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Challenges and Opportunities with Pyocyanin Pigment of *Pseudomonas aeruginosa*

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Abstract: Microbial secondary metabolites are the organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism but very useful for human health. These are formed during the stationary phase (idiophase) of culture, also known as idiolites such as pigments, toxins, alkaloids, antibiotics, etc, and have a very unusual structure. Production of secondary metabolites occurred when there is depletion of one or more nutrients in the culture medium. Microbial pigments such as carotenoids, melanin, prodigiosin, violacein, pyocyanin, etc, are natural colors that hold promising potential to meet various challenges in the pharmaceutical and nutraceutical sector. Pyocyanin is one of many natural pigments, a blue-colored redox-active secondary metabolite, which is produced by *Pseudomonas aeruginosa*. Several virulence factors are secreted by *P. aeruginosa* that can contribute to its pathogenicity and pyocyanin pigment is one of them. It causes oxidative stress to the host, disrupting host catalase, mitochondrial electron transport, induces apoptosis in neutrophils as well as inhibits the phagocytosis of apoptotic bodies by macrophages. It is challenging for anti-virulence drug intervention to control the pathogenesis of *P. aeruginosa* to some extent by targeting the biosynthetic pathway of pyocyanin. Besides this, pyocyanin pigment possesses antioxidant, antimicrobial, anti-inflammatory, anticancerous, and immunosuppressive properties. This article aims to describe challenges posed by pyocyanin pigment of *P. aeruginosa* and its potential applications in various fields of biotechnology.

Keywords: Secondary metabolites, microbial pigments, pyocyanin, virulence, antioxidant, anticancerous, immunosuppressive, biodegradability.

Introduction

Humans use secondary metabolites as medicines, flavorings, and biocontrol agents. Pigments are the secondary metabolites with numerous of importance in various industrial fields. The newfound awareness in human safety and environmental conservation has kindled fresh enthusiasm for natural sources of colors. Colors provide magnetize appearance to marketable products such as textiles, food products, and pharmaceutical products. *Pseudomonas aeruginosa* are Gram-negative, aerobic rod-shaped bacteria, motile by single polar flagellum [117]. It is an aquatic and soil bacterium [46,52,53,88,107,128] that can affect a range of organisms and an opportunistic pathogen capable of infecting immunocompromised human [104]. An important advantage of *P. aeruginosa* depends on its ability to secrete and release compounds with an inhibitory effect on other bacteria, fungi, protozoa, etc. These properties explain the capacity of *P. aeruginosa* to colonize a variety of niches and its persistence as a pathogen in humans and animal organisms [80,85,98,99]. Pyocyanin is a phenazine pigment, synthesize by the majority of strains of *P. aeruginosa* is a potential factor that may enhance the survival of *P. aeruginosa* by increasing its capacity to compete with other microorganisms. A decrease in pathoge-

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nicity of *P. aeruginosa* was observed *in vitro* when biosynthesis of pyocyanin was inhibited [61].
This suggests that pyocyanin is somewhat responsible for the initial colonization of *P. aeruginosa in vivo*. Pyocyanin pigment is a redox-active secondary metabolite and has a characteristic feature of inhibiting many bacterial colonization and fungal growth both *in vivo* and *in vitro* condition [111]. *P. aeruginosa* strains, isolated from infections have been well studied by researchers for the production of pyocyanin [62,63,114]. Pyocyanin is a blue-green pigment, also referred to as ''blue pus'' [122], produced in both solid and liquid culture media. Pyocyanin plays an important role in the metabolism of iron which considered as a crucial requirement for the growth of *P. aeruginosa*. The culture medium with low iron concentration produced pyocyanin abundantly [27].

Pyocyanin is a heterocyclic compound formed of two subunits of N-methyl-1-hydroxyphenazine as shown in (Figure 1).

The biosynthetic pathway which is used by the microorganism for the synthesis of various pigments in the shikimic acid pathway and shikimic acid act as a precursor for the biosynthesis of pyocyanin (Figure 2). This pathway is used by bacteria, fungi, algae, parasites, plants but absent in animals. Microorganisms and plants synthesize aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and various pigments by this pathway after the exponential phase of microbial growth [21].

Pyocyanin has various pharmacological effects on prokaryotic cells; its biological activity is related to similarity in the chemical structure to isoalloxazine, flavoproteins, flavin mononucleotide, and flavin adenine dinucleotide compounds [125]. It has been used to control phytopathogens [157]. In addition to this, as pyocyanin pigment is water-soluble, it has also been reported for its application in aquaculture [137]. Pyocyanin pigment can generate reactive oxygen species (ROS) and these ROS interferes with the topoisomerase I and II activities in eukaryotic cells (tumor cells) [58]. Pyocyanin has the potential to oxidize and reduce other molecules, as a result, can kill microbes competing against *P. aerugi-nosa*.

Pyocyanin is a redox-active compound, it has also got an application in biosensors because it can carry out the transfer of an electron between enzyme molecules and the electrode material. Such types of biosensors which are based on pyo-

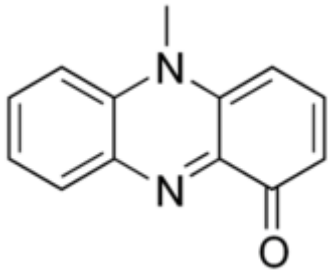


Figure 1. Structure of pyocyanin

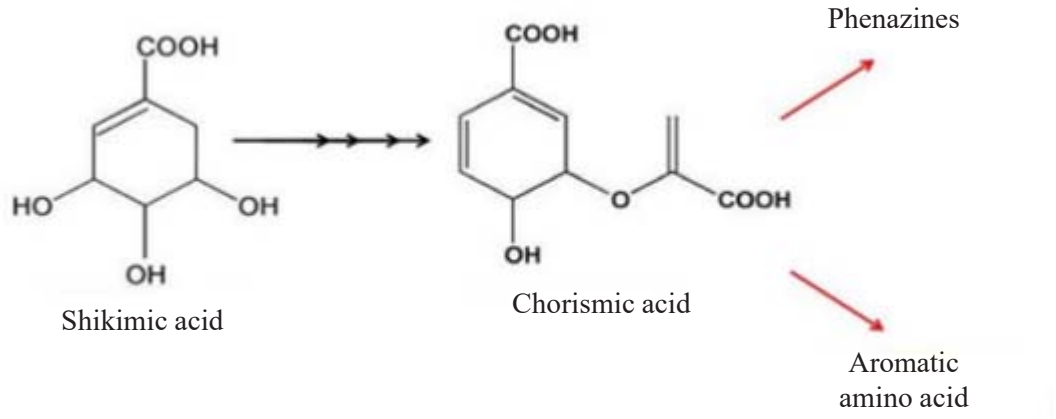


Figure 2. Phenazines from shikimic acid

cyanin were also expected in different fields such as pharmaceutical, agricultural, and environment [136]. Many researchers utilized phenazine compounds for the development of the sensor and also in nanotechnology; for example, a derivative of phenazine was used to develop a pH sensor based on luminescence [151]. An amperometric sensor was developed for the determination of hydrogen peroxide utilizing neutral red attached to multiwalled carbon nanotubes [74]. Pyocyanin has also been applied as biocontrol agents against many pathogens [70]. It also displays antimicrobial and antibiofilm activity because of its capability to arrest the electron transport chain of many microorganisms [81]. Antimicrobial resistance is an alarming issue to deal with, as most of the pathogen becoming resistant to their common treatable antibiotic. It has been reported that *S. aureus* and *E. coli* are multidrug-resistant bacteria. Inhibition of growth of these and some other pathogens by pyocyanin pigment shows its importance and potentiality as an antimicrobial agent. It can be used as a therapeutic agent to treat infections caused by these pathogenic bacteria. The pyocyanin pigment showed very high antioxidant activity at very minute concentrations, which is a favorable indication for the safe use of compound [100]. In addition to this, the pigment showed no cytotoxic effects on human red blood cells [131] and in cultured L929 cells [5]. Besides all, the antibiofilm activity of the compound against multiple antibiotic resistant food pathogens expand their efficacy for application in the food industry. This can be used to control several other active food pathogens if applied in the food.

