Review Article

Open Access Journal of
Biomedical Science
ISSN: 2690-487X

Biological activity and applications of pyocyanin produced by *Pseudomonas aeruginosa*

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ABSTRACT

There is growing interest in microbial pigments due to their natural character, safe to use, medicinal properties and rich in nutrients like vitamins. Production of these pigments is independent of season and geographical condition. Moreover, microbial pigments can be produced from waste material reducing water and environmental pollutions. *Pseudomonas aeruginosa* produce a wide variety of pigments as secondary metabolites, which play an important role in interactions between *Pseudomonas* species and other organisms. Four major different pigments have been described in *P. aeruginosa* produce variety of redox-active phenazine compounds, including pyocyanin, fluorescein, pyorubrin and pyomelanin. Pyocyanin is a chloroform soluble blue green phenazine pigment produced by active cultures of *P. aeruginosa*. Pyocyanin has antibiotic activity against bacteria, fungi and protozoa. About 90 to 95% of *P. aeruginosa* strains produce pyocyanin which was the main phenazine pigment associated with organism and had powerful antimicrobial, antioxidant and anticancer activities.

KEYWORDS: Pyocyanin; *Pseudomonas aeruginosa*; Antimicrobial; Antioxidant; Anticancer

ABBREVIATIONS: SSV: Soyabean Stunt Virus; MFCs: Microbial fuel cells; ROS: Reactive Oxygen Species; SAM: S-adenosyl Methionine; PCA: Phenazine-1-carboxylic Acid

PYOCYANIN

Pyocyanin is a blue redox-active secondary metabolite and a member of the large family of the tricyclic compounds, phenazines. They are secreted at the late stationary phase and provide a characteristic blue color to the medium. Because of its solubility in chloroform it can be easily isolated from culture medium. Chemically, pyocyanin is 5-methyl-1-hydroxyphenazine and can undergo complex series of oxidation-reduction reaction [1]. The elucidation of the structure of pyocyanin represents the first reported instance of the occurrence of the phenazine nucleus as a natural product. Pyocyanin can exist in either oxidized or reduced form, the latter being an unstable form of pyocyanin that reacts rapidly with molecular oxygen [2].

Biosynthesis of Pyocyanin

Two steps have been suggested the synthesis of pyocyanin from phenazine-1-carboxylic acid (PCA), which is the common precursor for many different species-specific phenazines. There are two reaction strategies by which PCA can be converted to pyocyanin. The first, PCA is first acted upon by the enzyme PhzM, S-adenosyl methionine (SAM) dependent methyltransferase and gets converted to 5-methylphenazine-1-carboxylic acid betaine

Quick Response Code:

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Received: December 20, 2019 **Published:** January 22, 2020

How to cite this article: Diaa A Marrez and Haitham SM. Biological activity and applications of pyocyanin produced by *Pseudomonas aeruginosa*. 2020 - 2(1) OAJBS.ID.000133.

DOI: 10.38125/OAJBS.000133

by transfer of a methyl group to an N atom of the phenazine-ring moiety. This is followed by the action of the enzyme PhzS, a FAD-dependent monooxygenase, which involves the hydroxylative decarboxylation of 5-methylphenazine-1-carboxylic acid betaine to pyocyanin [3]. On the other hand, in the other reaction strategy leading to the biosynthesis of pyocyanin, PCA first gets converted to hydroxyphenazine in presence of the enzyme PhzS followed by its methylation to pyocyanin in presence of the SAM dependent methyltransferase, PhzM [4].

The synthesis of pyocyanin is affected by carbon and nitrogen sources in growth media, but most nutrients support pyocyanin production as long as the phosphate ion concentration is low and there is adequate sulfate ion present. Synthesis of this pigment also appears to be under the control of iron concentration since addition of iron to a medium containing low phosphate stimulates the synthesis of pyocyanin and related phenazine pigments by other species of bacteria [5].

Mode of Action of Pyocyanin Against Target Cells

The potent antagonistic action of pyocyanin was identified as the result of its unique redox potential by accepting a single electron, yielding a relatively stable anion radical and readily undergoing a redox cycle. During respiration, pyocyanin becomes reduced and unvaliantly reduces oxygen to the toxic superoxide radical. Accordingly, the antibiotic action of pyocyanin might be an expression of the toxicity of the O_2- and of H_2O_2 produced in increased amounts in its presence [6]. The resistance of various bacteria to pyocyanin would therefore be dependent upon the levels of superoxide dismutase and catalase possessed by the organism and on the presence of oxygen [7].

