# Chapter 5
# Fermentative Production of Bacterial Phenazines

**Sudhir Chincholkar, Sandeep Patil, Prashant Sarode and Makarand Rane**

**Abstract** Phenazines, a nitrogen containing heterocyclic antibiotic biosynthesized by a diverse range of bacteria. Owing its enormous importance as (1) electron shuttles to alternate terminal acceptors in bacteria, (2) modify cellular redox states to modify host response, (3) contributing to biofilm formation and cell signaling, as well as (4) biotechnological applications such as environmental sensor, microbial fuel cell, antitumor, and biocontrol activity attracted attention of scientific community to target phenazine as lead molecule. Similarly, emerging application of phenazines insisted high productivity fermentative process. Current chapter focuses on sources of natural phenazines and impact of nutritional as well as environmental dynamics on fermentative production of phenazine in different bacteria.

## 5.1 Introduction

Phenazines cover nitrogen containing colored redox active heterocyclomers of biological and chemical origin. More than 6,000 phenazine derivatives have been described with one or other bioactivity (Mavrodi et al. 2006; Pierson and Pierson 2010). However, nearly 100 natural phenazines were reported exclusively from bacteria of diverse vicinity. Based on the types and position of functional groups present on structure, phenazines were known for a long time as pigments and antifungal/antibacterial compounds (Schoental 1941; Haynes et al. 1956; Mann 1970).

S. Chincholkar (✉) · S. Patil · P. Sarode · M. Rane
Department of Microbiology, School of Life Sciences, North Maharashtra University, Jalgaon, Maharashtra 425001, India
e-mail: sudhirchincholkar@gmail.com

S. Chincholkar and L. Thomashow (eds.), *Microbial Phenazines*,
DOI: 10.1007/978-3-642-40573-0_5, 

Chemical synthesis of phenazines renders toxic chemicals including, aniline, azobenzoate, lead oxide, o-phenylenediamine which are potentially toxic as well as this process, (1) shows relatively less productivity, (2) needs harsh reaction process, and (3) produce toxic by-product. Whereas, selective phenazine synthesis can be possible using bacterial fermentation with added advantage of noncytotoxicity and growth limiting potential for eukaryotes, hence can be used as effective therapeutic agent for eukaryotic organisms (Laursen and Nielsen 2004). Also natural phenazine derivative have proven to be the more effective biocontrol agent than synthetic one (Nansathit et al. 2009). Thus, natural phenazine can always be a choice of selection for wide applications.

Phenazine production has been studied in different bacterial strains including florescent *Pseudomonas* (Mavrodi et al. 2006; Maddula et al. 2008; Li et al. 2008; Shanmugaiah et al. 2010), *Streptomyces* sp. (Gebhardt et al. 2002; Clinton et al. 1993; Zendah et al. 2012; Ohlendorf et al. 2012; Fotso et al. 2010; Kondratyuk et al. 2012), *Bacillus* sp. B-6 (Kim 2000; Li et al. 2007), *Brevibacterium* (Podojilt and Gerber 1967), *Burkholderia* (Mavrodi et al. 2006), and archae *Methanosarcina mazei Gö*1 (Abken et al. 1998; Beifuss et al. 2000) and few others. Among all phenazine producers *Pseudomonas* sp. and *Streptomyces* sp. have been studied at metabolic and genomic level. It was observed that the structural complexity of phenazines increase from *Pseudomonas* sp. to S*treptomyces* sp. (Saleh et al. 2009) and *Methanosarcina mazei* (Beifuss et al. 2000) as long side chain where it served as final electron acceptor in electron transport chain (Abken et al. 1998; Beifuss et al. 2000). The change in structure related to change in phenazine properties.

The extra ordinary potential of phenazines is due to their physicochemical properties, i.e., oxidation–reduction (redox) and their bright pigmentation, which changes with pH and redox state (Pierson and Pierson 2010). These prosperities have been used for biotechnological applications such as (1) biocontrol agent (Rane et al. 2007a, b), (2) microbial fuel cell (Torres et al. 2010; Sanderson et al. 1987), (3) organic light emitting devices (OLED) like phenanthroline-fused phenazine (Chen and Xiao-Chang 2004), (4) antitumor agent (Laursen and Nielsen 2004; Mavrodi et al. 2006; Kondratyuk et al. 2012), (5) biosensor like glucose sensor (Ohfuji et al. 2004), $H_2O_2$ (Santos et al. 2005), (6) biocolor for dyeing silk fabrik (Saranya et al. 2012), (7) mineral reduction (Hernandez et al. 2004), (8) oil degradation (Norman et al. 2004), (9) anticandidal (Morales et al. 2010), (10) food colorent (Saha et al. 2008) etc.