Pigments produced by microorganisms

Microorganisms are the most versatile tools in biotechnology to produce a variety of molecules including enzymes, antibiotics, organic acids, and pigments. Several numbers of pigments have been secreted by microorganisms such as quinines, carotenoids, melanins, monascins, violancein, flavins [33]. The microbial pigments also possess various characteristics features *viz.*, antitumor, antioxidant, anti-inflammatory, antimicrobial besides act as a coloring agent in the cosmetic and food industry [169]. Microbial pigments are a promising source of food colorants [1,2]. Microorganisms mainly bacteria and fungi are widely studied for their probability as a source of food colorants. Natural pigments carry some fundamental desirable property of stability to light, heat, and pH [75]. Because of this, the food industry has become highly focused on the use of microbial sources to produce pigments for use in foods. It can also help to overcome the use of synthetic colors in food products as they hurt human health. On the other hand, besides the health benefit of human beings, the natural pigment will be a boon for the preservation of biodiversity as they are biodegradable. Natural pigments can also serve the dual need for visually appealing colors and probiotic health benefits in food products [119]. The production of synthetic colorants could be stopped as harmful chemicals released into the environment and contaminate the environment. Very few research studies are available on the exploration of pigment production from microorganisms especially in the Indian scenario which points towards exploring microbial pigments in more detail. There exist a diverse group of microbes that can produce pigments (Table 1).

Pseudomonas aeruginosa

Microorganisms are preferred sources for pigment production due to their advantages over plants in terms of availability, stability, cost efficiency, labor, yield, and easy downstream processing [75]. *P. aeruginosa*, a Gram-negative bacterium, can produce a variety of phenazine pigments (Table 2) [159]. The word "*Pseudomonas*" made up of two words Pseudo and monas. The prefix *Pseudo* means "false" and *mon* means germs. The word *aeruginosa* is a Latin word that means verdigris, referring to the bluish-green color of cultures. This blue-green color is due to the production of secondary metabolites by *P. aeruginosa* i.e, pyocyanin, which is responsible for the blue-green color of cultures [12]. *Pseudomonas aeruginosa* can cause disease in plants and animals including humans, comes under the family Psuedomonadaceae.

The preliminary identification of *P. aeruginosa* is often done by its pearlescent appearance and grape-like or tortilla-like odor *in vitro*. Definitive clinical identification of *P. aeruginosa* often in-

Table 1. List of pigments produce by microrganisms

No.	Microorganism	Pigment	Color	Function	Reference
1	*Fusarium sporotrichioides, Blakeslea trispora*	Lycopene	Red	Antioxidanst, Anti-cancer	32,50
2	*Janthinobacterium lividum, Pseudoalteromonas tunicate, Pseudoalteromonas spp. Chromobacterium violaceum*	Violacein	Purple	Antioxidant, detoxify ROS	35,87,109
3	*Pseudomonas aeruginosa*	Pyocyanin	Blue-green	Cytotoxicity, Antioxidant, Antimicrobial, Antibiofilm, Neutrophil apoptosis, Ciliary dysmotility, Pro-inflammatory	8
4	*Haematococcus pluvialis, Agrobacterium aurantiacum, Phaffia rhodozyma, Xanthophyllomyces, Dendrorhous*	Astaxanthin	Pink- red	Antioxidant, photoprotectant, Anti-cancer, Anti-inflammatory	40,41,140,147
5	*Staphylococcus aureus*	Zeaxanthin	Yellow	-	56
6	*Bradyrhizobium* Spp., *Monascus roseus*	Canthaxanthin	Orange	Antioxidant, Anti-cancer	20,24,33,34,102,108
7	*Corynebacterium insidiosum*	Indigoidine	Blue	Anti-microbial	29,156
8	*Serratia marcescens*, *Pseudoalteromonas rubra*	Prodigiosin	Red	Anti-cancer, DNA Cleavage, Immunosuppressant	31,39,115,165
9	*Ashbya gossypi*	Riboflavin	Yellow	Anti-cancer, anti-oxidant, protection against cardiovascular diseases, in vision	65,134,166
10	*Blakeslea trispora, Fusarium sporotrichioides, Mucor, Circinelloides, Neurospora crassa, Phycomyces, Blakesleeanus, Dunaliella salina*	β-Carotene	Yellow-orange	Anti-cancer, Antioxidant, suppressionof cholesterol synthesis	16,26,34,45,69 86,101,161
11	*Xanthomonas oryzae*	Xanthomonadin	Yellow	Protection against photodamage	139

Table 2. Pigments produced by *Pseudomonas aeruginosa*

Pigments	Colour of pigment
Pyocyanin	Blue-Green
Pyoverdine	Yellow-Green
Pyorubin	Red-Brown

cludes identifying the production of pyocyanin as well as its ability to grow at 42°C. It is citrate, catalase, and oxidase positive. It is a facultative anaerobe that grows not only in normal atmospheres but also in atmospheres deprived of adequate oxygen supply (hypoxic), therefore, it has colonized many natural and artificial environments. *P. aeruginosa* can achieve anaerobic growth with nitrate or nitrite as a terminal electron acceptor. It can ferment arginine and pyruvate by substrate-level phosphorylation when oxygen, nitrate, and nitrite are absent [154]. Adaptation to microaerobic or anaerobic environments is essential for certain lifestyles of *P. aeruginosa*. For example, during lung infection in cystic fibrosis patients, where thick layers of lung mucus and alginate surrounding mucoid bacterial cells can limit the diffusion of oxygen [25,59,173,177]. Pyocyanin pigment produced by *P. aeruginosa,* is involved in quorum sensing, virulence, and iron acquisition. In one of the studies, It has been reported that there is a decrease in *P. aeruginosa* pathogenicity *in vitro* when pyocyanin biosynthesis is inhibited [61]. This suggests that pyocyanin is most responsible for the initial colonization of *P. aeruginosa in vivo*.

