

Review article

An overview on biosynthesis and applications of extracellular pyocyanin pigment and its role in *Pseudomonas aeruginosa* pathogenesis

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Abstract

Microbial pigments are chemical moieties capable of absorbing light in the visible range. The demand for natural colorants is increasing in every day as the present trend throughout the world is shifting towards the use of eco-friendly and biodegradable commodities. Among the natural sources, the pigment producing microorganisms hold a promising potential to meet present day challenges. Pigments are compounds that come in a wide variety and extensively used in industries. Industrial production of natural pigments by microbial fermentation has several advantages over extraction of pigments of plant origin, such as easy availability of inexpensive raw materials for their production, independence from crop seasons and their seasonal variations, etc. The *Pseudomonas aeruginosa* has ability to produce a number of redox-active phenazine compounds including pyocyanin. The pyocyanin pigment possesses antimicrobial, anticancerous, antioxidant, anti-inflammatory and immunosuppressive properties. This review covers the challenges and new insights into pyocyanin from *P. aeruginosa* with emphasis on the role of pyocyanin in *P. aeruginosa* infection with special attention to applications of pyocyanin pigment.

Key words: *Pseudomonas aeruginosa*, pigment, pyocyanin, antagonistic property

1. Introduction

Pseudomonas aeruginosa is a Gram-negative rod shaped bacterium (Moore *et al.*, 2006), widely spread in soil, water and many other environments (Suthar *et al.*, 2009). It is a common opportunistic and nosocomial pathogen capable of infecting immunocompromised human (Lyczak *et al.*, 2000). *P. aeruginosa* produces variety of pigments as secondary metabolites with numerous of importance in various industrial fields. These secondary metabolites (pigments) are not essential for growth and proliferation of bacteria but for bacterial pathogenicity and biological control (Lau *et al.*, 2004; Muller *et al.*, 2009; Narsing Rao *et al.*, 2017). Humans use secondary metabolites as medicines, flavorings and as biocontrol agents. Pyocyanin is one of the extracellular phenazine pigment, synthesized by the majority of strains of *P. aeruginosa*. Nearly 90-95% of all isolates of *P. aeruginosa* produce pyocyanin pigment (Ran *et al.*, 2003). Pyocyanin is a heterocyclic water soluble compound composed of two subunits of N-methyl-1-hydroxyphenazine. Pyocyanin comes under the tricyclic phenazine class of compounds (Figure 1). Pyocyanin is a zwitter ion containing a phenol group and its zwitter ionic properties are believed to permit the toxin to easily permeate cell membranes (Hall *et al.*, 2016).

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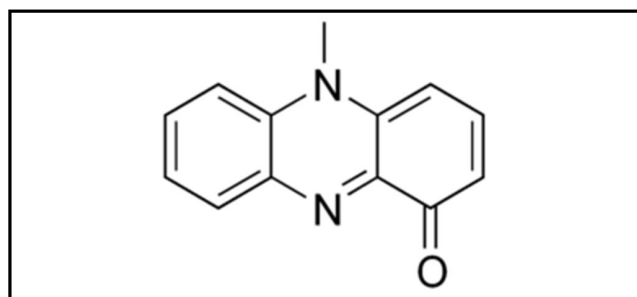


Figure 1: Structure of pyocyanin

The Shikimic acid pathway is used by the *P. aeruginosa* for the biosynthesis of pyocyanin where, shikimic acid acts as precursor (Figure 2). This pathway present only in bacteria, fungi, algae, parasites, plants but absent in animals. Microorganism and plants synthesize aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and various pigments by this pathway during the stationary phase of microbial growth (Chin *et al.*, 2001).

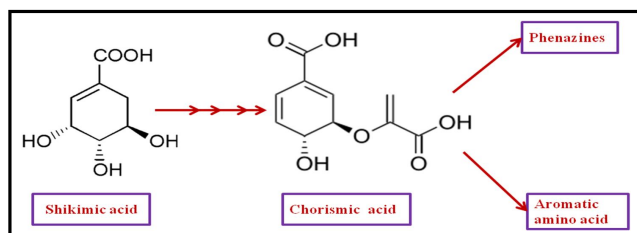


Figure 2: Phenazines from shikimic acid

Pyocyanin is a potential factor that may enhance the survival of *P. aeruginosa* by increasing its capacity to compete with other microorganisms. A decrease in pathogenicity of *P. aeruginosa* was observed *in vitro*, when biosynthesis of pyocyanin was inhibited (Ho Sui *et al.*, 2009). 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 colonizations and fungal growth both *in vivo* and *in vitro* condition (Pai *et al.*, 2010). *P. aeruginosa* strains, isolated from infections have been well studied by researchers for the production of pyocyanin (Hoadley *et al.*, 1981; Hoadley and Ajello, 1982; McGowan *et al.*, 1988).

Pyocyanin is a diffusible molecule which interacts with molecular oxygen intracellularly and disturbs redox cycling. These disturbances can result in the production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide which induce oxidative stress and damage of cells (Hassett *et al.*, 1992). This natural ability of pyocyanin to modulate redox cycle and rise in oxidative stress appears to play vital role in its antimicrobial, antioxidant and in other applications. Researchers explored that the antimicrobial action of pyocyanin produced by *Pseudomonas* sp. TBH2 aid in controlling biocorrosive bacterial biofilm made by *Bacillus* sp. (Narenkumar *et al.*, 2017). Pyocyanin has various pharmacological effects on prokaryotic cells (Ohfuji *et al.*, 2004). It has been used to control phytopathogens (Sudhakar *et al.*, 2013). It has also been reported for its application in aquaculture (Priyaja *et al.*, 2014). A number of phenazines, however, do not exert cytotoxic effects in eukaryote cell and have been considered as anticancer or anti-infective agents (Laursen and Nielsen, 2004; Mavrodi *et al.*, 2006). Pyocyanin pigment showed a more powerful interference in actively respiring cells such as tumor cells. It has been reported that phenazine derivatives can interfere with the function of topoisomerase I and II activities in eukaryotic cells (tumour cells) (Hassani *et al.*, 2012). Pyocyanin is a redox active compound; it has also got application in biosensors because it can carry out transfer of electron between the electrode material and enzyme molecules. Such types of biosensors which are based on pyocyanin were also expected in different fields such as pharmaceutical, agricultural, and environment (Priyaja, 2013). Many researchers used phenazine compounds for the development of sensors and also in nanotechnology; for example, a derivative of phenazine was used to develop a pH sensor based on luminescence (Ryazanova *et al.*, 2007). An amperometric sensor was

developed for determination of hydrogen peroxide utilizing neutral red attached to multiwalled carbon nanotubes (Jeykumari and Narayanan, 2007). It also displays antibiofilm activity because of its capability to arrest the electron transport chain of many microorganisms (Kerr, 1994). In previous research, it has been reported that secretions from *P. aeruginosa* also inhibit formation of biofilm by several fungi, as well as other bacterial pathogens (Holcombe *et al.*, 2010).