## 5.2 Phenazine Production

Broad range of bioactivity and applicability left impact on researcher to increase the productivity of phenazine from laboratory to fermentation scale production using potential phenazine producer. A wide variety of phenazines are biosynthesized by bacteria are given as follows.

### 5.2.1 Pseudomonad Phenazines

*Pseudomonas* is foremost phenazine producing bacteria with almost one-third of all known phenazines. Among all bio-chemo origin phenazines, pyocyanin was the first isolate one by Fordos in the 1850s from isolated *Pseudomonas*. Till date, fluorescent pseudomonads are the best studied phenazine producer which includes *Pseudomonas aeruginosa*, *P. fluorescens*, and *Pseudomonas chlororaphis.* Production profiling of *P. aeruginosa*, include (1) phenazine-1-carboxylic acid (PCA) (Rane et al. 2007a, b; Mavrodi et al. 2006); (2) phenazine-1-carboxamide (PCN) (Shanmugaiah et al. 2010), (3) pyocyanin (PYC) (Ra'oof and Latif 2010; Kaleli et al. 2006); (4) 1-hydroxyphenazine (1-OHPHZ) (Kerr et al. 1999), (5) Aeruginosin A and B (Holliman 1969; Herbert and Holliman 1969), etc. While, *P. chlororaphis* was reported for production of (1) PCA, (2) orange-colored 2-Hydroxyphenazine-1-carboxylic acid, (3) brick red-colored 2-Hydroxyphenazine. More than one phenazine derivative can be produced by *P. aeruginosa* and *P. chlororaphis* depending upon the genetic and environmental makeup (Mavrodi et al. 2006). *P. fluorescens* have found to produce only PCA production (Mavrodi et al. 2006). *P. aeruginosa* is the only known species capable of producing the very distinctive water-soluble pigment pyocyanin (Gohain et al. 2006), however pyocyanin negative *P. aeruginosa* strains are also reported (Mavrodi et al. 2006).

### 5.2.2 Streptomyces Phenazines

A vast diversity of phenazines was noticed in *Streptomyces* sp. like endophenazines (Gebhardt et al. 2002), diphenazines (Ding et al. 2011), phenazinomycin, D-alanylgriseoluteic acid (Giddens and Bean 2007), Geranylphenazinediol (Ohlendorf et al. 2012), esmeraldin, and saphenamycin (Clinton et al. 1993), also been studied till the date.

Apart from these major groups' phenazines like pelagiomicins A/B/C, myxin, PCA have been reported from *Pelagiobacter variabilis*, *Sorangium* sp. and *Bacillus* sp. respectively.

## 5.3 Phenazine Regulation and Environmental Factors

Phenazine biosynthesis is based on the phz gene expression, which turn on or off and allowing control of phenazine production (Linares et al. 2006). Different nutritional (carbon and nitrogen), metal (iron and phosphate), and environmental/process (pH, oxygen) parameters were found to regulate phz gene expression (Slininger and Jackson 1992; Slininger and Shea-Wibur 1995; Siddiqui and Shaukat 2004; van Rij et al. 2004).

Many reports had suggested, growth rate and phase-dependent phenazine production in pseudomonads, i.e., maximum phenazine production at late exponential and early stationary growth phase, while comparatively less product accumulation in early and mid-exponential phase (Chin-A-Woeng et al. 2001). With most important concern, quorum sensing, cell density dependant genome regulation are the most influencing factor for PCA and PCN production in *P. aureofaciens* (Pierson et al. 1995) and PCN in *P. chlororafis* (Chin-A-Woeng et al. 2005), respectively. The PCA molecule is thought to be the precursor for all other phenazine derivative (Gohain et al. 2006). Addition of exogenous PCA, as a precursor molecule in fermentation medium showed enhanced phenazine production in *P. chlororaphis* GP72, postulating that exogenous PCA may act as final electron acceptor and autoinducer providing more energy for bacterial growth and metabolite production (Huang et al. 2011).