History of pyocyanin

Since prehistoric times, the pigments have been used as coloring agents. Archaeologists uncovered various pieces of evidences which depict that early humans used paint for decorative purposes. Between 350,000 and 400,000 years back, pigments and grinding equipments were found in a cave at Twin Rivers, near Lusaka, Zambia, which further proves that pigments have been used from prehistoric time [78]. The blue-colored redox-active pyocyanin pigment (5-*N*-methyl-1-hydroxyphenazinium betaine) is the oldest known natural phenazine compound and was first reported when Fordos used chloroform to extract this microbial metabolite from purulent wound dressings in 1859 [42]. Production of a blue-green pigment by bacteria, albeit without identification of the responsible compound, had already been described by Schroeter in 1872 [155]. He called the responsible organism '*Bacteridium aeruginosum'* after 'aerugo', the Latin word for verdigris, the blue-green color that develops on the surface of copper exposed to air [155]. A few years later, in 1882, Gessard realized that pyocyanin was produced by anaerobic motile bacterium [48], which he subsequently named 'Bacillus pyocyaneus'. Gessard was, however, not the first to discover this organism. In 1900, Migula finally replaced these long names of the pyocyanin-producing species by '*Pseudomonas aeruginosa'*, which is the name still in use today [116]. By potentiometric studies, Jensen and Holten studied the zwitterion nature of the pigment and showed that pyocyanin acted as a reversible redox system in mixture with its reduced leuco derivative [73]. At acidic pH, color changes associated with progressive reduction of pyocyanin are red to yellow, to green, to colorless and at alkaline pH the color change is from blue to colorless [44]. In the absence of air, cultures of *P. aeruginosa* were observed to reduce pyocyanin to its colorless form and depending upon the pH value, the color varies according to the redox state of the system appeared to account for the shifting play of tints referred to as 'chameleon phenomenon'. Production of pyocyanin by *P. aeruginosa* was identified to be sensitive to the phosphate concentration in the growth media [43,84]. Moreover, Ingledew and Campbell (1969) reported that phosphate deficiency triggered pyocyanin production by *P. aeruginosa* [68]. Burton and his colleagues (1947) reported that amino acids could replace the peptone commonly claimed to be essential for good pigmentation and a medium

containing glycerol, leucine, glycine, or alanine and mineral salts was recommended for *P. aeruginosa* [13]. Since more than 100 different phenazine compounds of microbial origin have been reported in the literature [92,100].

Pyocyanin and its biosynthetic pathway

Pyocyanin comes under the category of phenazine compounds which are heterocyclic and secreted naturally by the bacterial species during the stationary phase of cell growth and substituted at different positions around their rings. Every color of the visible spectrum represented by phenazines, with a strong peak in the scale 250-290 nm and a weaker peak at 350-400 nm [47]. Oxidized, monovalent reduced or divalently reduced are three different states in which pyocyanin can exist.

Biosynthesis of pyocyanin pigment occurred by a metabolic pathway, known as the shikimic acid pathway (Figure 3). This pathway used by microorganisms (bacteria, fungi, algae, parasites) and plants for the synthesis of aromatic amino acid and various pigments. This pathway is absent in animals. Synthesis of pyocyanin pigment occurred by sequential modification of various molecules in the pathway. Shikimic acid pathway is also known as *aro* pathway.

Shikimic acid act as a precursor molecule for the synthesis of phenazines. Phenazine- 1,6-dicarboxylic acid believed to be the first phenazine structure in the pathway. It is formed by the condensation of two molecules of chorismic acid [95]. Aminodeoxyisochorismate (ADIC) synthase enzyme used in this step, which converts chorismic acid to 2-amino-2-deoxyisochorismic acid (ADIC) by doing amination of chorismic acid. ADIC is then lead to the formation of trans-2,3-dihydro-3-hydroanthranilic acid (DHHA). For the formation of a phenazine ring system, the condensation of two similar DHHA molecules is required. The two identical molecules react with

Phoshoenol pyruvate + Erythrose-4-phosphate
↓
3-Deoxy-D-arabino-heptulosonate
↓
5-Dehydroquinic acid
↓
5-Dehydroshikimic acid
↓
5-Phosphoshikimic acid
↓
3-Enol pyruvyl-5-phosphoshikimic acid
↓
Chorismic acid
↓ ADIC synthase
2-Amino-2-deoxyisochorismic acid (ADIC)
↓
Trans-2, 3-dihydroxyanthranilic acid (DHHA)
↓
2, 3-Dihydro-3-oxo-anthranilic acid
↓
Phenazine-1-dicarbolxylic acid (PCA)
↓PhzM
5-Methyl phenazinium-1-carboxylic acid betaine
↓PhzS
Pyocyanin

Figure 3. Shikimic acid pathway of pyocyanin biosynthesis in *P. aeruginosa*

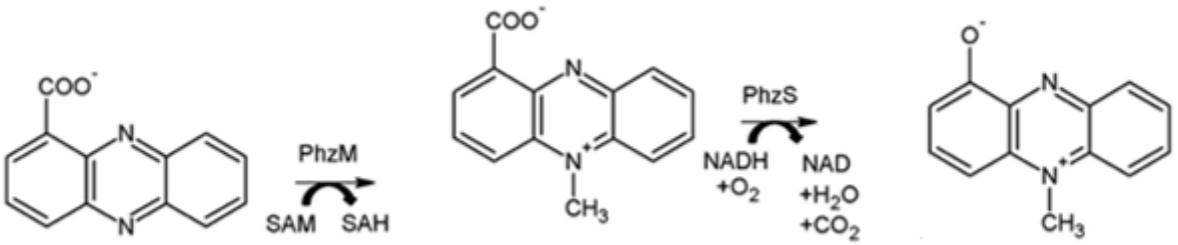
each other by nucleophilic addition, dehydration, and tautomerization to give 5,10-dihydroanthranilic acid, which then undergoes oxidation to form phenazine-1-carboxylic acid (PCA) (as shown in Figure 3).

The primary nitrogen source was glutamine and that the phenazine ring was constituted by a combination of two units of the same precursor for PCA biosynthesis [150]. The PCA leads to the synthesis of pyocyanin by hydroxylative decarboxylation mechanism. Mainly two steps involved in the pyocyanin synthesis from PCA (Figure 4). In the first step, PCA is converted to 5-methylphenazine-1-carboxylic acid betaine by the enzyme PhzM (an *S*-adenosylmethionine dependent methyltransferase). There is transfer of methyl group to the nitrogen atom of phenazine ring moiety. The second step leads to the formation of pyocyanin which is catalyzed by PhzS, a FAD-dependent monooxy-genase. This enzyme is responsible for hydroxy-lative decarboxylation of 5-methylphenazine-1 carboxylic acid betaine which leads to the formation of Pyocyanin [132]. PhzM alone had no methylation activity toward PCA, but pyocyanin was produced in the presence of PhzS and NADH. PhzS has also been shown to act directly on PCA to produce phenazin-1-ol, but this compound was not a precursor for pyocyanin and therefore PhzM must act before PhzS [132]. Formation of a complex between PhzM and PhzS would presumably prevent the release of 5-methylphena-zine-1-carboxylate.

Genetics of pyocyanin synthesis pathway and its regulation

Two specific genes must be functional for the production of pyocyanin by *Pseudomonas aeruginosa*. Mvfr is a gene that produces a transcription factor, which then activates phzAB genes. These genes produce the molecule quinolone which then regulates operons 1 and 2 of phzRABCDEFG which are the key to the synthesis of phenazine [110].

