ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1981, p. 814-820
0066-4804/81/120814-07$02.00/0

Vol. 20, No. 6

Antibiotic Action of Pyocyanin

STEPHEN S. BARON† AND JOHN J. ROWE*
Department of Biology, University of Dayton, Dayton, Ohio 45469

Received 9 June 1981/Accepted 21 August 1981

Biologically produced pyocyanin was purified, and the nature of its antibacterial action was determined for several bacteria. The pigment was shown to be bactericidal for all susceptible organisms. The bactericidal effect was dependent upon pyocyanin concentration and resulted in decreases in viability ranging from 1 to 8 log viable cells ml^{-1}. The gram-positive bacteria were more susceptible as a group to the antibiotic action than were the gram-negative bacteria. All apyocyanogenic pseudomonads tested were totally resistant to the pigment, suggesting that resistance may be a characteristic of the genus. *Pseudomonas aeruginosa*, the producer organism, was also essentially unaffected by high concentrations of pyocyanin. Facultative anaerobes were twofold or more times resistant to the action of the pigment under fermentative conditions; however, the antibiotic action did not require oxygen since denitrifying bacteria were more susceptible during anaerobic respiration than during aerobic respiration.

Pyocyanin is a water-soluble blue-green phenazine pigment produced in large quantities by active cultures of *Pseudomonas aeruginosa*. Pyocyanin has antibiotic activity against bacteria (4), fungi (8), and protozoa (9), but is of little therapeutic value because it is quite toxic to eucaryotic cells (18). The physiological significance of the pigment is not known, but because of its inhibitory action, it has been postulated that pyocyanin production may give *P. aeruginosa* a selective advantage in certain growth situations. The nature of bacterial inhibition by the phenazine is neither well understood nor well documented, and no information is available concerning the effect of pyocyanin on the producer organism *P. aeruginosa*. Recently, Hassan and Fridovich (10) proposed that the inhibitory action of pyocyanin is the result of its unique redox potential. They propose that, during respiration, pyocyanin becomes reduced and univalently reduces oxygen to the toxic superoxide radical. The resistance of various bacteria to pyocyanin would therefore be dependent upon the levels of superoxide dismutase and catalase possessed by the organism and on the presence of oxygen.

The purpose of this investigation was to determine the nature of the antibacterial action of pyocyanin and to characterize its inhibitory action on a variety of bacteria, including *P. aeruginosa*, under different environmental and physiological conditions. The results of our study demonstrate that the antimicrobial action of pyocyanin is bactericidal in nature and that the inhibitory action does not require the presence of oxygen as proposed by Hassan and Fridovich (10). However, inhibition by pyocyanin is dependent upon active aerobic or anaerobic respiration.

† Present address: CPC International, Biotechnology Department, Moffet Technical Center, Summit-Argo, IL 60501.

MATERIALS AND METHODS

Organisms. The organisms used for these studies were as follows: *Enterobacter aerogenes* CDC 659-66; *Bacillus licheniformis* ATCC 14580; *Staphylococcus aureus* ATCC 8325; *Bacillus subtilis* 168 *trpC2; Acinetobacter* HO1-N; *Pseudomonas aeruginosa* PAO; a reddish-brown clinical isolate identified as *P. aeruginosa*; *Pseudomonas denitrificans* ATCC 13867; *Pseudomonas fluorescens* ATCC 17513; *Pseudomonas perfectomarinus*, Zobell strain (21); *Paracoccus denitrificans* ATCC 2008; *Micrococcus luteus* ATCC 9341; and clinical isolates of *Escherichia coli* and *Proteus vulgaris*. All stock cultures were maintained on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) slants at room temperature.

Media and culture conditions. The semidefined medium used for most of the experimentation contained the following per liter of deionized water: K_2HPO_4, 7.0 g; $NaH_2PO_4 \cdot H_2O$, 3.0 g; $MgSO_4 \cdot 7H_2O$, 0.24 g; $(NH_4)_2SO_4$, 1.0 g; yeast extract, 1.0 g; and D-glucose, 1.0 g. The final pH was 7.0. A combination of the first three ingredients per liter of water will be referred to as basal salts solution (pH 7.0). The entire medium as described will be referred to as medium A. When needed, medium A was supplemented with 1% (wt/vol) KNO_3.