## 5.4 Nutritional Requirement for Phenazine Production

Media components of production media specify the productivity of the metabolite during fermentation. Pierson and Pierson (2010) had suggested phenazine production is depending upon the nutritional condition. Hence, qualitative and quantitative effect of nutrients in production medium has to be optimised during process optimization studies. Depending upon the type of phenazine producer nutrient condition was found to be changing. Although phenazine production is aged process, however, the assessments of its nutritional as well as fermentation parameters for its production are still not well documented.

### *5.4.1 Nutritional Factor for Pseudomonad*

Different media were studied earlier for pseudomonad phenazines production like (1) Pigment Production Medium D (Kluyver 1956), (2) Synthetic medium (Chang and Blackwood 1969), (3) Alanine medium (Frank and DeMoss 1959; Meyer and Abdallah 1978), (4) 1 % Casamino Acids-salts medium (Whooley and McLoughlin 1982), etc. Detailed of these media (Table 5.1) suggests requirement of sodium and potassium metal requirement during phenazine fermentation. Similarly, except 1 % Casamino Acids-salts medium other media comprising glycerol nutrition for phenazine production.

Yuan et al. (2008) showed glucose and soytone as influencing factors for PCA production in *Pseudomonas* sp. M-18q using Plackett Burman design (PBD) where, increasing the glucose concentration and decreasing the soytone concentration result in increasing the accumulation and secretion of PCA in growth medium (Yuan et al. 2008). Similarly, effect of carbon and nitrogen source on gacA-deficient *Pseudomonas* sp. M18G mutant suggest the glucose as influencing

**Table 5.1** Phenazine production media and producer

| Media | Ingredient | Organism | Phenazine | Reference |
|---|---|---|---|---|
| Pigment production medium D (PPMD) | Peptone, sodium chloride, glycerol, $KNO_3$ | *P. aeruginosa* ID 4365 | PCA | Rane et al. (2007a, b) |
| Synthetic medium | $MgCl_2.6H_2O$, $Na_2SO_4$, $FeSO_4.7H_2O$, $K_2HPO_4$, urea, glycerol | *P. aeruginosa* Mac 436 | PCA, PYC, oxychlororaphine | Chang and Blackwood (1969) |
| Alanine medium | $MgCl_2.6H_2O$, $Na_2SO_4$, ferric citrate, $K_2HPO_4$, DL-alanine, glycerol | *P. aeruginosa* | PYC | Frank and DeMoss (1959) |
| 1 % Casamino acids-salts medium | Casamino acids, NaCI, KC1, $K_2HPO_4$, $CaC1_2{\cdot}2H_20$, $MgSO_4{\cdot}7H_20$, tris (hydroxymethyl) methylamine, trace element | *P. aeruginosa* ATCC 10145 | PYC | Whooley and McLoughlin (1982) |
| NA | Glucose, glycerol, starch, corn steep powder, casein peptone, yeast extract, NaCl, $CaCO_3$ | *S. anulatus* | Endophenazine A–D PCA | Gebhardt et al. (2002) |
| TSB 10, modified trypticase soy broth | BD trypticase soy broth, NaCl, $CaCO_3$ | *Streptomyces* sp. HB202 | Streptophenazin | Mitova et al. (2008) |
| GYM1 | Glucose, yeast extract, malt extract | *Streptomyces* sp. HB202 | Streptophenazin | Mitova et al. (2008) |
| GYM2 | Glucose, yeast extract, malt extract | *Streptomyces* sp. HB202 | Streptophenazin | Mitova et al. (2008) |
| M1 | Peptone, meat extract | *Streptomyces* sp. HB202 | Streptophenazin | Mitova et al. (2008) |
| LB | Tryptone, yeast extract, NaCl | *Streptomyces* sp. HB202 | Streptophenazin | Mitova et al. (2008) |
| MB | Difco marine broth | *Streptomyces* sp. HB202 | Streptophenazin | Mitova et al. (2008) |
| Soybean–mannitol medium | Soybean flour, mannitol | *S. diastaticus* YIM DT26 | Phenazinolins A–E | Ding et al. (2011) |

(continued)