Pyocyanin biosynthesis begins with the conversion of chorismic acid to 2-amino-2-desoxyisochorismate (ADIC) by ADIC synthase, PhzE [97] (Figure 5). In this reaction the enzyme PhzE catalyzes the loss of the hydroxyl group from C4 of Chorismic Acid as well as the transfer of an amine group from glutamine to form glutamic acid and 2-amino-2-desoxyisochorismic acid (ADIC) [178]. Following this, PhzD catalyzes the hydrolytic removal of the pyruvate moiety from ADIC to form (5S,6S)-6-amino-5-hydroxy-1,3-cyclohexadieve-1-carboxylic acid (DHHA). In the next step, PhzF catalyzes two steps: the abstraction of a hydrogen from C3 of DHHA, delocalization of the double bond system, and reprotonation at C1 as well as enol tautomerization to form the highly unstable 6-amino-5-oxocyclohex-2-ene-1-carboxylic acid (AOCHC). From here two molecules of AOCHC are condensed by PhzB to form the tricyclic compound, hexahydrophenazine-1,6-dicarboxylic acid (HHPDC). The product of this reaction, HHPDC, is unstable and spontaneously undergoes oxidative decarboxylation in an uncatalyzed reaction to form tetrahydro phenazine-1,6-carboxylic acid (THPCA). In the final step of phenazine-1-carboxylic acid synthesis the enzyme PhzG catalyzes the oxidation of THPCA to dihydro-phenazine-1-carboxylic acid. This is the last catalyzed step in the production of PCA, the last step is an uncatalyzed oxidation of DHPCA to PCA [178]. The conversion of PCA to Pyocyanin is achieved in two enzymatic steps: firstly, PCA is methylated on N5 to 5-methyl-phenazine-1-carboxylate betaine by the enzyme PhzM using the cofactor S-adenosyl-L-methion-



Phenazine-1-carbolxylic acid 5-Methyl phenazinium-1-carboxylic acid betaine Pyocyanin

Figure 4. Role of PhzM and PhzS

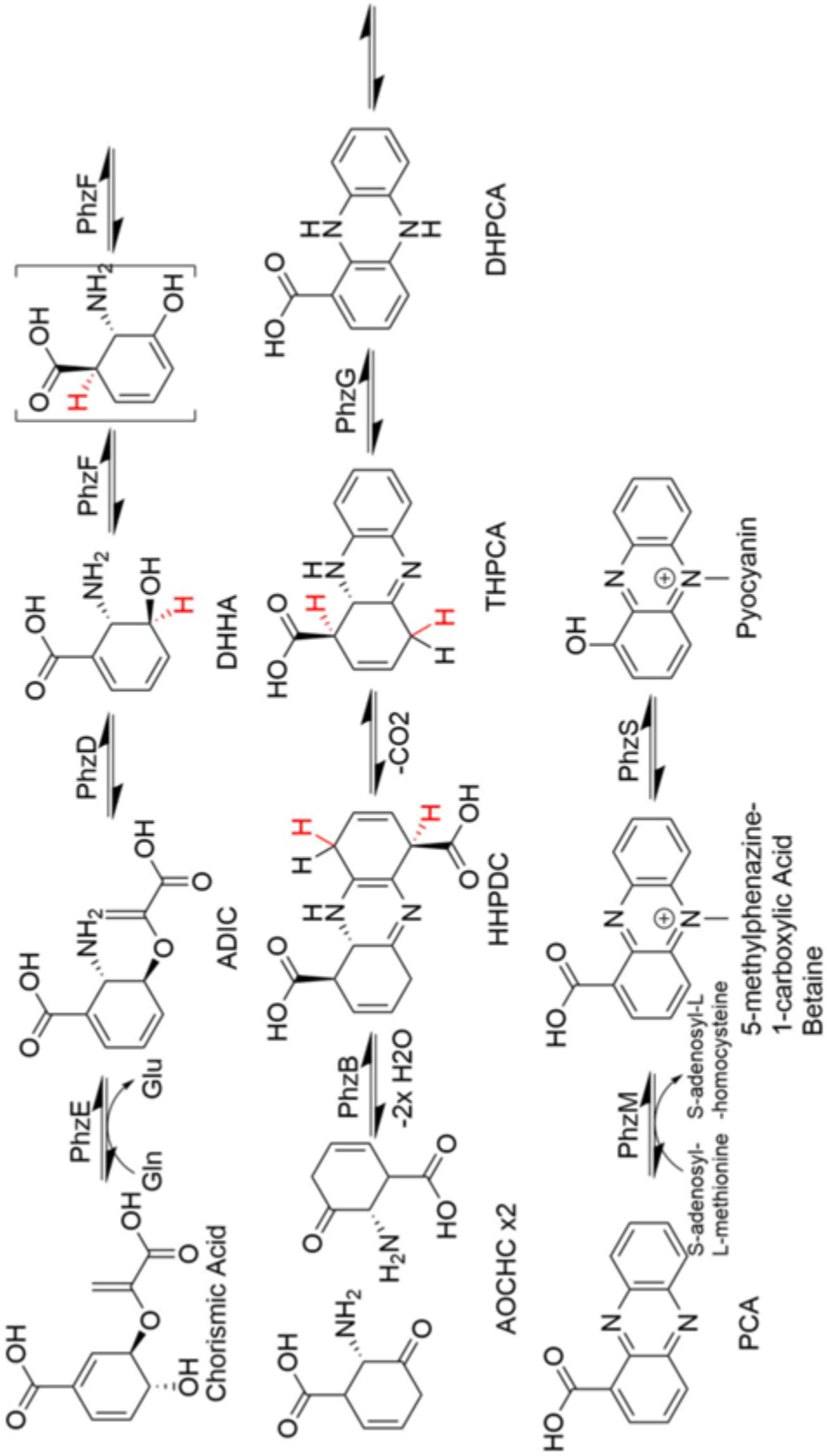


Figure 5 Biosynthetic pathway of phenazines in *P. aeruginosa*

ine and secondly, PhzS catalyzes the hydroxylative decarboxylation of this substrate to form the final product, pyocyanin [110].

The structure of phenazine polypeptides involved in pyocyanin biosynthetic pathway has been shown in Figure 6.

Role of pyocyanin in *Pseudomonas aeruginosa* pathogenicity

P. aeruginosa strains produce two distinct types of O antigen (O-Ag): a common polysaccharide antigen (A-band) composed of a homopolymer of d-rhamnose and an O-specific antigen (B-band) composed of a heteropolymer of three to five distinct sugars in its repeat units. So far, *P. aeruginosa* isolates have been classified into 20 serotypes by the International Antigenic Typing Scheme. The lipopolysaccharide (LPS) of *P. aeruginosa* is less toxic than that of other Gram-negative rods, facilitating its establishment of chronic infections by eliciting a low inflammatory response [135]. *P. aeruginosa* being opportunistic, occasionally colonize human body sites, with a preference for moist areas, such as the perineum, axilla, ear, nasal mucosa and throat as well as stools. The prevalence of colonization by *P. aeruginosa* in healthy subjects is usually low. It mainly infects patients with burns or those that are immunocompromised and it is one of the main causes of nosocomial infections [105]. According to data from the Centre for Disease Control and Prevention National Nosocomial Infection Surveillance System, in the USA, *P. aeruginosa* was the second most common cause of nosocomial pneumonia, the third most common cause of nosocomial urinary tract infections, and the seventh most common cause of nosocomial bacteremia [123]. In Europe, *P. aeruginosa* was found to be the third most common isolate from nosocomial infections in intensive care units [147]. Mortality rates ranging from 40 % to more than 60 % have been reported in bacteremic nosocomial pneumonia and ventilator-associated pneumonia [16,28,112,144]. *P. aeruginosa* uses several virulence factors to establish chronic respiratory infections in bronchiectasis, chronic obstructive pulmonary disease, and cystic fibrosis patients [22,118]. Cystic fibrosis is one of the most major and common fatal genetic disorders among the Caucasian population. It affects approximately 30,000 individuals in the United States alone. *P. aeruginosa* produces a large number of exoproducts (Table 3), including elastase,