Medium B, which was used for the production of pyocyanin, was a modification of the medium described by Burton et al. (3) and consisted of the following per liter of deionized water: L-aspartic acid, 14.0 g; glycerol, 10.0 g; K_2HPO_4, 0.4 g; $MgSO_4 \cdot 7H_2O$, 20.0 g; and $FeSO_4 \cdot 7H_2O$, 0.01 g.

All cultures were incubated at 35°C. Aeration was

achieved with a Precision Scientific water bath shaker (Precision Scientific Co., Chicago, Ill.) or with a New Brunswick MF214 10-liter fermentor (New Brunswick Scientific Co., New Brunswick, N.J.).

Preparation and purification of pyocyanin. Biologically produced pyocyanin was prepared from cultures of *Pseudomonas aeruginosa* according to a modified procedure which in part combined the methods described by Blackwood and Neish (2) and Ingledew and Campbell (11). A 12-h culture (400 ml) was used to inoculate 4 liters of medium B contained in a New Brunswick MF214 10-liter fermentor. The culture was maintained at 35°C under aerobic conditions (200 rpm with forced air) for 52 h. Molecular oxygen was required for pyocyanin biosynthesis since the pigment never appeared in cultures incubated under denitrifying conditions.

After being thawed, 1 liter of spent culture was titrated with KOH until a precipitate formed (pH 11 to 12); this precipitate was removed by filtration through cheesecloth. Potassium chloride (approximately 10 g 100 ml^{-1} of filtrate) was added to the filtrate, and the pyocyanin was extracted in a 0.5 volume of chloroform. The chloroform emulsion was centrifuged (15,000 × *g*, 25°C, 15 min) with a Sorvall GSA rotor (Ivan Sorvall Inc., Norwalk, Conn.), and the dark-blue chloroform layer was drawn off. The chloroform layer was washed three times with 0.2 volumes of water and extracted with a 0.33 volume of 0.1 N HCl. The acid layer was washed once with a 0.2 volume of chloroform and titrated with 1.0 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 11.0) until the blue color of pyocyanin reappeared. The pigment was extracted exhaustively from this solution with 0.2 volumes of chloroform, pooled in a flask containing about 2 g of anhydrous $MgSO_4$, filtered, and evaporated to dryness under vacuum. The resulting dark-blue residue was suspended in 10 ml of warm water, and the pyocyanin crystals were titrated with 4 N HCl until they completely dissolved. Small volumes (5 ml) of the now dark-red solution were passed through a column of Sephadex G-25 (particle size, 50 to 100 μm) with distilled water at a flow rate of 2.0 ml min^{-1}. The pyocyanin fraction was immediately titrated with 4 N HCl to a pH of 4.5 and evaporated with constant stirring on a hot plate-magnetic stirrer for the preparation of concentrated stock solutions. Pyocyanin concentrations were determined at 520 nm with an extinction coefficient of 2.46×10^6 cm^2 mol^{-1} in 0.2 N HCl (14). The average yield of pyocyanin was 2.3 mg 100 ml^{-1} of spent culture medium. Stock solutions of pyocyanin were refrigerated in the stable red acid form.

Thin-layer chromatography, ultraviolet absorption spectrophotometry, and melting point determination were used to estimate the purity of the pyocyanin preparations. Silica gel thin-layer chromatography was performed in two different solvent systems, chloroform-methanol (1:1) (system A) and ethyl acetate-acetic acid-water (3:2:1) (system B), as described by Knight et al. (13). After thin-layer chromatography in solvent system A, only a single light-blue, crescent-shaped spot with an R_f of 0.57 to 0.73 was observed. In solvent system B, a single wine-red spot with an R_f of 0.25 to 0.36 was observed, preceded by faint-blue tails representing pyocyanin which had remained in the alkaline form. The purified pyocyanin preparation in 0.2 N HCl showed absorption maxima at 388, 282, 246, and 215 nm, characteristic of a purified preparation (see Fig. 2) (7, 12, 16). Crystals from the dried samples appeared as long, fine needles when viewed microscopically and melted between 134 and 135°C. All of the methods (melting point determination, ultraviolet spectrophotometry, and thin-layer chromatography) we used for estimating purity indicated that the pyocyanin preparation was free of contaminants. The acidified pyocyanin was refrigerated in the dark to prevent decomposition (17, 20).