**Table 5.1** (continued)

| Media | Ingredient | Organism | Phenazine | Reference |
|---|---|---|---|---|
| GOT medium | Glycerol, oatmeal, tomato paste, $CaCO_3$ | *Streptomyces* sp. ICBB8198 | Griseolutein A<br>Griseoluteic acid<br>Compound 2<br>Compound 3 | Fotso et al. (2010) |
| M2 medium | Malt extract, yeast extract, glucose | *Streptomyces* sp. Ank 315 | Chromophenazines A–F | Zendah et al. (2012) |
| SPD medium | Starch, peptone from soymeal, artificial sea salt | *Streptomyces* sp. strain LB17 | Geranylphenazinediol | Ohlendorf et al. (2012) |
| NA | $KH_2PO_4$, $(NH_4)2SO_4$, $MgSO_4{\cdot}7H_2O$, $FeSO_4{\cdot}7H_2O$, biotin, octanoic acid | *Bacillus* sp. B-6 | PCA | Kim (2000) |
| Marine broth | Peptone, yeast extract, $FeSO_4$ | *Bacillus* strain 39 | Compound 1 | Li et al. (2007) |
| NA | $K_2HPO_4$, KH2PO4, $NH_4Cl$, $MgSO_4{\cdot}7H_20$, $CaCl_2{\cdot}2H_20$, NaCl, $FeSO_4{\cdot}7H_20$, resazurin, vitamin solution, trace elements, yeast extract (Difco), casitone (Difco), $NaHCO_3$, methanol, cysteine hydrochloride, $Na_2S{\cdot}9H_20$ | *Methanoscina mazei Göl* | Methanophenazine | Abken et al. (1998), Beifuss et al. (2000) |

*NA* not assign

carbon source; likewise yeast extract (0.28 %) was found most influencing factor for growth and soy peptone for maximum PCA production (He et al. 2008). The PCA biosynthesis at optimised conditions in *Pseudomonas flurescence* 2–79 have found to be accelerated by glucose as carbon source with unnoticeable influence of nitrogen source (Slininger and Shea-Wilbur 1995). Similarly, zinc sulfate, ammonium molybdate, and cytosine as micronutrition had shown increased PCA biosynthesis in *P. flurescence* 2–79 (Slininger and Jackson 1992).

Effect of individual amino acids on PCN production studied in *P. chlororaphis* PCL1391 illustrated the influence of aromatic amino acids and casamino acids (van Rij et al. 2004). In another study, Fusaric acid, a self defensive molecule of *Fusarium oxysporum* was shown to suppress the PCN production which can be overcome by presence of phenylalanine in *P. chlororaphis* PCL1391 (van Rij et al. 2005).

Labeyrie and Neuzil (1981) showed enhanced growth rate as well as enhanced pyocyanin secretion of *P. aeruginosa* A237 in amino acids (tyrosine and phenylalanine) supplemented media. The concentration of glycerol and paraffins was found to stimulate the production of pyocyanin and phenazine derivatives in *P. aeruginosa* (MacDonald 1967). The selective and increased production of PCA and PCN was achieved by Byng et al. (1979) using *m* or *p*-aminobenzoic acid as selective inhibitor of PYC specifically inhibit of phenazine methylation.

### *5.4.2 Nutritional Factors for Streptomyces*

As like pseudomonad phenazines various media were exploited for *Streptomyces* phenazine production which mainly includes, (1) soybean–mannitol medium (Ding et al. 2011), (2) GOT medium (Fotso et al. 2010), (3) M2 medium (Zendah et al. 2012), (4) SPD medium (Ohlendorf et al. 2012), (5) TSB 10, modified Trypticase Soy broth (Mitova et al. 2008), (6) GYM1 (Mitova et al. 2008), (7) GYM2 (Mitova et al. 2008), (8) M1 (Mitova et al. 2008), (9) LB (Mitova et al. 2008), (10) MB (Mitova et al. 2008). In all studied media (Table 5.1) a complex protein source was used like casein peptone, soy protein digest, yeast extract, and oatmeal. The change in streptophenazine biosynthesis in presence of antibiotics like, i.e., tetracycline and bacitracin in fermentation medium suggest two- to threefold increase in synthesis (Mitova et al. 2008). In marine *Streptomyces* sp., subinhibitory concentrations of antibiotics were found to enhance and modulate the production of new phenazines, i.e., streptophenazines A–H (Mitova et al. 2008).