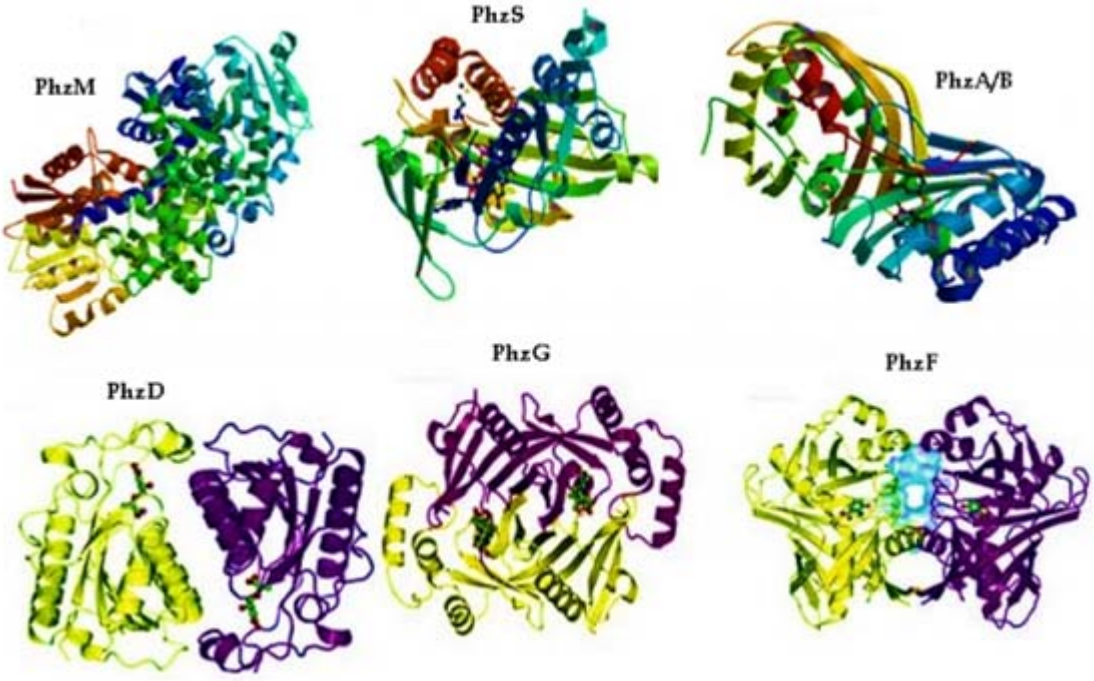


Figure 6. Structure of phenazine polypeptides involved in pyocyanin biosynthetic pathway [111]

Table 3. Factors enhancing the pathogenicity of *Pseudomonas aeruginosa*

No.	Factors	Role
1	Adhesions	Attachment
2	Alginate production	Mucoid layer
3	Exotoxin A	Inhibit host protein synthesis
4	Exoenzyme S	Interferes with phagocytic killing
5	Elastolytic activity	Degrades elastin
6	Phospholipase C	Damage tissue
7	Pyocyanin	Damage tissue by ROS
8	Antibiotic resistance	Complicates therapy

alkaline protease, the LasA protease, hemolysin, rhamnolipids, and pyocyanin [105] to disrupt the host immune responses and cause cytoskeletal reorganization.

Pyocyanin is a zwitterion that can easily penetrate biological membranes. Pyocyanin promotes virulence by interfering with several cellular functions in host cells including electron transport, cellular respiration, energy metabolism, gene expression, and innate immune mechanisms. It has been shown to act as an inhibitor of mitochondrial enzymes in mammalian tissue [4] and to cause disruption and cessation of ciliary beat on ciliated nasal epithelium. It is readily recovered in large quantities from the sputum of patients with CF infected by *P. aeruginosa* [175,176] and from ear secretions of *P. aeruginosa*-mediated chronic otitis media [143]. Despite this, the exact contribution of PCN in the pathogenesis of *P. aeruginosa*-mediated diseases has remained controversial. However, studies on the role of pyocyanin in virulence *in vivo* using alternative model hosts [15,90,106] and mice [91] have revealed that pyocyanin has crucial roles in *P. aeruginosa* infection.

Many studies have concluded that pyocyanin has a derogatory effect in cystic fibrosis (CF) which enables *P. aeruginosa* to persist in the CF lung. Pyocyanin *in vitro* can interfere with functions such as ciliary beating and therefore, cause epithelial dysfunction as the ciliary are needed to sweep mucus up the throat [76].

Additionally, neutrophil apoptosis [167], immunoglobulin release from B-lymphocytes, and interleukin (IL-8) release [30] and CCL5 are all impaired by pyocyanin causing the immune system of the lung to be weakened. *In vivo* studies have shown that the growth of the fungus is inhibited in the presence of pyocyanin. The fungicidal mechanism is the activation of NAD(P)H to induce a redox-active cascade to produce reactive oxygen intermediates. This allows *P. aeruginosa* to have a competitive advantage as it may dominate over other microorganisms in the CF lung [82]. The intracellular concentration of ATP is also diminished by pyocyanin causing further damage to CFTR which are already impaired in cystic fibrosis. CFTR channels rely on ATP for two main purposes. Firstly, the binding and hydrolysis of ATP has to occur at two nucleotide-binding domains for the channel to move between its open and closed conformation. Secondly, phosphorylation of CFTR by Protein kinase A-II should occur for the channel to be operational. PKA II is activated by cAMP which is produced from ATP. Both these processes are impaired when ATP is depleted by pyocyanin [126]. If *P. aeruginosa* uses pyocyanin production to its advantage in competing with other bacteria in the same ecological habitat, it must, therefore, have a mechanism to ensure its protection or immunity against the bactericidal agent it produces. This immunity could be via higher concentrations of SOD (sodium dismutase) and catalase or by lack of permeability. Hassan and Fridorich (1980) reported that *P. aeruguinsa* made a 62 % higher catalase when grown under conditions conducive for pyocyanin production. However, the level of SOD was slightly lower. They also checked the effect of pyocyanin on the rate of cyanide-resistant respiration in *P. aeruginosa* and found it to be non-

responsive to pyocyanin. The respiration of *P. aeruginosa* was generally resistant to cyanide. Thus, 8 to 10 mM was required to inhibit the respiration by 91.3 %, and 0.134 mM pyocyanin caused this cyanide-resistant respiration to rise from 8.7 to 14.8 %. This increase is very moderate compared to that observed in *E. coli*. These results tentatively indicate that *P. aeruginosa* is not as permeable to pyocyanin as is *E. coli*, and they show that the organism makes higher catalase to protect against H_2O_2 that might be generated outside the cells via extracellular autooxidation of pyocyanin. They presumed that *P. aeruginosa* actively secretes pyocyanin while keeping its intracellular level low by a combination of low permeability and active extrusion [57]. Pyocyanin also inhibits prostacyclin release and can inactivate human V-ATPases (involved in receptor-mediated endocytosis), a1-protease inhibitor (which modulates serine protease activity, including neutrophil elastase) and nitric oxide (which influences blood flow, blood pressure, and immune functions).

Challenges

P. aeruginosa is an opportunistic pathogen that causes infections in immune-compromised hosts, burn victims, individuals in intensive care, and patients with CF. The lungs of nearly all CF patients are chronically colonized by *P. aeruginosa*, which significantly reduces life expectancy and it is the leading cause of morbidity and mortality for CF patients. The ability of *P. aeruginosa* to cause infection depends on the secretion of agents termed, virulence factors, such as toxins and adhesion molecules, that actively cause damage to host tissues. Researchers have directed increasing attention in recent years to 'disarm' the pathogenicity of bacteria rather than kill them. This can be done by targeting virulence using anti-infective or anti-virulence drugs [141].