Agar disk diffusion. Agar disk diffusion was performed by the method of Matsen and Barry (15). Anaerobic incubation was carried out in a Brewer Anaerobic Jar (Becton, Dickinson & Co., Rutherford, N.J.), which was flushed with nitrogen gas and deoxygenated with a GasPak H_2-CO_2-generating system (BBL Microbiology Systems). All plates were checked after 48 h of incubation. The zones of inhibition were measured with a clear plastic ruler.

Kinetics of pyocyanin inhibition. A screw-capped tube (1.5 by 10 cm) containing 2.0 ml of medium A with 1% KNO_3 was inoculated with a loop from a 48-h Trypticase soy agar plate culture of the organism to be tested. The culture was incubated for 8 to 12 h. The contents were then used to inoculate 200 ml of medium A in a 1-liter Erlenmeyer flask, which was then incubated with shaking (80 strokes min^{-1}). When the cells reached the mid- to late exponential phase (absorbancy at 660 nm, 0.60 to 0.90), 18.0-ml samples were transferred to presterilized, cotton-stoppered, 125-ml Erlenmeyer flasks containing 2.0 ml of filter-sterilized pyocyanin at 10-fold the desired final concentration (made up in basal salts solution). The pH of the final mixture was approximately 7.0. The cultures were incubated with shaking (80 strokes min^{-1}) for 48 h. At selected times after treatment, 0.1-ml samples were withdrawn and diluted appropriately in 1.0% peptone. Six 0.1-ml samples of each dilution to be plated were spread onto plate count agar, the plates were incubated, and viable counts were determined as soon as possible. Logarithms of the viable counts obtained from 24-h samples of the control and treated cultures were subjected to a one-tailed Student *t* test to assess the significance of the treatment with pyocyanin. Logarithms of the viable counts obtained from 0- and 24-h samples of the treated cultures were similarly analyzed to determine whether the counts had decreased significantly over 24 h. Probabilities for the Student *t* test statistics obtained were considered significant if they were <0.05.

Chemicals. All chemicals used in this study were reagent grade or better. The water was purified by reverse osmosis, charcoal filtration, and double deionization (Culligan, Inc., Northbrook, Ill.).

RESULTS

Screening tests for susceptibility to pyocyanin. Agar disk diffusion tests were performed on 14 species of bacteria representing the *Pseudomonadaceae*, the *Enterobacteriaceae*, the *Bacillaceae*, the *Micrococcaceae*, and the gram-negative coccobacilli to determine

their susceptibilities to pyocyanin (Table 1). All of the pseudomonads tested, including *Pseudomonas aeruginosa* PAO, the producer of pyocyanin, were totally resistant to the level of pyocyanin tested. The gram-positive bacilli and cocci were highly susceptible as a group to pyocyanin (Table 1). The most susceptible of the gram-positive organisms was *M. luteus*, for which the minimal inhibitory concentration was 2 μg ml^{-1} (9.5 μM). The moderately susceptible organisms included *E. coli*, *P. vulgaris*, and *Acinetobacter* sp. The minimal inhibitory concentrations for *E. coli* and *M. luteus* were similar to those previously reported by Waksman and Woodruff (19).

The medium used clearly affected resistance to pyocyanin. In seven of eight susceptible bacteria, especially the gram-negative species, zones of inhibition on Trypticase soy agar were smaller than those on Mueller-Hinton agar (MHA) without glucose (Table 1). Zones of inhibition on MHA without glucose, as compared with those on MHA with glucose, were up to 56% smaller for the two gram-negative coccobacilli, up to 36% larger for three of the four gram-positive organisms, and 50% larger for *E. coli*.