2. Pigments

The pigments of natural origin gain more attention in the market due to the development of interest in customers toward natural pigment and hazardous effects of synthetic pigments. In previous years, there is elevation in use of natural pigments in cosmetics, dyestuff, foodstuff and in pharmaceutical industry (Unagul *et al.*, 2005). Natural color from flora and fauna are considered to be very safe because of non- carcinogenic, non-toxic and bio-degradable nature of pigments (Cristea and Vilarem, 2006). Plants and microorganism are the two main sources of natural pigments. Pigments come in various colors, some of which are water- soluble (Tibor, 2006) and some are solvent soluble. Large scale use of plants may lead to the loss of valuable species. Microbial pigments are preferred over the plant pigments because of their stable nature (Raisainen *et al.*, 2002) and availability throughout the year for cultivation (Parekh, 2000).

2.1 Microbial pigments

Microorganisms are the most powerful creatures in existence and known to produce a wide variety of pigments; therefore they are promising source of food colorants (Aberoumand, 2011; Ahmad *et al.*, 2012). Several numbers of pigments have been secreted by microorganisms (Fungi and bacteria) such as quinines, carotenoids, melanins, monascins, violancein, flavins (Duffose, 2006). Fungal (Table 1) and bacterial pigments (Table 2) have extensive applications, *viz.*, antitumour, antioxidant, anti-inflammatory, antimicrobial beside act as coloring agent in cosmetic and food industry (Venil and Lakshmanaperumalsamy, 2009). They have an enormous advantage over plant pigments, including easy and rapid growth in low cost medium, easy processing, and growth that is independent of weather conditions. Fungi play vital role in production of pigment that can be used safely. Moreover, fungi are reported to produce larger amount of pigments (Kirti *et al.*, 2014).

Table 1: Biologically active pigment produce by Fungi

S.No.	Fungi	Pigment	Color	References
1	<i>Fusarium oxysporum</i>	Anthraquinone	-	Nagia and El-Mohamedy, 2007
2	<i>Aspergillus versicolor</i>	Asperversin	-	Miao <i>et al.</i> , 2012
3	<i>Ashbya gossypi</i>	Riboflavin	Yellow	Unagul <i>et al.</i> , 2005; Hong <i>et al.</i> , 2008; Powers, 2003
4	<i>Talaromyces verruculosus</i>		Red	Chadni <i>et al.</i> , 2017
5	<i>Trichoderma, Aspergillus</i>	Anthraquinone	-	Duran <i>et al.</i> , 2002
6	<i>Monascus</i> sp.	Monascin, ankaflavin monascorubrin, rubropunctatin monascorubramine, rubropuntamine	Yellow Orange, Red	Feng <i>et al.</i> , 2012
7	<i>Stemphylium lycopersici</i>	Anthraquinone	-	Li <i>et al.</i> , 2017
8	<i>Penicillium herquei</i>	Atronenetin	Yellow	Takahashi and Carvalho, 2010
9	<i>Phycomyces blakesleeana</i>	b-carotene	Yellow-orange	Malik <i>et al.</i> , 2012
10	<i>Penicillium oxalicum</i>	Anthraquinone	Red	Atalla <i>et al.</i> , 2011
11	<i>Trichoderma virens</i>	Viridol	Yellow	Mukherjee and Kenerley, 2010

Among microorganisms, bacteria have great potential to secrete diverse bioproducts (Figure 3). 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 (Nagpal *et al.*, 2011). Pigment producing bacteria are ubiquitous and present in various ecological niches such as soil (Zhu *et al.*, 2007), fresh water (Asker *et al.*, 2008), rhizospheric soil (Peix *et al.*, 2005), marine samples (Franks *et al.*, 2005) and desert sand (Liu *et al.*, 2009). Various researchers have been investigated the production and application of bacterial pigments (Joshi *et al.*, 2003; Ahmad *et al.*, 2012). By finding cheap and suitable growth medium which can reduce the cost of pigment production and increase its applicability for industrial production (Ahmad *et al.*, 2012), the work on the bacterial pigments should be intensified.

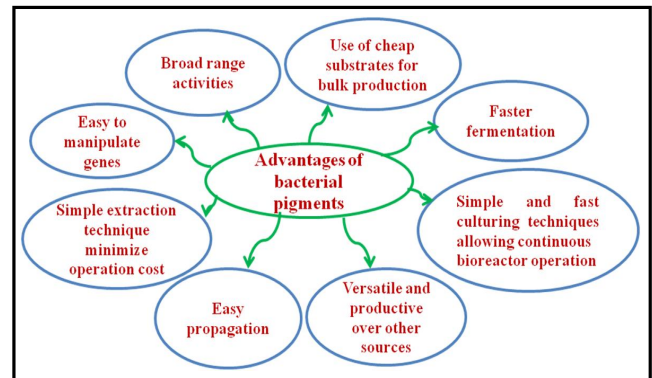


Figure 3: Various advantages of bacterial pigments

Table 2: List of some of bacterial pigments

S. No.	Bacteria	Pigment	Color	References
1	<i>Chromobacterium</i> sp. NIIST (MTCC 5522)	Violacein		Sasidharan <i>et al.</i> , 2015
2	<i>Serratia rubidaea</i>	Prodigiosin	Red	Moss, 2002
3	<i>Pseudomonas aeruginosa</i>	Pyocyanin	Blue	El-Fouly <i>et al.</i> , 2015
4	<i>Chromobacterium violaceum</i> , <i>Janthinobacterium lividum</i> <i>Pseudoalteromonas luteoviolacea</i>	Violacein		Yada <i>et al.</i> , 2007