The phenazine production media for *Bacillus* sp. as mentioned in Table 5.1 by Kim (2000) and Marine broth (Li et al. 2007) stipulate iron (Ferrous) requirement for phenazine secretion apart from other nutrition factors. Phenazine production using *Bacillus* strain 39 deep sea sediment isolate, has also been studied in marine broth by Li et al. (2007).

Very few reports have been seen in case of methanophenazine (Abken et al. 1998; Beifuss et al. 2000) where a complex media (Table 5.1) was tried for phenazine production from *Methanoscina mazei Gö1*.

## 5.5 Fermentative Conditions of Phenazine Production

Environmental and process parameters, i.e., temperature, pH, and dissolved oxygen, respectively affect growth and hence secondary metabolites secretion. Change in pH condition, i.e., from neutral to slight acidic or alkaline minimizes the phenazine production while phenazine production at pH 7 has given considerable phenazine yield, while optimum pH-control during late phase of fermentation has been studied with effective PCA production (Li et al. 2010). pH and temperature sensitive PCA production was detected in *P. flurescence* 2–79, where optimum productivity was recorded at pH 7 and 25–27 °C with greater cell density (Slininger and Jackson 1992).

Influenced of abiotic environmental factors, i.e. pH, $O_2$ exchange and temperature on PCN production in *P. chlororaphis* PCL1391, showed PCN productivity was increased at 1 % oxygen and at low magnesium concentrations, while noticeable decrease observed at pH 6 and temperature 16 °C. Also, production of autoinducer during cell growth in pseudomonas is influenced by cell density, which directly affects the PCN production positively by increasing the PCN yield during fermentation process (Rij et al. 2004; Mavrodi et al. 2006).

In current scenario insufficient known knowledge is available about DO requirement for phenazine production during fermentation. However, reports claimed that the higher productivity of PCA fermentation yielded at 20 % of DO with optimized agitation and aeration condition. However, increased in DO (50 %) drastically decrease the PCA yield during fermentation, which might be due to cell lysis caused by increased agitation condition and unsuitable pH (Li et al. 2010).

## 5.6 Phenazine Productivity

PCA production in *Pseudomonas* sp. M18G gacA mutant showed 30-fold increase productivity from 0.02 to 0.6 $gL^{-1}$ compared to the wild-type strain (Ge et al. 2004). With optimum glucose and yeast extract in fermentation media increased PCA production from 673.3 up to 966.7 $\mu gmL^{-1}$ in *Pseudomonas* sp. M18G gacA mutant (He et al. 2008). The same strain at optimized fermentation conditions after 60 h of incubation showed 1.89 $gL^{-1}$ of PCA production (Li et al. 2008). Rane et al. (2007a, b) had recovered 18 gm of crystalline PCA at large-scale fermentation (125 L working volume in synthetic medium) from *P. aeruginosa* ID 4365. Phenazine production in *Bacillus* sp. B-6 yielded 400 $\mu gmL^{-1}$ PCA in chemically defined medium (Kim 2000).

## 5.7 Conclusion

Characteristics of phenazine and its derivative opened its applicability in different biotechnological segments. Current research in evaluation of new properties and its exploitation of same warrants economic fermentative production of phenazines. In the same regard, this chapter rationalizes the factors influencing phenazine fermentation. To a great extent, nutritional and environmental factors supporting growth as well as phenazine production have been discussed in different Pseudomonads and *Streptomyces* strains. The future debate on alone phenazine gene expression will certainly lead to techno economic production of selective phenazines.

## References

Abken HJ, Tietze M, Brodersen J et al (1998) Isolation and characterization of methanophenazine and function of phenazines in membrane-bound electron transport of *Methanosarcina mazei* Gö1. J Bacteriol 180:2027–2032

Beifuss U, Tietze M, Bäumer S et al (2000) Methanophenazine: structure, total synthesis, and function of a new cofactor from methanogenic archaea. Angew Chem Int Ed 39:2470–2472

Byng GS, Eustice DC, Jensen R (1979) Biosynthesis of phenazine pigments in mutant and wild-type cultures of *Pseudomonas aeruginosa*. J Bacteriol 138:846–852

Chang PC, Blackwood AC (1969) Simultaneous production of three phenazine pigments by *Pseudomonas aeruginosa* Mac 436. Can J Microbiol 15:439–444