Kinetics of pyocyanin activity. Figures 1 through 5 show the effects of various concentrations of pyocyanin on mid- to late-exponential-phase cultures of *M. luteus*, *S. aureus*, *B. licheniformis*, *E. coli*, and *Pseudomonas aeruginosa*. A preliminary experiment revealed that the growth of cultures treated with the minimal inhibitory concentration of pyocyanin determined by the tube tests for each organism was not significantly inhibited by the pigment. Therefore, pyocyanin concentrations higher than the tube test minimal inhibitory concentrations were used.

TABLE 1. *Spectrum of antibacterial activity*

Organism	Zone of inhibition[a] (mm) on:		
	MHA	MHA plus 0.5% glucose	TSA[b]
Escherichia coli	13	3	2
Proteus vulgaris	3	—[c]	0
Enterobacter aerogenes	0	—	0
Pseudomonas aeruginosa (PAO)	0	—	0
Pseudomonas aeruginosa (reddish-brown strain)	0	—	0
Pseudomonas denitrificans	0	—	0
Pseudomonas fluorescens	0	—	0
Pseudomonas perfectomarinus	0	—	0
Micrococcus luteus	23	19	21
Staphylococcus aureus	15	8	17
Bacillus licheniformis	19	10	18
Bacillus subtilis	9	—	8
Acinetobacter HO1-N^{-1}	1	10	0
Paracoccus denitrificans	6	13	6

[a] Susceptibility was determined by the agar disk diffusion test. The zone of inhibition was measured from the edge of the disk, and each disk contained 2.9 μg of pyocyanin.

[b] TSA, Trypticase soy agar.

[c] —, Not determined.

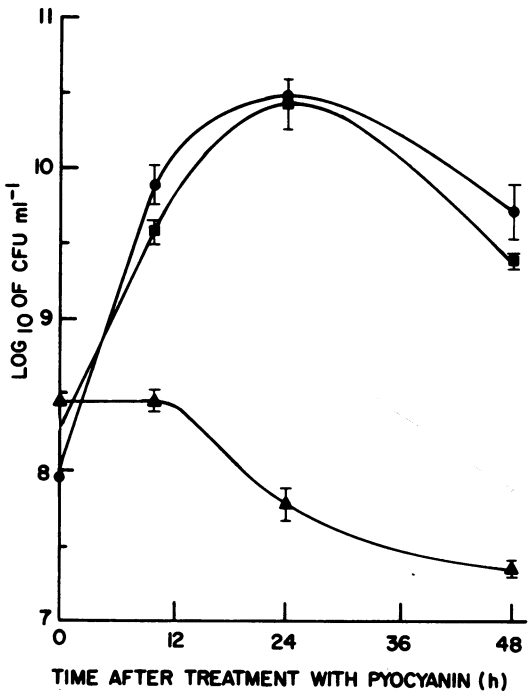

FIG. 1. *Effect of pyocyanin on growth and viability of M. luteus. The cultures were grown aerobically in medium A supplemented with 1.0% potassium nitrate and treated with pyocyanin during the midexponential phase. Symbols: ●, control; ■, 5 μg of pyocyanin ml^{-1} ▲, 25 μg of pyocyanin ml^{-1}. CFU, Colony-forming units.*

A decrease in viability occurred in cultures of all susceptible organisms treated with pyocyanin (Fig. 1 through 4). The bactericidal effect of pyocyanin was most pronounced for *B. licheniformis*, for which an 8 log decrease in viability was observed (Fig. 3).

As shown in Fig. 5, 200 μg of pyocyanin ml^{-1} did not affect the growth of *Pseudomonas aeruginosa*. However, during the stationary phase (after about 18 h), the pigment slightly decreased the viability of the culture, as determined by statistical analysis of the viable counts. The magnitude of this effect, however, was much