3. *P. aeruginosa* and its pigments

P. aeruginosa is a Gram-negative bacterium, classified as an opportunistic pathogen, which cause diseases mostly in patients which are immunocompromised with cancer and patients suffering from severe burns and cystic fibrosis (CF). *P. aeruginosa* grows at optimum temperature of 25° to 37°C and has the capacity to grow at 42°C which distinguish it from other *Pseudomonas* spp. It has the ability to grow under varieties of environmental conditions. It is facultative anaerobe that grows in normal atmosphere as well as in atmosphere deprived of adequate oxygen supply (hypoxic), therefore has colonized many natural and artificial environments. *P. aeruginosa* can achieve anaerobic growth with nitrate or nitrite as a terminal electron acceptor. By substrate-level phosphorylation, it is able to ferment arginine and pyruvate when oxygen, nitrate and nitrite are absent (Schobert and Jahn, 2010). Adaptation to microaerobic or anaerobic environments is required for certain lifestyles of *P. aeruginosa*. For example, thick layers of lung mucus and alginate surrounding mucoid bacterial cells can limit the diffusion of oxygen during lung infection in cystic fibrosis patients (Hassett, 1996; Worlitzsch *et al.*, 2002; Cooper *et al.*, 2003; Williams *et al.*, 2007). It has few nutritional requirements and can adapt to conditions not tolerated by other microorganisms. The genome of *P. aeruginosa* is very large for a prokaryote, it provides an understanding of the metabolic and pathogenic mechanisms that underlie the success of this versatile pathogen, and it has become a model for understanding microbial genomic variation and evolution in chronic disease. The success of *P. aeruginosa* as an opportunistic pathogen is substantially due to the versatility and adaptability encoded in its genome (Paul, 2018).

Pseudomonas species is known for their ubiquity in the natural world and capacity to utilize a striking variety of organic compounds as energy sources and production of a remarkable array of secondary metabolites (Gross and Loeper, 2009). It produces different pigments (Figure 4) like pyocyanin (blue-green), pyoverdin

(yellow-green and fluorescent), pyomelanin (light-brown) and pyorubin (red-brown) (Meyer, 2000). Pyoverdin is an iron binding molecule, produced by *Pseudomonas* species, that competes with transferring for host iron. It contributes to the virulence of *Pseudomonas* infections. Pyoverdin are fluorescent siderophores produced by pseudomonads. Pyomelanin synthesized from the catabolism of tyrosine or phenylalanine. Pyomelanin is formed abiotically outside the cell when excreted homogentisic acid auto-oxidizes to form benzoquinone acetic acid. Pyorubin is a bright red, water soluble, non fluorescent pigment produced by some strains of *P. aeruginosa*.

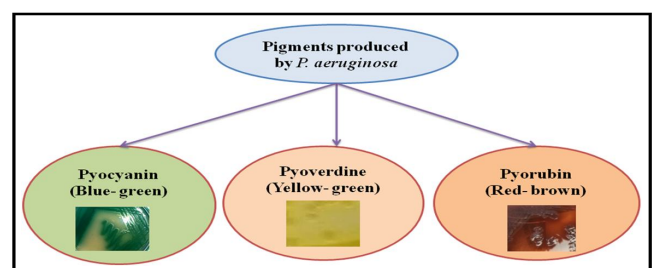


Figure 4: Pigments produced by *P. aeruginosa*

4. Pyocyanin: history and its structure

Since prehistoric times, the pigments have been used as coloring agents. Archaeologists uncovered various evidences which depict that early humans used pigments for decorative purposes, which prove that pigments has been used from pre-historic time (Kassinger, 2003). The pyocyanin pigment was first reported by Fordos from purulent wound dressings in 1859 (Fordos, 1859). Production of a blue-green pigment by bacteria, albeit without identification of the responsible compound, has been described by Schroeter (Schroeter, 1872). Gessard found that pyocyanin was produced by an aerobic motile bacterium (Gessard, 1882), which he named '*Bacillus pyocyaneus*'. In 1900, Migula finally renamed pyocyanin-producing species by '*Pseudomonas aeruginosa*', which is the name still in use today (Migula, 1900). By potentiometric

studies, it has been found that zwitter ion nature of the pigment showed that pyocyanin acted as a reversible redox system in mixture with its reduced leuco derivative (Jensen and Holten, 1949). It also shows chameleon phenomenon due to its redox active states (Friedheim and Michaelis, 1931). Production of pyocyanin by *P. aeruginosa* was identified to be sensitive to the phosphate concentration in the growth media (King *et al.*, 1954; Frank and DeMoss, 1959). Burton and his colleagues (1947) reported that amino acids could replace the peptone commonly claimed to be essential for good pigmentation. Since then more than 100 different phenazine compounds of microbial origin have been reported in the literature (Laursen and Nielsen, 2004; Mavrodi *et al.*, 2006; Pierson and Pierson, 2010).

5. Biosynthetic pathway

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 (Gerber, 1973). Oxidized, monovalently reduced or divalently reduced are three different states in which pyocyanin can exist. Biosynthesis of pyocyanin pigment occurred by a metabolic pathway, known as shikimic acid pathway (Figure 5). This pathway used by microorganism (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 pathway (Herrmann and Weaver, 1999). Shikimic acid pathway is also known as aro pathway. Shikimic acid act as precursor molecule for 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 (Leisinger and Margraff, 1979). Amino deoxy isochorismate (ADIC) synthase enzyme used in this step, which convert 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 phenazine ring system, the condensation of two similar DHHA molecules is required. The two identical molecules react with each other by nucleophilic addition, dehydration and tautomerization to give 5, 10-dihydroanthranilic acid, which is then undergo oxidation to form phenazine-1-carboxylic acid (PCA) (Figure 5).