Pyocyanin acts as both a virulence factor and a quorum-sensing signaling molecule for *P. aeruginosa* [77,91]. It has been identified by some researchers that pathogen-associated proteins have homology only with pathogenic bacteria and not with non-pathogens [61]. Such types of proteins are more likely to have virulence-related functions. The list of identified pathogen-associated proteins has been included in components of the phenazine biosynthesis pathway. Therefore, pyocyanin biosynthesis is an attractive target for anti-infective drug intervention.

Biodegradation, detoxification, and inactivation of pyocyanin

The environmental degradation of the residual pyocyanin became an important factor as pyocyanin has been used in the aquaculture system. Yang and his colleagues reported the biodegradation of PCA, the precursor of pyocyanin by soil organisms *Sphingomonas* sp. DP58. *Sphingomonas* sp. DP58 consume PCA as the sole source of carbon and nitrogen and completely degrade it within 40h [179]. Hill and Johnson [60] reported the microbial transformation of phenazines by *Aspergillus sclerotiorum.* Chen and his colleague conducted the study on intermediates or metabolites produced out of this degradation [19].

Biodegradation of pyocyanin is esteemed by the presence of a phenolic character in the compound and phenolics are the best substrates for peroxidases. The oxidation of pyocyanin leads to its inactivation and becomes non-toxic and the reaction is irreversible [146]. The study on photosensitized oxidation and inactivation showed that pyocyanin could be partially inactivated through photochemical oxidation. The resulting product is a poorer free radical generator and therefore a less efficient stimulant of oxidative processes. These results suggest that photosensitization could be a potentially useful method for inactivation and possibly for detoxification [146].

Applications of pyocyanin

Phenazine compounds produced by *P. aeruginosa* were known to possess a broad spectrum of antibiotic activity toward bacteria, fungi, and eukaryotic cells. Many of phenazine compounds showed antitumor, antimalaria and antiparasitic activities [92]. The antimicrobial activities of phenazine had been investigated through their inhibition of some bacterial and fungal growth [11]. So, these compounds suitable to restrain microbes in agricultural and pharmaceutical applications. Aunchaleeb and colleagues [6] found that phena-

zine had antimicrobial activity against some pathogenic bacteria. Pyocyanin has been applied as biological control agents in agriculture against pathogenic fungi and bacteria. This antagonistic property of pyocyanin to various pathogenic microorganisms have been studied extensively [3,7,89,142]. The application of pyocyanin pigment at concentration 5-10 mg/mL does not show any deleterious or pathological effect in the eukaryotic system and can be used as a biocontrol agent against fungal and bacterial pathogens in agriculture and aquaculture against *Vibrios* [137].

Pyocyanin as antimicrobial agent

Since the year 1940, it has been reported that pyocyanin possesses antibacterial properties [174]. Pyocyanin pigment was also known as Colicin because it inhibits the growth of *E. coli*. During lysis of bacteria, protein fraction was released which showed the antimicrobial properties of pyocyanin pigment [180]. According to Hassan and Fridorich [57], exposure of *E. coli* cultures exposed to pyocyanin pigment caused the depletion of oxygen supply to the cells which leads to the production of hydrogen peroxide (H_2O_2) and also divert the electron flow, causing toxicity to the cells.

Earlier reports on antibiotic action of pyocyanin pigment suggested that pigment inhibits the growth of bacteria by interrupting the metabolic transport in the cell through the interaction with the respiratory chain. The production of stable anion radical takes place upon uptaking of an electron by pyocyanin and thereafter undergo a redox cycle that is responsible for its antibiotic action. Pyocyanin itself gets reduced and reduces oxygen to superoxide radicals which are very toxic and these radicals are also responsible for its antimicrobial action [57].

The bactericidal effect of purified pyocyanin pigment depends on its concentration in all cases [8]. Baron and Rowe [8] reported that only 2.9 µg of purified pigment is sufficient for the inhibition of bacterial growth. Pyocyanin showed its antibacterial action on both Gram-negative as well as Gram-positive bacteria. It was reported that pyocyanin has a negative influence on the mechanism of active transport of many microorganisms [9]. Nearly 90-95 % of the antimicrobial actions of *P. aeruginosa* strains were due to the secretion of the extracellular secondary metabolite pyocyanin. It showed bactericidal action against many pathogenic bacteria like *Salmonella paratyphi*, *E. coli,* and *Klebsiella pneumonia* [152]. The pigment showed very effectual activity against organisms like *Acinetobacter, S. aureus, E. coli,* and *Streptococcus pneumonia* [160]. Pyocyanin pigment also showed antibacterial action on food spoilage bacteria like *L. monocytogenes* and *B. cereus*. It has been reported in various papers that Gram-positive bacteria were more susceptible than Gram-negative bacteria. El-Fouly and colleagues in 2015 [36] reported that the highest minimum inhibitory concentration (MIC) of purified pyocyanin was 50 mg/mL whereas the lowest MIC was 20 mg/mL against *E. coli.*

Pyocyanin pigment has antimicrobial potential to be used as antifungal, antibacterial, and antiprotozoal agents. The mechanism of action of the pigment on fungus is the same as that of the mechanism of antibacterial action. Several groups reported the *in vitro* inhibition of yeast growth by *P. aeruginosa* [67,81] and there are reports which suggest that inhibition of yeast growth by *P. aerugi-nosa in vivo* in patients with [67] and without cystic fibrosis [81]. Pyocyanin obtained from *P. aerugi-nosa,* isolated from the sputum of CF patients, also seize the growth of fungi like *Candida albicans* and *Aspergillus niger.* Inhibition of growth of *A. fumigatus* by pyocyanin was reported to be dose-dependent but occurred at much higher levels (>19 mg/well). In contrast to its effect on *C. albicans*, pyocyanin caused complete inhibition of the growth of *A. fumigatus* [83]. Pal and his colleagues in 2006 [130] reported that pyocyanin showed antibiotic activity *in vivo* on *Candida* sp. grown on Sabroud's dextrose agar. Growth of additional yeast species known to cause human infection (*C. krusei, C. keyfr, C. guillermondii, C. tropicalis, C. glabrata, C. lusitaniae, C. parapsilosis, C. pseudotropicalis,* and *S. cerevisiae*) was inhibited in the well plate assay by pyocyanin (0.6 mg/well) and 1-hydroxyphenazine (9.5 mg/well).

It was observed that pyocyanin and pyorubin obtained from *P. aeruginosa* have distinct anti-

bacterial activity against *Citrobacter* sp., which are usually associated with urinary tract and wound infections [121]. Pyocyanin pigment could be a promising anti-tyrosinase agent and a new active compound against *Trichophyton rubrum*, which could be a major causative agent of tinea corporis. Purified pyocyanin showed high inhibitory activity against tyrosinase and *T. rubrum*.

In vivo, topical treatments with pyocyanin ointment established the efficiency of pyocyanin (MIC 2000 µg/mL) to cure tinea corporis compared to fluconazole. Also, the results showed complete healing and disappearance of hyperpigmentation by testing the safety of pyocyanin ointment and its histopathological efficiency in the skin treatment without any significant toxic effect [38]. These findings cover the continuous demand for novel and natural antimycotic agents against troublesome fungal infections.