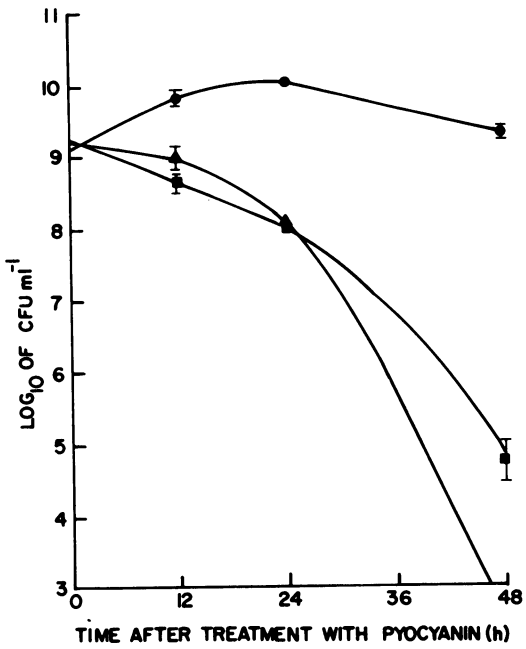

FIG. 2. *Effect of pyocyanin on growth and viability of S. aureus. The cultures were grown aerobically in medium A supplemented with 1.0% potassium nitrate and treated with pyocyanin during the late exponential phase. Symbols: ●, control; ■, 12.5 μg of pyocyanin ml^{-1}; ▲, 37.5 μg of pyocyanin ml^{-1}. CFU, Colony-forming units.*

less than that observed with any of the other four bacteria tested.

Demonstration of the respiration dependence of pyocyanin toxicity. To determine whether the antibacterial effect of pyocyanin was dependent upon respiration, agar disk diffusion plates were inoculated with the test organisms and incubated aerobically, anaerobically with nitrate (denitrifying conditions), or anaerobically without nitrate (fermentative conditions).

Results of the tests are shown in Table 2. As expected, *Pseudomonas aeruginosa* PAO was totally resistant to the pigment under both aerobic and denitrifying conditions. *E. coli*, *S. aureus*, and *B. licheniformis* incubated under fermentative conditions demonstrated zones of inhibition at least less than one-half the size of those observed under aerobic conditions. On the other hand, zones of inhibition for *B. licheniformis* and *Paracoccus denitrificans* grown under denitrifying conditions were 85 and 8%, respectively, larger than those observed under aerobic conditions. Two concentric rings of growth were observed next to the paper disk within the zones of inhibition on the plates of *B. licheniformis* incubated aerobically.

DISCUSSION

The experiments illustrated in Fig. 1 through 4 clearly establish that the antimicrobial action of pyocyanin is bactericidal in nature. Although this appears to be in conflict with an early report by Waksman and Woodruff (19) in which they categorize pyocyanin as a bacteriostatic agent, it is not. These investigators used the term bacteriostatic to indicate inhibition of bacterial growth on an agar plate and, therefore, in their context, a bacteriostatic agent could be bactericidal in action. The bactericidal effect of pyocyanin that we observed was concentration dependent in all cases. The viability of *Pseudomonas aeruginosa* did not significantly change after treatment with high concentrations (200 μg ml^{-1}) of the pigment (Fig. 5). The gram-positive bacteria were much more susceptible to pyocyanin than were the gram-negative bacteria

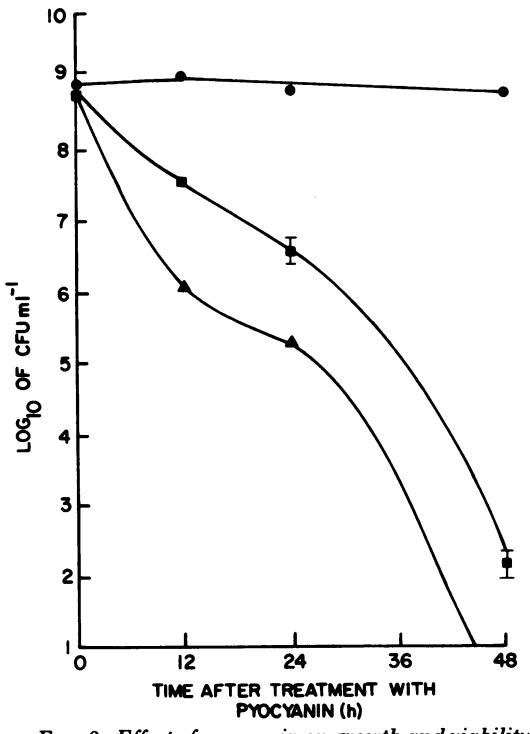

FIG. 3. *Effect of pyocyanin on growth and viability of B. licheniformis. The cultures were grown aerobically in medium A supplemented with 1.0% potassium nitrate and treated with pyocyanin during the early stationary phase. Symbols: ●, control; ■, 20 μg of pyocyanin ml^{-1}; ▲, 150 μg of pyocyanin ml^{-1}. CFU, Colony-forming units.*