Hassan & Fridovich [6] described a mechanism for the toxicity of pyocyanin whereby electron flow from biological pathways is diverted to increase the production of intracellular O_2 reduction products, leading to cell death. It is of interest that *P. aeruginosa*, a "strict" aerobe, is itself insensitive to pyocyanin and seemingly escapes free-radical injury during production of or exposure to this compound [2]. Physiological studies have shown that *P. aeruginosa* resists the toxicity of this compound with increased superoxide dismutase and catalase activities under pyocyanin-producing conditions [8]. Baron & Rowe [7] reported that the antimicrobial action of pyocyanin was bactericidal in nature and the effect was dependent on pyocyanin concentration.

Biological Activity of Pyocyanin

Pyocyanin is a good example of a secondary metabolite, which has antibiotic activities and able to co-ordinate the response of microbial communities to changes in the environment. The mechanism by which pyocyanin inhibits bacterial growth was investigated and it was concluded that, pyocyanin interacts with the cell membrane respiratory chain resulting in the inability of the bacterial cells to perform their active metabolic transport process [8,9]. Pyocyanin has variety of pharmacological effects on eukaryotic and prokaryotic cells [10] and, had antimicrobial activity against bacteria, fungi and protozoa [11]. The pyochelin and pyocyanin act synergistically to produce active oxygen species that cause cell damage and it subsequently leads to induced resistance [12].

Antibacterial activity of Pyocyanin

Saha et al. [13] reported that nearly 90-95% of antimicrobial inhibitions of *P. aeruginosa* strains were due to production of the water-soluble secondary metabolite pyocyanin. It showed antagonistic activity against pathogenic bacteria like *Salmonella paratyphi, E. coli, Klebsiella pneumonia*. Pyocyanin isolated from *P. aeruginosa* 4B strain showed antibiotic activities against various pathogens and food spoilage bacteria like *Listeria monocytogens* and *Bacillus cereus*. The secondary metabolite along with various enzymes like haemolysin and hydrolytic enzymes played a key role for their antimicrobial activities [14].

Rahman et al. [15] revealed that pyocyanin from *P. aeruginosa* DSO-129 has antimicrobial effect on organisms like *S. aureus, Staphylococcus epidermis, Bacillus subtilis, Micrococcus luteus* and *Saccharomyces cerevisiae*. There has been another report related to the antibiotic activity of pyocyanin against different pathogens. The pigment produced by the strain showed very effective activity against organisms like *E. coli*, *Acinetobacter, S. aureus* and *Streptococcus pneumonia* [16]. Pyocyanin from *P. aeruginosa* has antibacterial activity toward competing bacteria including indole-producing *E. coli* [2]. Also, Sudhakar et al. [17] reported that pyocyanin from *P. aeruginosa* SU1against *E. coli, S. aureus, Proteus* sp., *Klebsiella* sp. and *Pseudomonas* sp. The maximum activity was seen against *E. coli, S. aureus, Proteus* sp., *Klebsiella* sp., whereas *Pseudomonas* sp. showed resistance.

Antifungal Activity of Pyocyanin

Pyocyanin inhibit the growth of various *Candida* spp. and *Aspergillus fumigatus*; pyocyanin may also inhibit yeast-mycelial transformation in *C. albicans* [18]. Pyocyanin induced triggering systemic resistance against Fusarium wilt of tomato [12]. Pyocyanin has antifungal activity against *A. niger* [18]. Pyocyanin isolated from *P. aeruginosa* also inhibited the growth of fungi like Aspergillus fumigatus and Candida albicans isolated from the sputum of CF patients [19]. There was a clinical evidence that pyocyanin suppressed the growth of different species of *C. albicans* in patients with lung infection. Reoccurring of *C. albicans* was noticed after the suppression of pyocyanin. Substances like pyocyanin, pyrrolnitrin and pseudomonic acid produced by *P. aeruginosa* showed antibiotic actions *in vivo* on *Candida* species grown on Sabroud's Dextrose Agar [20,21]. Signal mediated interactions between *P. aeruginosa* and *C. albicans* in CF patients was found. The presence of N-acyl homoserine lactones (HSLS) produced by *P. aeruginosa* affected the morphology of *C. albicans*. In the same way *C. albicans* inhibited the swarming motility of *P. aeruginosa*. When *P. aeruginosa* was cocultured with *C. albicans*, the former synthesised large amounts of pyocyanin and even the growth of *C. albicans* was inhibited [22-24]. Sudhakar *et al*. [17] reported the production of pyocyanin from *P. aeruginosa* WS1 and its antagonistic activity against commonly encountered phytopathogens. The MIC of pyocyanin was further analysed against phytopathogens and was found to be 64 µg ml^{-1} against *Aspergillus flavus* and *Aspergillus fumigates*, and 128 µg ml^{-1} against *Candida* species.