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 (Romer and Herbert, 1982). The PCA leads to the synthesis of pyocyanin by hydroxylative decarboxylation mechanism. Mainly two steps involved in the pyocyanin synthesis from PCA (Figure 6). In 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 nitrogen atom of phenazine ring moiety. Second step lead to the formation of pyocyanin which is catalyzed by PhzS, a FAD-dependent mono-oxygenase. This enzyme is responsible for hydroxylative decarboxylation of 5-methylphenazine-1 carboxylic acid betaine which leads to the formation of pyocyanin (Parsons *et al.*, 2007). 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 (Parsons *et al.*, 2007). Formation of a complex between PhzM and PhzS would presumably prevent release of 5-methylphenazine-1-carboxylate.

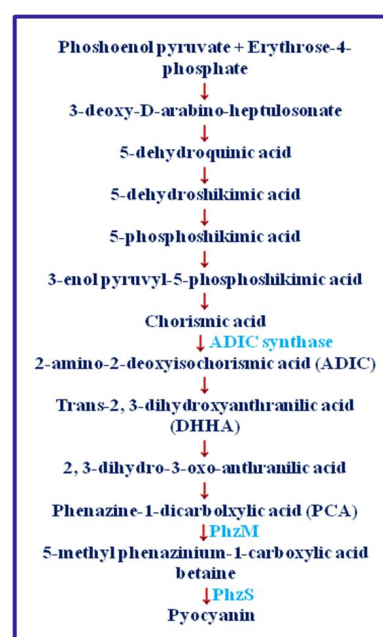


Figure 5: Shikimic acid pathway of pyocyanin biosynthesis in *P. aeruginosa*

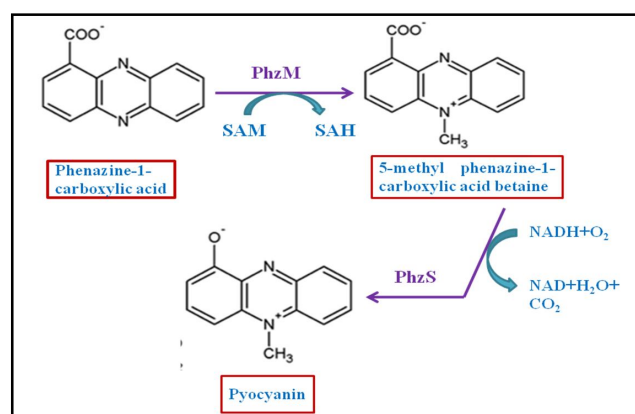


Figure 6: Role of PhzM and PhzS on pyocyanin synthesis

6. Genetics and regulation of pyocyanin pathway

Two specific genes must be functional for the production of pyocyanin by *P. aeruginosa*. Mvfr gene, produces a transcription factor, which then activates phzAB genes. These genes produce the molecule quinolone which then regulates operons 1 and 2 of PhzRABCDEF which are the key to the synthesis of phenazine (Mavrodi *et al.*, 2001). Pyocyanin biosynthesis begins with the conversion of chorismic acid to 2-amino-2-desoxyisochorismate (ADIC) by ADIC synthase, PhzE. 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) (Wulf and Parson, 2014). Following this, PhzD catalyzes the hydrolytic removal the pyruvate moiety from ADIC to form (5S,6S)-6-amino-5-hydroxy-1,3-cyclohexadiene-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 tetrahydrophenazine-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 (Wulf and Parson, 2014). The conversion of PCA to pyocyanin is achieved in two enzymatic steps: firstly, PCA is methylated on N5 to 5-methylphenazine-1-carboxylate betaine by the enzyme PhzM using the cofactor S-adenosyl-L-methionine and secondly, PhzS catalyzes the

hydroxylative decarboxylation of this substrate to form the final product, pyocyanin (Mavrodi *et al.*, 2001).

7. Pyocyanin in *P. aeruginosa* infection

The isolates of *P. aeruginosa* 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 (Prince, 2012). *P. aeruginosa* is a metabolically versatile bacterium that can cause a wide range of severe opportunistic infections in patients with serious underlying medical conditions. *P. aeruginosa* 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 (Lyczak *et al.*, 2002) (Table 3).

Table 3: Common infection caused by *P. aeruginosa*

S. No.	Pseudomonal Infection	Major risk factors
1	Urinary tract	Use of urinary catheter
2	Soft tissues	Burns, open wounds, postsurgery
3	Respiratory/pneumonia	Old age, chronic obstructive pulmonary disease, cystic fibrosis, mechanical ventilation
4	Otitis externa (swimmer's ear)	Tissue injury, water blockage in ear canal
5	Bacteremia	Immunocompromised
6	Keratitis (corneal infection)	Extended contact lens wear, contaminated contact lens solution
7	Otitis media folliculitis (hot tub rash)	Improperly cleaned hot tubs

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 bacteraemia (NNIS, 1999). In Europe, *P. aeruginosa* was found to be the third most common isolate from nosocomial infections in intensive care units (Vincent *et al.*, 1995). Mortality rates ranging from 40% to more than 60% have been reported in bacteremic nosocomial pneumonia and in ventilator associated pneumonia (Crouch Brewer *et al.*, 1996; Mayhall, 1997; Rello *et al.*, 1997). In the United States, *P. aeruginosa* is among the most common hospital pathogens and is the second most common pathogen isolated from patients with ventilator-associated pneumonia (VAP) (Hidron *et al.*, 2008).

There is substantial difference in phenotype of *P. aeruginosa* isolated from acute infections and from chronic infections (Smith *et al.*, 2006). Isolates from acute infections express a wealth of virulence factors, while in contrast, many isolates from chronic CF lung infections lack some of the most inflammatory bacterial features, such as flagella and pili, and downregulate other virulence mechanisms such as the type 3 secretion system (Hogardt and Heesemann, 2010). *P. aeruginosa* uses several virulence factors to establish chronic respiratory infections in bronchiectasis, chronic obstructive pulmonary disease and cystic fibrosis patients (Murphy, 2008; Cohen and Prince, 2012). 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 4), including elastase, alkaline protease, the LasA protease, hemolysin, rhamnolipids and pyocyanin (Lyczak *et al.*, 2002) to disrupt the host immune responses and cause cytoskeletal reorganization.