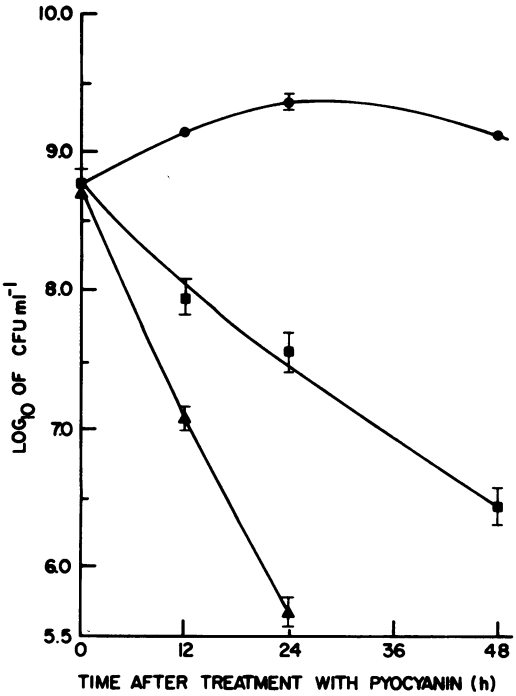

FIG. 4. *Effect of pyocyanin on growth and viability of E. coli. The cultures were grown aerobically in medium A supplemented with 1.0% potassium nitrate and treated with pyocyanin during the late exponential phase. Symbols: ●, control; ■, 50 μg of pyocyanin ml⁻¹; ▲, 200 μg of pyocyanin ml⁻¹. CFU, Colony-forming units.*

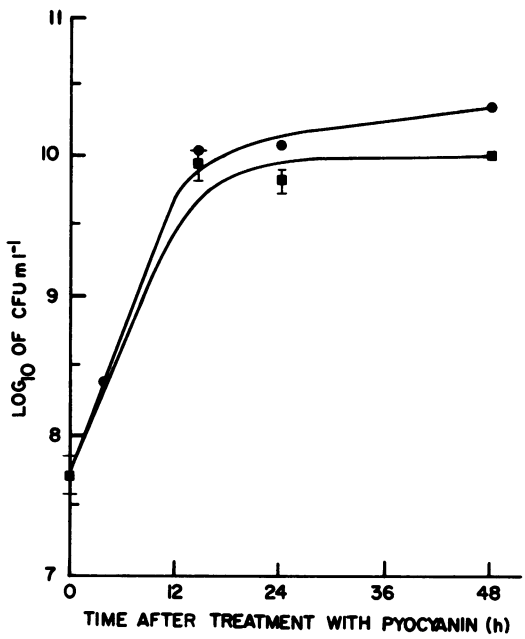

FIG. 5. *Effect of pyocyanin on growth and viability of Pseudomonas aeruginosa PAO. The cultures were grown aerobically in medium A supplemented with 1.0% potassium nitrate and treated with pyocyanin during the midexponential phase. Symbols: ●, control; ■, 200 μg of pyocyanin ml⁻¹. CFU, Colony-forming units.*

(Table 1). *Pseudomonas aeruginosa* was resistant to the highest level of pyocyanin tested. Interestingly, the four apyocyanogenic pseudomonads tested were also highly resistant to the pigment (Table 1), suggesting that resistance to pyocyanin may be characteristic of the entire genus. Because of the distribution of resistant organisms, it is difficult not to speculate that the mechanism of resistance might be related to the outer membrane, since this structure is only found in gram-negative organisms and is known to play a role in antibiotic resistance.