Antioxidant Activity of Pyocyanin

Rada et al. [25] indicated that pyocyanin has several toxic effects on host cells, including depletion of NADH, glutathione, decomposing hydrogen peroxidase and other antioxidant in the host cell an alteration of the redox status and generation of oxygen radicals. Laxmi & Bhat [26] reported that pyocyanin isolated from *P. aeruginosa* (BTRY1) were significant as higher radical scavenging activities at concentration very much lower than the ascorbic acid.

Anticancer Activity of Pyocyanin

Hassani et al. [27] reported that the cytotoxicity of pyocyanin produced by both strains *P. aeruginosa* PHA-1 and mutant S300-8, against cancer cell line RD and normal cell line REF revealed that low concentrations (7.81–31.25 µg ml^{-1}) of had low activity on RD cell line with inhibition rate ranged from 28 to 36% after 24 hrs of incubation. Also, it noticed that toxic efficiency of pyocyanin against RD cell line was increased at the highest concentrations (250 and 500 µg ml^{-1}), the inhibition rate was 60% and 64%, respectively after 72 hrs of incubation. While, pyocyanin produced by mutant strain S300-8 was more efficient against growth of cancer cell line RD that produced by PHA-1. Pyocyanin inhibit growth of RD and achieved 65% of dead cells at low concentration, 7.81 µg ml^{-1}, and notable an increase on growth inhibition of RD cell line 88% after 72 hrs of incubation at pyocyanin concentration, 500 µg ml^{-1}. In contrast, pyocyanin either produced by PHA-1or S300-8 had no effectiveness on the viability of REF normal cells at all period of incubation.

Zhao et al. [28] revealed that pyocyanin had significantly inhibition against HePG2 cancer cells line proliferation and triggered the production of large amounts of reactive oxygen species (ROS), thereby up regulating superoxide dismutase (SOD) and catalase. In addition to depleted reduced glutathione (GSH) and decrease the GSH/oxidized GSH ratio. Muller et al. [29] reported that pyocyanin inhibited the proliferation of human dermal fibroblasts cell line. Also, Laxmi & Bhat [26] indicated that pyocyanin isolated from *P. aeruginosa* (BTRY1) had reduced hemolytic activity even at concentration 1 µg ml^{-1}. Also, reported that that the pyocyanin was not cytotoxic against normal cells line at concentrations ranged from 6.25 to 100 µg ml^{-1}. It was observed that the cells showed almost 90% viability after pyocyanin treatment 6.25 µg ml^{-1}. The cell viability of around 80% even at high concentrations indicating its safety of use in food consumption for human.

Applications of Pyocyanin

Biological applications

Phenazine compounds that produced by *Pseudomonas* spp. were known to possess a wide spectrum antimicrobial activity toward bacteria, fungi and eukaryotic cells. Also, several phenazine compounds showed antitumor, antimalaria and antiparasitic activities [30-31]. The antimicrobial activities of phenazine had been used in sustainable agriculture as a biocontrol agent against some food spoilage and pathogenic bacteria and fungi [32,33]. So, these compounds suitable to restrain microbes in agricultural and pharmaceutical application [34]. Phenazine was used to suppression of *Erwinia amylovora* which causes fire blight disease in apple flowers [35] and used as natural suppression of *Fusarium* wilt disease [36].

Pyocyanin was required for prevention of disease symptoms in plants and killing of nematode *Caenohabditis elegans* and the fruit fly *Drosophila melanogaster* [37,38]. Various plant roots colonizing *Pseudomonas* spp. had been shown to be potent microbiological control agents in various plant pathogen systems [39]. In 1986 found that phenazine compounds inhibit mycelia growth of several fungal pathogens of plants, phenazine produced by the root colonizing bacteria *P. fluorescens* and *P. aureofaciens*, had a dominant role in the control of take all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* [40,41]. phenazine had broad spectrum action against active and dormant structure of fungal phathogen *Pythium aphanidermatum* and root knot nematode *Meloidogyne inconita*. Phenazine derivatives were also used to reduce the chemical pesticides in agriculture which could be used either alone or in combination with pesticides to lower the doses of chemicals needed to obtain a profitable crop yield [42].

In medical applications, phenazine and their derivatives used in antifungal activity against variety of microorganisms e.g., *Candida albicans* and *Aspergillus fumigatus*, the risk factor of pulmonary candidiasis in patients [19]. Phenazine also known as tubermycin B because its antibiotic activity against *Mycobacterium tuberculosis*, which causing pneumonia and often fatal infections in susceptible patient population [43]. Also, Allen et al. [44] indicated that phenazine production leads to reduced chemokine and cytokine production by reduced neutrophil numbers and accelerated neutrophil apoptosis, which were associated with impaired bacterial clearance. Active proliferation of human lymphocytes was inhibited by pyocyanin [45]. The development of synthetic anticancer phenazine derivatives was an ongoing area of research aimed at combining known phenazine biological activities with increased target specificity towards cancer cells [46].