Table 4: Factors enhancing the pathogenicity of *P. aeruginosa*

S. 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

Pyocyanin is a zwitter ion that can easily penetrate biological membranes. By interfering with several cellular functions in host cells, pyocyanin promotes virulence including electron transport, cellular respiration, energy metabolism, gene expression and innate immune mechanisms. It causes oxidative stress to the host, disrupting host catalase and mitochondrial electron transport (Lau *et al.*, 2004). Purified pyocyanin induced apoptosis *in vitro* in neutrophils as well as inhibit the phagocytosis of apoptotic bodies by macrophages (Lau *et al.*, 2004; Bianchi *et al.*, 2008). It is also able to modulate the expression of the chemokines IL-8 and RANTES by airway epithelial cells (Denning *et al.*, 1998) and suppress cilia beating.

It is readily recovered in large quantities from the sputum of patients with CF infected by *P. aeruginosa* (Wilson *et al.*, 1988) and from ear secretions of *P. aeruginosa* mediated chronic otitis media (Reimer, 2000). In spite of this, the exact contribution of pyocyanin 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 (Mahajan-Miklos *et al.*, 1999; Cao *et al.*, 2001; Lau, 2003) and mice (Lau *et al.*, 2004)

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* has the ability to interfere with functions such as ciliary beating and, therefore cause dysfunction of epithelium as the ciliary are required for sweeping mucus up the throat (Kanthakumar *et al.*, 1993). Additionally, neutrophil apoptosis, (Usher *et al.*, 2002) immunoglobulin release from B-lymphocytes and interleukin (IL-8) release (Denning *et al.*, 1998) and CCL5 are all impaired by pyocyanin causing the immune system of the lung to be weakened. *In vivo* studies have shown that in the presence of pyocyanin, the fungal growth is inhibited. The fungicidal mechanism is the activation of NAD(P)H to induce a redox active cycle to produce reactive oxygen intermediates. This allows *P. aeruginosa* to have a competitive advantage as it may dominate over other micro-organisms in the CF lung (Kerr *et al.*, 1998). The intracellular level of ATP is also reduced by pyocyanin causing further damage to CFTR which are already impaired in cystic fibrosis. CFTR channels depend on ATP for two main purposes. First, the binding and hydrolysis of ATP has to occur at two domains of nucleotide binding for the channel to move between its open and closed conformation. Second, phosphorylation of CFTR by protein kinase A II should occur in order for the channel to be operational. PKA II is activated by cAMP which is secreted from ATP. Both these processes are impaired when ATP is depleted by pyocyanin (Ostedgaard *et al.*, 2001). If *P. aeruginosa* utilize pyocyanin to its advantage in competing with other bacterial strains in the same ecological niche, it must, therefore, have a mechanism for its own 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. aeruginosa* made 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 nonresponsive 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 H₂O₂ that might be generated outside the cells *via* extracellular auto-oxidation 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 (Hassan and Fridorich, 1980). 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).

8. Antimicrobial resistance of *P. aeruginosa* infection

Antimicrobial resistance is the capacity of microbes to stop an antimicrobial substance such as antibiotics, antivirals and antimalarials from working against it. Because of this standard

treatments become ineffective, infections persist and may spread to others. The *P. aeruginosa* is a leading source of infection in hospitals, with few available treatment options. The infections caused by *P. aeruginosa* are very difficult to treat due to its intrinsic ability to resist many classes of antibiotics as well as its ability to acquire resistance. There are three mechanisms responsible for resistance and all these known mechanisms of antibiotic resistance can be displayed by this bacterium (intrinsic, acquired and adaptive); sometimes all within the same isolate. Despite the use of combination of drug therapies, resistance rates increased rapidly (Moore and Flaws, 2011). There are many new drugs available in the market to treat *P. aeruginosa* infections but there has been a return to the use of older drugs such as polymyxins that had previously fallen out of favor due to wide reports of toxic side effects (Livermore, 2002). Despite the reports of nephrotoxicity and neurotoxicity, for patients with CF suffering recurrent infections of multidrug-resistant bacteria, colistin (a polymyxin drug) has been routinely administered *via* inhalation for the past 15 years (Falagas and Kasiakou, 2006), demonstrating that the antibiotic resistance problem has been influencing therapeutic choices for many years.

9. Inhibition of pyocyanin production to combat *P. aeruginosa* infection

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 colonised by *P. aeruginosa*, which significantly reduces life expectancy and it is the leading cause of morbidity and mortality for CF patients. *P. aeruginosa* is a versatile pathogen, possessing a number of adaptations such as; an outer membrane of low permeability, a multitude of efflux pumps and various degradative enzymes that disable antibiotics. These features combine to limit the range of effective treatment options. The ability of *P. aeruginosa* to cause infection is depend on the secretion of agents termed, virulence factors, such as toxins and adhesion molecules, that actively cause damage to host tissues. Pyocyanin produced by several clinical strains of *P. aeruginosa* from pulmonary and extra-pulmonary infections (Schaber *et al.*, 2004; García-Contreras *et al.*, 2015; Guendouze *et al.*, 2017) as well as in environmental strains (Grosso-Becerra *et al.*, 2014) and is found in high concentrations up to 100 M (Caldwell *et al.*, 2009) in the lung of cystic fibrosis patients.

Pyocyanin act as both a virulence factor and a quorum sensing signalling molecule for *P. aeruginosa* (Lau *et al.*, 2004; Karatuna and Yagci, 2010). In *P. aeruginosa*, virulence is controlled through quorum sensing (Allen *et al.*, 2014). Virulence and quorum sensing are connected by signaling pathways. Quorum sensing pathways rely on the production, release and detection of small molecule signals that regulate virulence genes (Ng and Bassler, 2009). It has been identified by some researcher that pathogen-associated proteins have homology only with pathogenic bacteria and not with non-pathogens (Ho Sui *et al.*, 2009). 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. 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.

It has been reported that thiolactone 1 which is a structural analog of the native acyl-homoserine lactone, is a potent inhibitor of pyocyanin production *in vivo*. The thiolactone 1 interacts with both LasR and RhlR but the inhibition through RhlR results in the key anti-virulence effects of the compound (O'Loughlin *et al.*, 2013). LasR is a transcriptional activator in *P. aeruginosa*, required for the transcription of the genes for elastase and LasA protease associated with virulence whereas, RhlR is a regulatory gene encodes the transcriptional regulator RhlR which has central role in quorum sensing response.