Whatever the molecular mechanism(s) for resistance or susceptibility, it is likely that, once elucidated, the physiological role of the pigment in the metabolism of *Pseudomonas aeruginosa* will also be pinpointed. Some reports (4, 10) indicate that pyocyanin stimulates respiration in eucaryotes and procaryotes, whereas other reports (1, 5, 6, 18) claim that respiration in these organisms is inhibited. Whatever the case, it appears that pyocyanin does alter normal electron transport through the respiratory chain. With this in mind, Hassan and Fridovich (10)

TABLE 2. *Comparison of the antibacterial action of pyocyanin under aerobic, fermentative, and denitrifying conditions*

Organism	Zone of inhibition[a] (mm) under given conditions		
	Aerobic incubation[b]	Denitrification[c]	Fermentation[d]
Escherichia coli	9	—[e]	0
Staphylococcus aureus	13	—	6
Bacillus licheniformis	16	35	5
Paracoccus denitrificans	20	22	—
Pseudomonas aeruginosa	0	0	—

[a] Susceptibility was determined by the agar disk diffusion test. The zone of inhibition was measured from the edge of the disk, and each disk contained 5.8 μg of pyocyanin.

[b] Aerobic incubation of MHA cultures with 0.5% glucose.

[c] Anaerobic incubation of MHA cultures with 0.5% glucose and 1.0% potassium nitrate.

[d] Anaerobic incubation of MHA cultures with 0.5% glucose.

[e] —, *E. coli* and *S. aureus* do not denitrify. *Paracoccus denitrificans* and *Pseudomonas aeruginosa* cannot grow by fermentation.

proposed that pyocyanin diverts electron flow from some point in the respiratory chain and consequently generates the superoxide radical and hydrogen peroxide. Organisms which have high levels of superoxide dismutase would thus be protected from the effects of pyocyanin, whereas organisms unable to produce adequate amounts of superoxide dismutase would be susceptible to the action of the pigment. According to this hypothesis, pyocyanin toxicity would necessarily be oxygen dependent. Hassan and Fridovich (10) demonstrated that *E. coli* was not affected by pyocyanin when incubated under fermentative conditions, whereas aerobically incubated *E. coli* cultures were susceptible. We observed a similar phenomenon with *S. aureus*, *B. licheniformis*, and *E. coli*. In fact, facultative anaerobes may be able to partially circumvent the toxicity by switching from respiratory to fermentative metabolism if the proper substrate is available. In further support of such a hypothesis, we noted that, under aerobic conditions, pyocyanin was less toxic to facultative organisms when 0.5% glucose was used as a carbon and energy source than when nonfermentable substrates were present (Table 1). *Acinetobacter* sp. and *Paracoccus* sp., which are unable to ferment, were more susceptible to pyocyanin when glucose was used as an energy source. We have determined, however, that the cause of inhibition is not just a result of the formation of the superoxide radical. The data shown in Table 2 demonstrate that pyocyanin is more toxic to *B. licheniformis* and *Paracoccus denitrificans* under denitrifying conditions than under aerobic conditions; therefore, the toxicity is not necessarily dependent on the presence of oxygen. Because pyocyanin is active under aerobic and denitrifying condtions, but not under fermentative conditions, its antibacterial action requires active respiration, but not necessarily the presence of oxygen. It is possible that, under denitrifying conditions, a toxic nitrogen oxide radical is formed via reduced pyocyanin in a way similar to the formation of the superoxide radical proposed by Hassan and Fridovich (10). It is also possible that the formation of such toxic radicals does not represent the mechanism by which pyocyanin acts. The pigment has a redox potential such that it could act as a competitor for electrons at several levels of respiration, ultimately reducing oxygen itself or reducing oxygen via a terminal oxidase and bypassing the vectoral passage of electrons necessary for active transport and oxidative phosphorylation. The pigment could also simply divert electron flow at some specific point in the respiratory chain, bypassing several necessary carriers. Any of these hypotheses could account for the antimicrobial action of pyocyanin during both aerobic and anaerobic respiration.

ACKNOWLEDGMENT

This work was supported in part by a grant from the Research Council of the University of Dayton.