Pyocyanin as an antioxidant agent

Antioxidants are natural molecules that may prevent or delay cell damage. It inhibits the oxidation of other molecules. Oxidation is a process that releases free radicals, leading to a chain of reactions that may destroy cells. Free radical scavenging activity of pyocyanin was estimated by DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) radical scavenging assay [100]. It is a free radical, contain an unpaired electron. This assay is very simple and rapid to perform and very effective for the evaluation of antioxidants by spectrophotometry [66]. DPPH contains an odd electron because of which it has a strong absorption maximum at 517 nm and is purple in color. The conversion of color from purple to yellow is observed when an odd electron of DPPH paired with hydrogen from a radical scavenging oxidant to form the reduced DPPH-H (2,2-diphenyl-1-picrylhydra-zine).

It has been reported that pyocyanin produced from *P. aeruginosa* BTRY1 strain has higher free radical scavenging activity of 80 % at 0.2 µg/mL, even at a very much lower concentration than that of ascorbic acid [93]. This is a positive indication for the safe use of products as the compound showed very high antioxidant activity at a very minute concentration of pyocyanin [100]. Chandran (2014) evaluated the antioxidant activity of pyocyanin pigment and it was found to be 55 % at 500 mg/ml concentration [17].

Antibiofilm activity of pyocyanin

Any group of microorganisms, in which cells adhered to each other and often also to the surface comprises biofilm and these cells become enclosed in a slimy extracellular matrix that is formed of extracellular polymeric substances (EPS). Many disease outbreaks were found to be linked with biofilms formation and there is always a threat to human health because of foodborne diseases. Biofilms became a major problem in the food industry. It has been reported that biofilm formed by many fungi and other pathogenic bacteria was inhibited by secretion from *P. aeruginosa* [64]. Pigments produced by bacteria with the ability to control biofilm are reported to have antioxidant activity which makes them beneficial in the food industry for control of foodborne disease or infections [94].

The formation of biofilm *in vitro* and their inhibition was examined by using a microtitre plate assay with crystal violet staining. Generally, 96 well microtitre plates were used for antibiofilm assay [149]. The antibiofilm activity of the compound was expressed using two techniques: scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

Laxmi and Bhat (2016) [93], reported that pyocyanin pigment at very low concentration (2*10-2 ng/mL biofilm inhibitory concentration) has anti-biofilm activity against biofilms formed by *Vibrio diabolicus* and *Salmonella enteritidis* and more than 80 % reduction in biofilm was achieved. Pyocyanin (1.245 mg/mL) was able to bring about 62 % inhibition in the EPS production by *B. casei* BTDF1 and *S. warneri* BTDF2, followed by 59 % in case of *B. altitudinis* BTMW1, *B. pumilus* BTMY2 and *B. niacini* BTDP3. Around 41 % reduction in biofilm was observed in the case of *M. luteus* BTDF3 and *G. staerothermophilus* BTFF2 followed by a 40 % reduction in *Bacillus* sp BTSD1. Azithromycin used as the positive control antibiotic to which most of the biofilm producers were sensitive. The BIC values of the bioactive compounds used in the study were in nanogram quantities against the tested food pathogens. This indicates the im-

mensely potent strength of the pyocyanin in biofilm control compared to the current antibiofilm strategies like antibiotic treatments.

Agricultural applications of pyocyanin

Biological control of plant diseases has been considered a valuable alternative method to manage various plant diseases. The microbial agent that suppresses the pathogen is referred to as the biological control agent. Biocontrol agents are microbial antagonists to suppress plant diseases as well as they are host specific pathogens to control weed populations. Phenazine has widely been used in agricultural applications. It was used for suppression of *Erwinia amylovora* which causes fire blight disease in apple flowers [49] and also used as natural suppression of *Fusarium* wilt disease in soils of France [113]. *P. aeruginosa* in soil produces pyocyanin which exists in the rhizosphere and soil, it promotes plant growth and protects plants from the phytopathogens [23,51]. The production of pyocyanin by *P. aeruginosa* was required for generation of disease symptoms in plants and the killing of nematode *Caenohabditis elegans* and the fruit fly *Drosophila melanogaster* [106]. The *Pseudomonas* sp. which colonize the various plant roots were found to act as potent microbiological control agents for various plant pathogens [163]. It was found that phenazine compounds inhibit mycelia growth of several fungal pathogens of plants. The 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* [55]. The phenazine had broad spectrum action against active and dormant structure of fungal pathogen *Pythium aphanidermatum* and root-knot nematode *Meloidogyne inconita* [79]. The phenazine derivatives were also selected to reduce the use of chemical pesticides in agriculture. These pigments could either be alone or in combination with some pesticides to lower the doses of chemicals needed to obtain a profitable crop yield [21].

Antitumour/ anticancerous action of pyocyanin

Cytotoxicity is defined as the quality of being toxic to the cells. A compound is cytotoxic if it kills the cells. The L929 fibroblast cell line was derived from the normal subcutaneous areolar and adipose tissue of the mouse. It has been reported that after exposure of pyocyanin pigment at 6.25 mg/mL concentration, their cells showed 90 % of viability. However with cancerous cell lines, pyocyanin pigment showed its cytotoxic effect. That is why this can be used as an anticancerous compound. Zhao and his colleagues (2014), reported that pyocyanin pigment shows a cytotoxic effect on HepG2 cancerous cell line [182]. Priyaja and colleagues [137], demonstrated the cytotoxic effect of pyocyanin pigment on L132, RTG2, and Sf9 cell lines. Among these cell lines L132, a human embryonic lung epithelial cell line showed the highest response to pigment than others. Further, 80 % of cell viability remains even at high concentrations of pigment indicate that it is safe to consume the food supplemented with pyocyanin [93]. The cytotoxic effect of pyocyanin pigment was checked by MTT assay [5].

Pyocyanin act as a cytotoxic compound by increasing the intracellular reactive oxygen species. Pigment generates superoxide (O2-) and hydrogen peroxide (H2O2) reactive oxygen species on reduction by NADH and NADPH in the cells and the reduced form of pigment transfer electron to O2. This is known as intracellular redox cycling of pyocyanin. The reactive oxygen species formed around and within the mitochondria [146]. Pyocyanin prevents the breakdown of hydrogen peroxide (H2O2) by inhibiting the activity of catalase by reducing the expression of gene encoding catalase. This leads to an increase in the level of reactive oxygen species indirectly [148]. A very toxic reactive nitrogen species (RNS) are formed when superoxide reacts with nitric oxide. The RNS and ROS both act together to damage proteins, DNA, phospholipids of cells, and finally cause death to the cells [122].

The measurement of cell viability after treatment with different concentrations of pyocyanin to establish the cytotoxic effect has been done by MTT (Diphenyltetrazolium Bromide) assay [5]. This is a colorimetric assay method that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase to formazan.

The MTT enters the cells and passes into the mitochondria where it has been reduced to an insoluble, dark purple colored formazan product.

The pyocyanin extracted from *Pseudomonas sp.* MCC 3145 has cytostatic potential and was found to arrest the growth of Hep-G2, SK-MEL-2, A-549, and HeLa cancer cells. The DNA intercalation-based cytostatic activity of pyocyanin on various human cancer cell lines suggests that this molecule could be explored for use in therapeutics [133].