Chen JP, Xiao-Chang CL (2004) Organic light-emitting device having phenanthroline-fused phenazine. US Patent 6713781

Chin-A-Woeng TFC, van den Broek D, de Voer G et al (2001) Phenazine-1-carboxamide production in the biocontrol strain *Pseudomonas chlororaphis* PCL1391 is regulated by multiple factors secreted into the growth medium. Mol Plant Microbe Interact 14:969–979

Chin-A-Woeng TFC, van den Broek D, Lugtenberg BJJ et al (2005) The *Pseudomonas chlororaphis* PCL1391 sigma regulator psrA represses the production of the antifungal metabolite phenazine-1-carboxamide. Mol Plant Microbe Interact 18:244–253

Clinton W, Mocek U, Floss HG (1993) Biosynthesis of the phenazine antibiotics, the saphenamycins and esmeraldins, in *Streptomyces* an *tibioticus*. J Org Chem 58:6576–6582

Ding ZG, Li MG, Ren J et al (2011) Phenazinolins A–E: novel diphenazines from a tin mine tailings-derived *Streptomyces* species. Org Biomol Chem 9:2771–2776

Fotso S, Santosa DA, Saraswati R et al (2010) Modified phenazines from an Indonesian *Streptomyces* sp. J Nat Prod 73:472–475

Frank L, DeMoss R (1959) On the biosynthesis of pyocyanine. J Bacteriol 77:776–782

Ge YH, Huang XQ, Wang SL et al (2004) Phenazine-1-carboxylic acid is negatively regulated and pyoluteorin positively regulated by gacA in *Pseudomonas* sp. M18. FEMS Microbiol Lett 237:41–47

Gebhardt K, Schimana J, Krastel P et al (2002) Endophenazines A-D, new phenazine antibiotics from the arthropod associated endosymbiont *Streptomyces anulatus.* I. Taxonomy, fermentation, isolation and biological activities. J Antibiot 55:794–800

Giddens SR, Bean DC (2007) Investigations into the in vitro antimicrobial activity and mode of action of the phenazine antibiotic D-alanylgriseoluteic acid. Int J Antimicrob Agents 29:93–97

Gohain N, Thomashow LS, Mavrodi DV et al (2006) The purification, crystallization and preliminary structural characterization of PhzM, a phenazine-modifying methyltransferase from *Pseudomonas aeruginosa*. Acta Crystallogr, Sect F: Struct Biol Cryst Commun 62:887–890

Haynes WC, Stodola FH, Locke JM et al (1956) *Pseudomaons aureofaciens* Kluyver and phenazine α-carboxylic acid, its characteristic pigment. J Bacteriol 72:412–417

He L, Xu YQ, Zhang XH (2008) Medium factor optimization and fermentation kinetics for phenazine-1-carboxylic acid production by *Pseudomonas* sp. M18G. Biotechnol Bioeng 100:250–259

Herbert RB, Holliman FG (1969) Pigments of *Pseudomonas* species. Part II. Structure of aeruginosin B. J Chem Soc C 19:2517–2520

Hernandez ME, Kappler A, Newman DK (2004) Phenazines and other redox-active antibiotics promote microbial mineral reduction. Appl Environ Microbiol 70:921–928

Holliman FG (1969) Pigments of *Pseudomonas* species. Part I. Structure and synthesis of aeruginosin A. J Chem Soc C 19:2514–2516

Huang L, Chen MM, Wang W et al (2011) Enhanced production of 2-hydroxyphenazine in *Pseudomonas chlororaphis* GP72. Appl Microbiol Biotechnol 89:169–177

Kaleli I, Demir M, Cevahir N et al (2006) Serum neopterin levels in patients with replicative and nonreplicative HBV carriers. BMC Infect Dis 6:157

Kerr JR, Taylor GW, Rutman A et al (1999) *Pseudomonas aeruginosa* pyocyanin and 1-hydroxyphenazine inhibit fungal growth. J Clin Pathol 52:385–387

Kim KJ (2000) Phenazine 1-carboxylic acid resistance in phenazine 1-carboxylic acid producing *Bacillus* sp. B-6. J Biochem Mol Biol 33:332–336

Kluyver AJ (1956) *Pseudomonas aureofaciens* nov. spec., and its pigments. J Bacteriol 72:406–411