LITERATURE CITED

1. **Armstrong, A. V., D. E. S. Stewart-Tull, and J. S. Roberts.** 1971. Characterization of the *Pseudomonas aeruginosa* factor that inhibits mouse-liver mitochondrial respiration. J. Med. Microbiol. **4**:249–262.
2. **Blackwood, A. C., and A. C. Neish.** 1957. Pyocyanine formation from labelled substrates by *Pseudomonas aeruginosa*. Can. J. Microbiol. **3**:165–170.
3. **Burton, M. O., J. J. R. Campbell, and B. A. Eagles.** 1948. The mineral requirements for pyocyanine production. Can. J. Res. **C26**:15–22.
4. **Caltrider, P. G.** 1967. Pyocyanine, p. 117–121. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics. Vol. 1. Springer-Verlag New York Inc., New York.
5. **Campbell, J. J. R., A. M. MacQuillan, B. A. Eagles, and R. A. Smith.** 1957. The inhibition of keto-acid oxidation by pyocyanine. Can. J. Microbiol. **3**:313–318.
6. **Case, E. M., and H. McIlwain.** 1951. Respiration and phosphorylation in preparations from mammalian brain. Biochem. J. **48**:1–32.
7. **Chang, P. C., and A. C. Blackwood.** 1969. Simultaneous production of three phenazine pigments by *Pseudomonas aeruginosa* Mac 436. Can. J. Microbiol. **15**:439–444.
8. **Costa, A. L., and V. Cusmano.** 1975. Antimycotic activity of pyocyanin *in vitro* and *in vivo* on a pathogenic strain of *Candida albicans*. G. Batteriol. Virol. Immunol. **66**:297–308.
9. **Dive, D.** 1973. Action of exocellular pigments secreted by *Pseudomonas aeruginosa* on the growth and division of *Colpidium campylum*. Protistologica **9**:315–318.
10. **Hassan, H. M., and I. Fridovich.** 1980. Mechanism of the antibiotic action of pyocyanine. J. Bacteriol. **141**: 156–163.
11. **Ingledew, W. M., and J. J. R. Campbell.** 1969. Evaluation of shikimic acid as a precursor of pyocyanine. Can. J. Microbiol. **15**:535–541.
12. **Kanner, D., N. N. Gerber, and R. Bartha.** 1978. Pattern of phenazine pigment production by a strain of *Pseudomonas aeruginosa*. J. Bacteriol. **134**:690–692.
13. **Knight, M., P. E. Hartman, Z. Hartman, and V. M. Young.** 1979. A new method of preparation of pyocyanin and demonstration of an unusual bacterial sensitivity. Anal. Biochem. **95**:19–23.
14. **Kurachi, M.** 1958. Studies on the biosynthesis of pyocyanine. I. On the cultural conditions for pyocyanine formation. Bull. Inst. Chem. Res. Kyoto Univ. **36**:163–173.
15. **Matsen, J. M., and A. L. Barry.** 1974. Susceptibility testing: diffusion test procedures, p. 418–427. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
16. **Morrison, M. M., E. T. Seo, J. K. Howie, and D. T. Sawyer.** 1978. Flavin model systems. I. The electrochemistry of 1-hydroxyphenazine and pyocyanine in aprotic solvents. J. Am. Chem. Soc. **100**:207–211.
17. **Propst, C., and L. Lubin.** 1979. Light-mediated changes in pigmentation of *Pseudomonas aeruginosa* cultures. J. Gen. Microbiol. **113**:261–266.
18. **Stewart-Tull, D. E. S., and A. V. Armstrong.** 1972. The effect of 1-hydroxyphenazine and pyocyanin from *Pseudomonas aeruginosa* on mammalian cell respira-

tion. J. Med. Microbiol. **5**:67–73.
19. **Waksman, S. A., and H. B. Woodruff.** 1942. Selective antibiotic action of various substances of microbial origin. J. Bacteriol. **44**:373–384.
20. **Wrede, F., and E. Strack.** 1929. Uber das Pyocyanin, den blauen Farbstoff des *Bacillus pyocyaneus*. Z. Physiol. Chem. **181**:58–62.
21. **Zobell, G. E., and H. C. Upham.** 1944. A list of marine bacteria including descriptions of sixty new species. Bull. Scripps Inst. Oceanogr. Univ. Calif. **5**:239–292.