H	H	Cl	H
fasamycin U:	H	H	Br	H
fasamycin V:	H	H	Br	Br
fasamycin W:	H	Me	Cl	H
fasamycin X:	H	Me	Br	H
fasamycin Y:	Cl	Me	Br	H

Figure 3. Enzymatic halogenation of fasamycins to produce new congeners.

In another study, a chemoenzymatic approach was used to introduce the chlorine substituent in naphthacemycin B_1 to form halogenated PNPs [37]. The retrosynthesis of naphthacemycin B_1 was carried out in a convergent manner by combining two fragments in a Sammes annulation strategy followed by the installation of the pendant phenyl ring by Suzuki coupling. Naphthacemycin B_1 was then subjected to an enzymatic halogenation reaction using FasV and ForV halogenases in combination with five FAD reductases, namely Fre, SfFre (a Fre homolog from *S. formicae*), SsuE, CtcQ, and HpaC. Mass spectrometric analysis of the reaction mixture revealed monochlorinated and *di*chlorinated PNPs with fasamycin A as the major product as confirmed by comparison with an authentic standard. Among the combinations tested, the FasV/CtcQ pair showed the highest conversion to fasamycin A though the overall chlorination yield was very low.

4. Challenges and Future Opportunities

Phenylnaphthacenoid polyketides possess potent antibacterial and anticancer activities with a high barrier to the development of resistance, which demonstrates immense potential as lead candidates for investigation as a new structural class of antibiotics. However, the inadequate supply of naturally derived antimicrobials has been a major limiting factor in their preclinical and clinical development. In another case, the expression of PNPs in liquid cultures has been a significant hurdle in *S. formicae* [20]. Indeed, this has been a bottleneck since the majority of industrial antibiotic production is produced in bioreactors in liquid cultures rather than in solid media [38]. Engineering microbial cell factories offer

tremendous potential in inducing biosynthesis in liquid cultures, improving product titers, and expanding the chemical space. The deletion of MarR regulatory genes, *accJ* and *forJ*, in accramycin and formicamycin biosynthesis, respectively, enabled the production of PNPs in higher yields and the expression of new analogs [1,2,20]. In addition, engineered *S. formicae* strains produced PNPs not only in solid but also in liquid cultures.

Refactoring of complex biosynthetic pathways in heterologous hosts has also enabled the generation of cell factories that are optimized for producing new PNP congeners [3,16]. Biocatalysts, such as halogenases, have produced new chlorinated and brominated PNP derivatives although, in some cases, the yield is very low. Future study is therefore geared towards engineering enzymes and understanding their optimum reaction conditions. It is noteworthy that the bromination (and in most cases chlorination) of PNP molecules rendered them more bioactive [6,12]. Hence, the characterization of halogenases that can selectively introduce a halogen atom into desired positions in the PNP scaffold is of significant interest to access antimicrobials with increased potencies.

Two is better than one as demonstrated in the combined activity of naphthacemycin and imipenem against β-lactam-resistant MRSA [18,21]. Such drug combinations offer better efficacy than single agents and are therefore an avenue for further exploration to combat multidrug-resistant superbugs. Further research is also needed to understand the molecular mechanisms underlying them. In the face of the growing threat of AMR with a dry antibiotic pipeline, it is more critical than ever to find other treatment options [23,39].

Looking forward, further characterization of PKS-encoding genes involved in PNP pathways will uncover new antibiotics, pave the way for a greater understanding of their complex biosynthetic mechanisms, and establish new strategies for metabolic engineering. The characterization of TCS genes could unveil cryptic antibiotics that are not expressed under standard laboratory conditions. Understanding the novel mechanism involved in reductive ring contraction catalyzed by ForY in formicamycin biosynthesis could produce further improved molecules with potent bioactivities.

The biosynthesis of nanoparticles in *Streptomyces* is an emerging trend in bionanotechnology [40,41]. Nanoparticles have wide applications in therapeutics and medicine, drug and gene delivery, bioimaging and biosensors, cancer nanotechnology, and the chemical industry [42,43]. Because *Streptomyces* is the largest antibiotic-producing genus to date [44], it comprises efficient candidates for the biological synthesis of metal nanoparticles [40]. Exploiting the biosynthetic potential of PNP-producing *Streptomyces* to produce diverse nanomaterials is therefore an exciting field to explore! We expect that with the recent advances in science and technology, there will be increasing momentum in the discovery and development of nanoparticle systems in the coming years.

5. Conclusions

Taken together, the discovery, structural diversity, bioactivity, and cell factory for metabolite production highlighted in this review demonstrate the huge potential of the phenylnaphthacenoid class of natural products in modern medicine. PNPs could prove invaluable in the fight against resistant superbugs. More than 100 members of PNPs with diverse structures and biological activities have been isolated from various *Streptomyces* species to date. These compounds have been obtained from native strains, mutant strains, heterologous expression, and biocatalytic modifications. There are exciting opportunities ahead!

Conflicts of Interest: The authors declare no conflict of interest.

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