Kondratyuk TP, Park EJ, Yu R et al (2012) Novel marine phenazines as potential cancer chemopreventive and anti-inflammatory agents. Mar Drugs 10:451–464

Labeyrie S, Neuzil E (1981) Addition de tyrosine ou de phénylalanine aux cultures de *Pseudomonas aeruginosa*: influence sur la croissance microbienne et la pigmentation. Ann Microbiol 132:31–40

Laursen JB, Nielsen J (2004) Phenazine natural products: biosynthesis, synthetic analogues, and biological activity. Chem Rev 104:1663–1685

Li D, Wang F, Xiao X et al (2007) A new cytotoxic phenazine derivative from a deep sea bacterium *Bacillus* sp. Arch Pharmacal Res 30:552–555

Li YQ, Jiang HX, Xu YQ et al (2008) Optimization of nutrient components for enhanced phenazine-1-carboxylic acid production by gacA-inactivated *Pseudomonas* sp. M18G using response surface method. Appl Microbiol Biotechnol 77:1207–1217

Li Y, Jiang H, Dua X et al (2010) Enhancement of phenazine-1-carboxylic acid production using batch and fed-batch culture of gacA inactivated *Pseudomonas* sp. M18G. Bioresour Technol 101:3649–3656

Linares JF, Gustafsson I, Baquero F et al (2006) Antibiotics as intermicrobial signaling agents instead of weapons. Proc Natl Acad Sci USA 103:19484–19489

MacDonald JC (1967) Pyocyanine. In: Gottlieb D, Shaw PD (eds) Antibiotics. Biosynthesis. Springer, Berlin, pp 52–65

Maddula VS, Pierson EA, Pierson LS (2008) Altering the ratio of phenazines in *Pseudomonas chlororaphis* (*aureofaciens*) strain 30-84: effects on biofilm formation and pathogen inhibition. J Bacteriol 190:2759–2766

Mann S (1970) Zur identifizierung und redoxfunktion der pigmente von *Pseudomonas aureofaciens* und *P. iodine*. Arch Microbiol 71:304–318

Mavrodi DV, Blankenfeldt W, Thomashow LS (2006) Phenazine compounds in fluorescent *Pseudomonas* spp. biosynthesis and regulation. Annu Rev Phytopathol 44:417–445

Meyer JM, Abdallah MA (1978) The fluorescent pigment of *Pseudomonas fluorescens*: biosynthesis, purification and physicochemical properties. J Gen Microbiol 107:319–328

Mitova MI, Lang G, Wiese J et al (2008) Subinhibitory concentrations of antibiotics induce phenazine production in a marine *Streptomyces* sp. J Nat Prod 71:824–827

Morales DK, Jacobs NJ, Rajamani S et al (2010) Antifungal mechanisms by which a novel *Pseudomonas aeruginosa* phenazine toxin kills *Candida albicans* in biofilms. Mol Microbiol 78:1379–1392

Nansathit A, Apipattarakul S, Phaosiri C et al (2009) Synthesis, isolation of phenazine derivatives and their antimicrobial activities. Walailak J Sci Tech 6:79–91

Norman RS, Moeller P, McDonald TJ et al (2004) Effect of pyocyanin on a crude-oil-degrading microbial community. Appl Environ Microbiol 70:4004–4011

Ohfuji K, Sato N, Hamada-Sato N et al (2004) Construction of a glucose sensor based on a screen-printed electrode and a novel mediator pyocyanin from *Pseudomonas aeruginosa*. Biosens Bioelectron 19:1237–1244

Ohlendorf B, Schulz D, Erhard A et al (2012) Geranylphenazinediol, an acetylcholinesterase inhibitor produced by a *Streptomyces* species. J Nat Prod 75:1400–1404

Pierson LS, Pierson EA (2010) Metabolism and function of phenazines in bacteria: impacts on the behavior of bacteria in the environment and biotechnological processes. Appl Microbiol Biotechnol 86:1659–1670

Pierson LS 3rd, Gaffney T, Lam S et al (1995) Molecular analysis of genes encoding phenazine biosynthesis in the biological control bacterium. *Pseudomonas aureofaciens* 30-84. FEMS Microbiol Lett 134:299–307