10. Biodegradation of pyocyanin pigment

The environmental degradation of the residual pyocyanin became an important factor as pyocyanin has been used in 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 40 h (Yang *et al.*, 2007). Hill and Johnson (1969) 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 (Chen *et al.*, 2008).

Biodegradation of pyocyanin is esteemed by the presence of phenolic character in the compound and phenolics are the best substrates for peroxidases. The oxidation of pyocyanin leads to its inactivation and become non-toxic and the reaction is irreversible (Reszka *et al.*, 2004). 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 (Reszka *et al.*, 2004).

11. Applications of pyocyanin

11.1 Pyocyanin as an anti-oxidant

Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging (Kanwar *et al.*, 2009; Chiavaroli *et al.*, 2011). 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 chain of reaction that may destroy cells. Free radical scavenging activity of pyocyanin was estimated by DPPH (2, 2- diphenyl-1-picrylhydrazyl hydrate) radical scavenging assay (Liyana and Shahidi, 2005). 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 (Huang *et al.*, 2005). DPPH contains an odd electron because of which it has 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-picrylhydrazine).

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 very much lower concentration than that of ascorbic acid (Laxmi and Bhat, 2016). This is a positive indication for the safe use of product as compound showed very high antioxidant activity at very minute concentration of pyocyanin (Liyana and Shahidi, 2005). Chandran (2014) evaluated the antioxidant activity of pyocyanin pigment and it was found to be 55% at 500 µg/ml concentration. Pyocyanin extracted from *P. aeruginosa* CGR-3 showed 38% of free radical scavenging activity at 4.25 µg/ml pyocyanin concentration (Rani *et al.*, 2018). The phenolic compounds present in plants also shown to have antioxidant potential. The polyphenols like methanolic extract of *Abutilon indicum* leaves showed very strong antioxidant activity *in vitro* (Das *et al.*, 2019).

11.2 Pyocyanin as an antibacterial compound

P. aeruginosa, which exerts broad antagonistic activity against other bacterial and fungal pathogens through the production of a secondary metabolite-pyocyanin. Although Pseudomonads are well reported for their pathogenicity, the ability of this bacterium to produce antimicrobial pigment opened the door of using this as biological control agent (Devnath *et al.*, 2017). Pyocyanin has antibiotic activities and it is also able to coordinate the response of microbial communities to changes in the environment. Small attempts have been made to analyze the relation between pyocyanin and its inhibitory action. Since year 1940, it has been reported that pyocyanin possess antibacterial properties (Waksman and Woodruff, 1940). Pyocyanin pigment was also known as Colicin because it inhibit the growth of *E. coli*. During lysis of bacteria, protein fraction was released which showed the antimicrobial properties of pyocyanin pigment (Young, 1947). According to Hassan and Fridorich (1980), 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 (H₂O₂) and also divert the electron flow, causing toxicity to the cells.

Pyocyanin, a water soluble bio-active compound, has the capacity to arrest the electron transport chain, exhibits antifungal activity (Wilson *et al.*, 1987) and antibacterial activity. Pyocyanin increases intracellular oxidant stress and exhibits a redox cycle under aerobic conditions. This leads to reactive oxygen species (ROS) production such as superoxide and hydrogen peroxide; these ROS compounds are capable of inhibiting microbial growth (Denning *et al.*, 1998; Das and Manefield, 2012). The bactericidal effect of purified pyocyanin pigment depends on its concentration in all cases (Baron and Rowe, 1981). Baron and Rowe (1981) 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.

Pyocyanin showed bactericidal action against many pathogenic bacteria like *Salmonella paratyphi*, *E. coli* and *Klebsiella pneumonia* (Saha *et al.*, 2008). 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. Variation in the lipid content of cell wall of Gram-positive and Gram-negative bacteria may be responsible for the variation in the sensitivity of pyocyanin

antibiotic. El-Fouly and colleagues in (2015) reported that highest minimum inhibitory concentration (MIC) of purified pyocyanin was 50 µg/ml whereas lowest MIC was 20 µg/ml against *E. coli*. Pyocyanin from *P. aeruginosa* DSO-129 demonstrated for antibacterial effect on organisms like *S. aureus*, *Staphylococcus epidermis*, *Bacillus subtilis*, *Micrococcus luteus* and *S. cerevisiae* (Rahman *et al.*, 2009). There has been another report related to antimicrobial action of pyocyanin against pathogens. The pigment showed very effective against organisms like *E. coli*, *Acinetobacter*, *S. aureus* and *Streptococcus pneumonia* (Sweden, 2010).

The mechanism of action of the pigment on fungus is same as that of mechanism of antibacterial action. Pyocyanin yield obtained from *P. aeruginosa* OSh1 was recorded 76.11 µg/ml showed higher antibacterial activities at 28.3, 37.3 and 21 mm against Gram-positive, Gram-negative bacteria and fungi respectively (Barakat *et al.*, 2015). Pyocyanin obtained from culture supernatants of *P. aeruginosa* isolates (grown in peptone water liquid medium) reached about 62.8 µg/ml and showed more antimicrobial action on Gram-positive bacteria (inhibition zones = 15 mm in diameter) than on Gram-negative bacteria (inhibition zones = 14 mm in diameter) (El-Shouny *et al.*, 2011). The antimicrobial activity of pyocyanin of *P. aeruginosa* CGR-3 inhibited *E. coli*, *Salmonella paratyphi*, *Klebsiella* sp. *Alternaria* sp. but *Aspergillus niger* remain resistant to pyocyanin (Rani *et al.*, 2018).

Several groups reported the *in vitro* inhibition of yeast growth by *P. aeruginosa* (Hughes *et al.*, 1973; Kerr, 1994) and there are reports which suggest that inhibition of yeast growth by *P. aeruginosa* *in vivo* in patients with (Hughes *et al.*, 1973) and without cystic fibrosis (Kerr, 1994). Pyocyanin obtained from *P. aeruginosa*, isolated from the sputum of CF patient, also seize the growth of fungi like *Candida albicans* and *Aspergillus niger*. When *P. aeruginosa* was co-cultured with *C. albicans*, the *P. aeruginosa* synthesized large amounts of pyocyanin and even the growth of *C. albicans* was inhibited (Hogan and Kolter, 2002; Gibson *et al.*, 2009; Hassanein *et al.*, 2009). Inhibition of growth of *A. fumigatus* by pyocyanin was reported to be of dose dependent but occurred at much higher levels (>19 µg/well). Pal and his colleagues in (2006) reported that pyocyanin showed antibiotic activity *in vivo* on *Candida* sp. grown on Sabroud's dextrose agar. Pyocyanin (0.6 µg/well) and 1-hydroxyphenazine (9.5 µg/well) inhibited the growth of additional yeast species known to cause human infection in the well plate assay (*C. krusei*, *C. keyfr*, *C. guillermondii*, *C. tropicalis*, *C. glabrata*, *C. lusitaniae*, *C. parapsilosis*, *C. pseudotropicalis* and *S. cerevisiae*).