Pyocyanin in aquaculture

Aquaculture is defined as the farming of fish, molluscs, crustaceans, etc. and it is the cultivation of saltwater and freshwater populations under controlled conditions. Aquaculture is the multi-billion dollar industry on a global scale and fastest-growing food sector and, looked upon as the high protein resource to meet the nutritional requirements of the increasing population. The swing in aquaculture development is towards magnification [10]. However, commercial production by aquaculture is obstructed by diseases caused by bacteria, fungi, parasites, viruses, and other undiagnosed and emerging pathogens. In this context, antibiotics engaged the central stage as control agents. This common strategy to control diseases over a period. There is antibiotic resistance and horizontal transfer of resistant genes from fish pathogens to humans. In this scenario, several alternatives remedies for the prevention and control of diseases in aquaculture have been put forth, such as immunostimulants, vaccines, and probiotics [52].

A large number of microorganisms harm the aquaculture environment which includes Gram-negative species (*Aeromonas, Flavobacterium, Pseudomonas, Achromobacter,* and *Vibrio*) and Gram-positive (*Corynebacterium*, *Micrococcus,* and *Bacillus*). Vibriosis is a major disease caused by *Vibrio* spp. which is ubiquitous in aquaculture associated with all cultured species including fish, molluscs and crustaceans [72,162,168,169].

Pseudomonads are a usual occupant of the aquatic environment including shrimp culture ponds [127] and are often related to gills, skin, and intestinal tract of live fish [14]. However, many bacterial isolates which are common members of the non-pathogenic microflora of fish and shellfish culture systems have been shown to inhibit fish and prawn pathogens *in vitro* [71]. It was stated that certain strains of bacteria associated with *Artemia* and prawn culture systems can control pathogens through competitive exclusion or by the secretion of inhibitory compounds [170]. The disease prevention has received much attention to control the fish and shellfish pathogenic vibrios [172], particularly by the use of non-pathogenic bacterial isolates [145,158].

Pyocyanin can be applied as an eco-friendly drug in the aquaculture system as it is biodegradable and can be readily oxidized. For marine prawn, *Pseudomonas* acts as a potent probiotic as it caused growth inhibition of several pathogens such as *Salmonella*, *Photobacterium demenselae*, *Staphylococcus aureus*, *Vibrio vulnificus, V. parahaemolyticus*, *V. harveyi* [129], *V. fluvialis* and *Aeromonas* [124,171]. Hai and Fotedar [54], reported that *P. synxantha* and *P. aeruginosa* are the most effective probiotic in inhibiting bacteria isolated from *Penaeus latisulcatus.*

Pyocyanin as an anticorrosive agent

Varieties of extracellular pigments produced by microbes possess unique properties including anticorrosive abilities. The *P. aeruginosa* produced a high amount of water-soluble, blue-green pyocyanin pigments [36] which has applications in various industrial sectors such as pharmaceutical, control of phytopathogens, aquaculture, etc. [125,136,157]. Apart from this, a study has evaluated the anti-corrosion property of the bioactive pyocyanin produced by *P. aeruginosa*. The researchers explored a new antibacterial property of pyocyanin produced by *Pseudomonas* sp. TBH2 in controlling the biocorrosive bacterial biofilm formed by *Bacillus* sp.[120]. This study gains importance as the production of pyocyanin from the indigenous bacteria is not an expensive process and it is environment friendly. The anticorrosion role of pyocyanin towards the control of microbially influenced corrosion (MIC) on Cu metal surface in a cooling water system (CWS) was supported by various characterization techniques. Generally MIC of Cu metal occurs due to

the biofilm formation which leads to the oxidation of copper metal into metal oxides. However in the presence of pyocyanin adsorbed on the metal surface, inhibition of biofilm formation was noted due to its antibacterial activity. The functional nitrogen-based group of pyocyanin coordinates with Cu metal from as Cu-N complex which acts as a protective layer on the metal surface. The corrosion inhibition efficiency of pyocyanin was observed to be 72 % [120] and this finding was supported by earlier reports too [37]. These results strongly suggest that the pyocyanin act as an effective biofilm inhibitor as this compound was found to reduce the corrosion rate of Cu metal in CWS.

Other applications

Phenazines can promote electron transfer and have many recognized and potential biotechnological applications. Phenazines had been used as colorimetric redox indicators and the pH indicator neutral red was among the best known. The phenazines had been utilized for the development of sensors and in nanotechnology and a derivative of phenazine was used to develop a luminescence-based pH sensor [151]. An amperometric sensor for hydrogen peroxide determination was developed using neutral red attached to multiwalled carbon nanotubes [74]. A novel mediator-free method based on genetically modified bacteria was also developed for detecting water toxicity. The genetically modified *P. aeruginosa* was selected as the biosensor strain and pyocyanin produced by this strain was used as the indicator. The toxicity response of this *P. aeruginosa* to 3,5-dichlorophenol was measured electrochemically and spectroscopically [181]. This study provided a convenient, sensitive, and cost-effective method for water toxicity detection and extended the biosensing application of the genetically modified bacterium.

Microbial fuel cells (MFCs) use microorganisms to catalyze the conversion of chemical energy into electrical energy [164]. An ongoing issue with MFCs was that the slow rate of electron transfer from the microorganism to the anodic electrode which limits the MFC efficiency. It was demonstrated that phenazine methosulfate or phenazine methosulfate served as good electron acceptors in photoelectron chemical cells [153]. It was observed that other phenazines also contributed to the rates of electron transfer in MFCs. The addition of pyocyanin to MFC-containing *Brevibacillus* sp PTH1 doubled the rate of electron transfer [138]. The addition of a phenazine-1-carboxylic acid-producing *P. chlororaphis* or a derivative that produces high levels of pyocyanin to a mixed MFC resulted in higher electron transfer rates. A strain of *P. aeruginosa* isolated from MFC was maintained in a batch mode for over a year that supported 352 mV using 1500 mg/l glucose as fuel [103]. Phenazines conjugated to other compounds such as a phenanthroline-fused phenazine offer potential as components of organic light-emitting devices (OLED) [18]. OLEDs were gaining popularity due to their low voltage requirements, wide color range, and lightweight. OLEDs were organic semiconductors containing an emissive layer placed between a transparent anode (e.g., transparent indium tin oxide) and a metal cathode (e.g., Mg, Al and Ag). When a bias was applied across the electrodes, the 'holes' (areas lacking electrons) and electrons combine in the emissive layer resulting in light emission [96].

Conclusion

Pyocyanin is one of the virulence factors produced by the *P. aeruginosa* for enhancing its pathogenesis. Pyocyanin enhances the pathogenesis by inhibiting the growth of competitive microorganisms of *P. aeruginosa*. Besides this, pyocyanin gain attention due to its various applications in pharmaceutical, agriculture, biosensors, environmental, etc. Many new antibiotics have been developed by pharmaceutical industries, but finding new broad-spectrum antimicrobial agents is still a priority because of resistant bacterial infections. The pyocyanin possesses effective antimicrobial activity against many multidrug-resistant pathogens. Inhibition of such pathogens by pyocyanin pigment showed its importance and potentiality as an antimicrobial drug. Researchers explored this antibacterial property of pyocyanin produced by *Pseudomonas* sp. TBH2 in controlling biocorrosion bacterial biofilm formed

by *Bacillus* sp. In addition to this, pyocyanin pigment showed a cytotoxic effect *in vitro* on cancerous cell lines at very minute concentration but it has been reported that pyocyanin has no toxic effect on normal cell lines. The anti-biofilm activity of the pyocyanin against multiple antibiotic resistant food pathogens expands their efficacy for applications in the food industry. This can be used to control several other active food pathogens if applied in the food. More applications need to be explored with*in vivo* studies to establish the positive effects of pyocyanin in clinical and therapeutic science.

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