Podojilt M, Gerber NN (1967) The biosynthesis of 1,6-phenazinediol 5, lO-dioxide (iodinin) by *Brevibacterium iodinum*. Biochemistry 6:2701–2705

Rane MR, Sarode PD, Chaudhari BL, Chincholkar SB (2007a) Foliar application of *Pseudomonas* metabolite protects *Capsicum annum* (chilli) from fungal phytopathogens. Bionano Frontier 1:46–53

Rane MR, Sarode PD, Chaudhari BL, Chicholkar SB (2007b) Detection, isolation and identification of phenazine-1-carboxylic acid produced by biocontrol strains of *Pseudomonas aeruginosa*. J Sci Ind Res 66:627–631

Ra'oof WM, Latif IAR (2010) *In vitro* study of the swarming phenomena and antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from different human infections. Eur J Sci Res 47:405–421

Saha S, Thavasi R, Jayalakshmi S (2008) Phenazine pigments from *Pseudomonas aeruginosa* and their application as antibacterial agent and food colourent. Res J Microbiol 3:122–128

Saleh O, Gust B, Boll B et al (2009) Aromatic prenylation in phenazine biosynthesis: dihydrophenazine-1-carboxylate dimethylallyltransferase from *Streptomyces anulatus*. J Biol Chem 284:14439–14447

Sanderson DG, Gross EL, Seibert M (1987) A photosynthetic photoelectrochemical cell using phenazine methosulfate and phenazine ethosulfate as electron acceptors. Appl Biochem Biotechnol 14:1–12

Santos AS, Dura′n N, Kubotaa LT (2005) Biosensor for $H_2O_2$ response based on horseradish peroxidase: effect of different mediators adsorbed on silica gel modified with niobium oxide. Electroanalysis 17:1103–1111

Saranya R, Jayapriyaa J, Tamilselvi A (2012) Dyeing of silk fabric with phenazine from *Pseudomonas* species. Color Technol 128:440–445

Schoental R (1941) The nature of the antibacterial agents present in *Pseudomonas pyocyanea* culture. Brit J Exp Pathol 22:137–147

Shanmugaiah V, Mathivanan N, Varghese B (2010) Purification, crystal structure and antimicrobial activity of phenazine-1-carboxamide produced by a growth promoting biocontrol bacterium, *Pseudomonas aeruginosa* MML2212. J Appl Microbiol 108:703–711

Siddiqui IA, Shaukat SS (2004) Liquid culture carbon, nitrogen and inorganic phosphate source regulate nematicidal activity by fluorescent pseudomonads in vitro. Lett Appl Microbiol 38:185–190

Slininger PJ, Jackson MA (1992) Nutritional factors regulating growth and accumulation of phenazine 1-carboxylic acid by *Pseudomonas fluorescens* 2–79. Appl Microbiol Biotechnol 37:388–392

Slininger PJ, Shea-Wibur MA (1995) Liquid-culture pH, temperature, and carbon (not nitrogen) source regulate phenazine productivity of the take-all biocontrol agent *Pseudomonas fluorescens* 2–79. Appl Microbiol Biotechnol 43:794–800

Torres CI, Marcus AK, Lee HS et al (2010) A kinetic perspective on extracellular electron transfer by anode-respiring bacteria. FEMS Microbiol Rev 34:3–17

Van Rij ET, Wesselink M, Chin-A-Woeng TF et al (2004) Influence of environmental conditions on the production of phenazine-1-carboxamide by *Pseudomonas chlororaphis* PCL1391. Mol Plant-Microbe Interact 17:557–566

Van Rij ET, Girard G, Lugtenberg BJJ et al (2005) Influence of fusaric acid on phenazine-1-carboxamide synthesis and gene expression of *Pseudomonas chlororaphis* strain PCL1391. Microbiology 151:2805–2814

Whooley MA, McLoughlin AJ (1982) The regulation of pyocyanin production in *Pseudomonas aeruginosa*. Eu J Appl Microbiol and Biotechnol 15:161–166

Yuan LL, Li YQ, Wang Y et al (2008) Optimization of critical medium components using response surface methodology for phenazine-1-carboxylic acid production by *Pseudomonas* sp. M-18Q. J Biosci Bioeng 105:232–237

Zendah I, Riaz N, Nasr H et al (2012) Chromophenazines from the terrestrial *Streptomyces* sp. Ank 315. J Nat Prod 75:2–8