11.3 Pyocyanin as antibiofilm agent

Bacteria within biofilm are thousand times more resistant to conventional antibiotic treatment and responses of host immune. This makes it extremely difficult to eradicate biofilm. 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 slimy extracellular matrix that is formed of extracellular polymeric substances (EPS). An EPS is a sugars, proteins and nucleic acids (such as DNA) network. It helps the microbes to stick together in a biofilm. Many disease outbreaks were found to be linked with biofilms formation and there is always threat to human health because of food borne diseases. Biofilms became a major problem

in food industry. The reason that biofilm formation is a great cause of concern is that, within a biofilm, bacteria are more resistant to antibiotics and other major disinfectants that we used to control them. Therefore, searching for novel compounds or strategies to inhibit biofilm formation or disperse preformed biofilm is needed (Wu *et al.*, 2015). It has been reported that biofilm formed by many fungi and other pathogenic bacteria was inhibited by secretion from *P. aeruginosa* (Holcombe *et al.*, 2010). There are many reports that *P. aeruginosa* itself can inhibit growth and biofilm formation. Bacterial pigments used for controlling biofilm are reported to have antioxidant activity along, which in addition to absence of cytotoxicity, make them useful in food industry for control of food borne pathogens.

Microtitre plate assay with crystal violet staining was used to test *in vitro* biofilm formation and its inhibition. Generally, 96 well microtitre plates were used for antibiofilm assay. The antibiofilm activity of compound was expressed using two techniques: scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). Biofilm inhibitory concentration (BIC) is defined as the lowest concentration of the compound which inhibits biofilm formation. Laxmi and Bhat (2016), reported that pyocyanin pigment at very low concentration (2×10^{-2} ng/µl biofilm inhibitory concentration) has antibiofilm activity against biofilms formed by *Vibrio diabolicus* and *Salmonella enteritidis* and more than 80% reduction in biofilm was achieved. Pyocyanin (1.245 µg/ml) from *P. aeruginosa* BTRY1 was able to bring about 80% reduction of biofilm in *S. warneri* BTDF2, followed by 60% inhibition in the EPS production of *B. casei* BTDF1, 40% in *B. pumilus* BTMY2, 36% in *Bacillus* sp. BTSD1, 28% in *B. niacini* BTDP3 and 11.6% in case of *B. altitudinis* BTMW1. However, biofilm formation by *P. aeruginosa*, *Micrococcus luteus* and *Geobacillus stearothermophilus* could not be controlled.

Pyocyanin (2×10^{-2} ng/µl) in combination with bacteriocin (3.8 ng/µl) showed around 70% biofilm inhibition in case of *B. casei* BTDF1 and *S. warneri* BTDF2 and 30–40% in case of *Micrococcus luteus* and *Geobacillus stearothermophilus*. Whereas, with rhamnolipids (1.2 ng/µl) it has been reported that both *B. casei* BTDF1 and *B. pumilus* BTMY2 were inhibited to 70%, but it does not inhibit the biofilm of *P. aeruginosa* BTRY1. The BIC values of the bioactive compounds used in the study were in nanogram quantities against the tested food pathogens. This clearly indicates the immensely potent strength of the pyocyanin in biofilm control compared to the current antibiofilm strategies like antibiotic treatments. Besides pigments, phytochemicals has been also used as antibiofilm agent (Kanwar *et al.*, 2018) alone or in combination with antibiotics.

11.4 Pyocyanin as antitumour agent

Pyocyanin act as cytotoxic compound by increasing the intracellular reactive oxygen species. Pigment generates superoxide (O_2^-) and hydrogen peroxide (H_2O_2) reactive oxygen species on reduction by NADH and NADPH in the cells and the reduced form of pigment transfer electron to O_2 . This is referred as intracellular redox cascade of pyocyanin. The reactive oxygen species formed around and within the mitochondria (O'Malley *et al.*, 2003). Pyocyanin prevent the breakdown of hydrogen peroxide (H_2O_2) by inhibiting the activity of catalase by reducing the expression of gene encoding catalase. This leads to the increase in level of reactive oxygen species

indirectly (Ricciardolo *et al.*, 2006). 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 (Nishi and Forgac, 2002).

Hassani and his colleagues (2012) also studied the cytotoxic effect of pyocyanin from mutant and wild type strains of *P. aeruginosa* on RD cells. Zhao and his colleagues (2014) studied the proliferation of HepG2 cells in the presence of 10 µg/ml pyocyanin. According to their study, a nine day treatment by pyocyanin led to 67% decline in HepG2 cell numbers compared to the control. They demonstrated that cell death induced by pyocyanin arises from oxidative stress due to ROS augmentation, damage to DNA, activation of caspase-3 and the acceleration of senescence and apoptosis. Aylin and his colleague (2018) reported the cytotoxic effects of pyocyanin on human pancreatic cancer cell line (Panc-1). According to their study there was inhibition of 98.69 ± 0.23 and $89.88 \pm 1.86\%$ of 6 mg/ml of pyocyanin extracted from clinical and soil isolates of *P. aeruginosa* respectively. Priyaja and colleagues (2014), 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 highest response to pigment than others. Further, 80% of cell viability remain even at high concentration of pigment indicate that it is safe to consume the food supplemented with pyocyanin (Laxmi and Bhat, 2016). The cytotoxic effect of pyocyanin pigment was checked by MTT assay (Arung *et al.*, 2009). 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 (Patil *et al.*, 2017).

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 (Arung *et al.*, 2009). This is a colorimetric assay method which 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.