Biotechnological Applications

Microbial fuel cells (MFCs) use microorganisms to catalyze the conversion of chemical energy into electrical energy [47]. An ongoing issue with MFCs was that the slow rate of electron transfer from the microorganism to the anodic electrode limits MFC efficiency. Sanderson et al. [48] reported that phenazine methosulfate or phenazine ethosulfate served as good electron acceptors in photoelectrochemical cells. Rabaey et al. [49] revealed that addition of pyocyanin to MFC-containing *Brevibacillus* sp. PTH1 doubled the rate of electron transfer. The addition of a PCA-producing *P. chlororaphis* or a derivative that produces high levels of PCN to a mixed MFC also resulted in higher electron transfer rates [50].

Beneficial *Pseudomonas* species reach root surfaces chemotactically by flagella motility and colonise them. It was found that *Pseudomonas* also promotes the plant growth. The root and rhizosphere offer an ecological niche [51]. The interaction of *P. aeruginosa* with plants as a beneficial association was found to be quite common. *P. aeruginosa*, produces pyocyanin which is present in the rhizosphere soil and other sources. In soil it promotes direct plant growth and protects plants from the phytopathogens [52-54]. Ali Siddiqui et al. [55] reported the use of rhizobacteria in the control of root rot and root knot disease complex of mungbean. The organism isolated from the rhizosphere was found to be *P. aeruginosa* which acted as a bio-control agent in inhibiting the growth of *Macrophomina phaseolina*, *Fusarium solani* and *Rhizoctonia solani*. *Pseudomonas* also showed nematicidal activity in killing the second stage larvae of *Meloidogyne javanica*.

The role of pyocyanin from *P. aeruginosa* 7NSK2 strain, in inducing resistance to *Botrytis cinerea* that causes infection in tomato and grapevine was demonstrated by Audenaert et al. [56]. Also, Anjaiah et al. [57] reported that pyocyanin from *Pseudomonas* species isolated from rhizosphere soil were used as biocontrol agent against *Fusarium*, the causative agent of wilt of chickpea and Pythium damping of bean. Sunish kumar et al. [58] revealed that pyocyanin produced by particular strains of *P. aeruginosa* strain PUPa3 showed biocontrol activity against a wide range of phytopathogenic fungi that infect rice, groundnut, tobacco, chilli,

mango, sugarcane, tea, cotton and banana crops. The minimal inhibitory concentration of pyocyanin was found to be 29 µg ml^{-1} for *Sclerotium rolfsii* NCM1084, which also inhibited the growth of phytopathogens such as *Aspergillus niger* NCIM 1025, *Fusarium oxysporum* NCIM 1008, *S. rolfsii* NCIM1084 and *Colletotricum falcatum* [43].

De Vleesschauwer et al. [59] reported the use *P. aeruginosa* 7NSK2 strain as a biocontrol agent against the leaf blast (*Magnaporthe grisea*) and sheath blight (*R. solani*) in the monocot model rice plant. *P. aeruginosa* 7NSK2 was treated in root which protected rice against leaf blast leaving unprotected from sheath blight. The pyocyanin produced by 7NSK2 enhanced the production of H_2O_2 in roots and leaves, which degraded the toxic enzyme from the plants. Onbasli & Aslim [60] found that pyocyanin from Pseudomonas inhibits the *E. coli* isolates from sugar beet molasses. *Pseudomonas* strains isolated from the rhizosphere plant were used to treat against various species of *Fusarium, Ralstonia* and *Meloidogyne* which cause wilting disease in *coleus* and *ashwagandha* species [61]. The mechanism involved was studied and it was suggested that siderophore, HCN, indole acetic acid, pyocyanin and other volatile metabolites synthesized by *Pseudomonas* strain exhibits the biocontrol effect and they can also act as plant growth enhancers.

Khamdan & Suprapta [62] found that *P. aeruginosa* act as very good biopesticides against soyabean stunt virus (SSV). *P. aeruginosa* has been isolated from rhizospheres of soyabean and formulated in various forms such as liquid formulation, together with polyacrylamide hydrogel. The formulations were effective in plant growth and increased resistance against SSV also increases the yield, chlorophyll content and peroxidase activity. The study concluded that the application of *P. aeruginosa* formulation was effective against SSV.

CONCLUSION

Pigments that produced as secondary metabolites to protect microorganisms from injurious effect of light rays of visible and near ultraviolet range had also several biological activities. Pyocyanin showed antibacterial and antifungal activity against several pathogenic bacteria and mycotoxigenic fungi also possess antioxidant and anticancer activity against cancer cell lines. These finding can be used as base for food industries as food preservation and in pharmaceuticals applications.

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