11.5 Pyocyanin in biosensor

Biosensors are analytical devices that convert a response into an electrical signal with biological system. For more accurate determination of glucose concentration, an amperometric biosensor system using a screen printed electrode and pyocyanin as mediator were also developed. Pyocyanin which exists in the oxidized form was reduced by the reaction between glucose oxidase and glucose. The reduced form was a linear relationship between sensor output currents and glucose concentrations (Ohfuji *et al.*, 2004). For detecting water toxicity, a novel mediator-free method based on genetically modified bacteria was also developed where genetically modified (G) bacteria, *P. aeruginosa* was selected as the biosensor strain was used as the indicator. An amperometric biosensor system using pyocyanin as a mediator was developed by (Ohfuji *et al.*, 2004) for a more accurate determination of glucose. Therefore, the biosensors using pyocyanin was also expected to apply to some

fields such as medicine, food and environment. Quintessentially biosensors must be highly specific, independent of physical parameters such as pH and temperature and should be reusable (Mehrotra, 2016). The toxicity response of *G. P. aeruginosa* to 3, 5-dichlorophenol (3, 5-DCP) was measured electrochemically and spectroscopically (Yu *et al.*, 2017). The present study provided convenient, sensitive method for water toxicity detection and extended biosensing supplication of the genetically modified bacterium.

11.6 Microbial fuel cells

The devices which convert chemical energy into electricity are known as microbial fuel cells (MFCs). In MFCs, electrochemically active bacteria (EAB) oxidized a series of carbon source including organic substrate or even pollutants in wastewater and transfer the generated electron to anodes (Rabaey and Rozendal, 2010; Logan and Rabaey, 2012; Lovley, 2012). EAB play an important role in the power generation of MFCs for its ability of generating electrons during metabolism and transferring electrons through the cell membrane from the cell to the anode (Liu *et al.*, 2012; Yong *et al.*, 2013). Bacteria used in MFCs system *Shewanella*, *Pseudomonas* and *Geobacter*. Out of these bacteria, *P. aeruginosa* gets much attention, since it can produce highly redox-active endogenous electron shuttles such as pyocyanin (Rabaey *et al.*, 2005; Yong *et al.*, 2014a, Yong *et al.*, 2014b). Riboflavin and pyocyanin electron shuttles has been proved to be more fruitful (Hernandez and Newman, 2001; Rabaey *et al.*, 2005; Wen *et al.*, 2011). Pyocyanin excreted by *P. aeruginosa* is responsible for carrying electron through cell membrane due to which its concentration in the anodic culture directly effect the power generation efficiency (Yong *et al.*, 2011).

It has been reported that sophorolipid was an effective additive to improve the performance of *P. aeruginosa* inoculated MFC. The improvement is due to the increased electron shuttle (pyocyanin) production and the enhanced membrane permeability, which directly promoted the electron shuttle, mediated extracellular electron transfer (EET) (Shen *et al.*, 2014). MFCs has been studied for their various applications such as treatment of waste (Yong *et al.*, 2014b), biosensors (Mansfeld, 2007), renewable energy (Logan, 2009) *etc.* Despite the applications, poor energy yield has been a serious issue hindering the efficiency of MFCs. There was a study which has been attempted to increase pyocyanin yield from environmentally isolate strain of *P. aeruginosa*. Physiochemical and nutritional parameters were tinkered and yield of pyocyanin was enhanced by formulating bacterial growth media. The study can help in improvement MFC bioelectricity yield by enhanced pyocyanin production in a *P. aeruginosa* inoculated MFC (Rashid and Andleeb, 2018).

11.7 Application of pyocyanin in aquaculture

Aquaculture is defined as the farming of fish, molluscs, crustaceans, *etc.* and it is the cultivation of populations of saltwater and freshwater under controlled conditions. Aquaculture is the multibillion dollar industry on 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 (Bondad-Reantaso *et al.*, 2005). However, the commercial production by aquaculture are 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 of time 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 (Gomez *et al.*, 2007).

A large number of microorganisms harm the aquaculture environment which include 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 (Verdonck *et al.*, 1997; Thompson *et al.*, 2001; Vandenberg *et al.*, 2003; Jayaprakash *et al.*, 2006).

Pseudomonads are usual occupant of the aquatic environment including shrimp culture ponds (Otta *et al.*, 1999) and are often related with gills, skin and intestinal tract of live fish (Cahill, 1990). However, *in vitro* inhibition of fish and prawn pathogens have been shown by the many bacterial isolates which are common members of the non pathogenic microbiota of fish and shellfish culture systems (Jayaprakash *et al.*, 2005). It was stated that certain strains of bacteria have the ability to control pathogens by means of competitive exclusion or by the secretion of inhibitory compounds for, *e.g.*, bacteria associated with *Artemia* and prawn culture systems (Verschuere *et al.*, 2000). The disease prevention has received much attention in order to control the fish and shellfish pathogenic vibrios, particularly by use of non pathogenic bacterial isolates (Sugita *et al.*, 1998; Rengpipat *et al.*, 2000).

Pyocyanin can be applied as eco-friendly drug in 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 a number of pathogens such as *Salmonella*, *Photobacterium demerselae*, *Staphylococcus aureus*, *Vibrio vulnificus*, *V. parahaemolyticus*, *V. harveyi*, *V. fluvialis* and *Aeromonas* (Oblinger and Kreft, 1990; Vijayan *et al.*, 2006). Hai and Fotedar (2009), reported that *P. synxantha* and *P. aeruginosa* are most effective probiotic in inhibiting bacteria isolated from *Penaeus latissulcatus*.

12. Conclusion

Pyocyanin is one of the toxins produced by the *P. aeruginosa* for enhancing its pathogenesis. Pyocyanin enhance 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 pathogens which are multidrug resistant. 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 biocorrosive bacterial biofilm formed by

Bacillus sp. In addition to this, pyocyanin pigment showed cytotoxic effect *in vitro* on cancerous cell line at very minute concentration but it has been reported that pyocyanin has no toxic effect on normal cell lines. The antibiofilm activity of the pyocyanin against multiple antibiotic resistant food pathogens expands their efficacy for applications in 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 in order to establish firmly the positive effects of pyocyanin in clinical and therapeutics science.

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Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. Both the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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