**Journal of Industrial Microbiology & Biotechnology (1997) 19**, 130–133


# Phenol degradation by the marine cyanobacterium *Phormidium valderianum* BDU 30501

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**Bioremediation is a cost-effective alternative to conventional disposal methods and is a new technology which emphasizes the detoxification and destruction of the pollutants by acclimatized microorganisms. Cyanobacteria are in a more advantageous position than heterotrophic bacteria because of their trophic independence for nitrogen as well as carbon. Phenol, the toxic constituent of several industrial effluents, was found to be effectively removed and degraded by the marine cyanobacterium *Phormidium valderianum* BDU 30501. The organism was able to tolerate and grow at a phenol concentration of 50 mg $L^{-1}$ and remove 38 mg $L^{-1}$ within a retention period of 7 days. The removal and degradation were confirmed by changes in the ultraviolet absorption spectra in the culture filtrate, colorimetric estimation of residual phenol and measuring the intracellular activity of the inducible polyphenol oxidase and laccase enzymes. This opens up the possibility of treating a variety of phenol-containing industrial effluents using this organism.**

**Keywords:** bioremediation; phenol; laccase; polyphenol oxidase; *Cyanobacterium*

## Introduction

Effluents discharged from petrochemical, textile, tannery and coal gasification units generally contain phenolic compounds ranging from 6–2000 mg $L^{-1}$; their admissible limit is only 3 mg $L^{-1}$ in the receiving waterbodies [22]. These compounds are toxic even at low levels and pose a threat to the biosphere because of their recalcitrant nature. Bioremediation is a cost-effective alternative to conventional disposal methods and is a new technology which emphasizes the detoxification and destruction of the pollutants by acclimatized microorganisms [4,15]. Biodegradation of phenolics by certain anaerobic and as well as aerobic bacteria and fungi has long been reported [1–3,6,12,17]. Biodegradation of aromatic compounds is by the hydroxylation of the ring by oxygen-requiring enzyme systems [13]. Among prokaryotes, the photosynthetic oxygen-evolving cyanobacteria would be ideal for the treatment of effluents containing aromatic compounds since they would hasten the process of biodegradation through oxygenation and reduce BOD, unlike heterotrophic microorganisms. In addition, they possess advantages over other bacteria and green algae by their trophic independence for nitrogen as well as carbon [7]. Marine cyanobacteria have already been reported to be useful in treating effluents from industries producing antibiotics [27], ossein [30] and chlor-alkali [31]. Identification of phenol-degrading cyanobacteria is important in view of their pivotal role in effluent treatment and recycling processes. There are reports on the cyanobacterial degradation of aromatic hydrocarbons [8–11,13,24] and xenobiotics [18,28]. Except for a solitary report on phenol degradation by two freshwater cyanobacterial species using $^{14}C$-labelled phenol and measuring the $^{14}CO_2$-released [11], there has been no further report on the usefulness of these organisms for bioremediation. This is the first report of a marine cyanobacterium, *Phormidium valderianum* BDU 30501, showing the production of inducible intracellular phenol-degrading enzymes.

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Received 8 August 1996; accepted 16 June 1997

## Materials and methods

### Organism

*P. valderianum* BDU 30501, a marine filamentous, non-heterocystous cyanobacterium was from the germplasm of the National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India. The cultures were made axenic by repeated antibiotic treatments.

### Culture conditions

The cultures were maintained in ASN III medium and incubated at 25 ± 2°C under white fluorescent light at an intensity of 13.7 W $m^{-2}$ [26]. The exponentially grown cultures of *P. valderianum* BDU 30501 were inoculated into ASN III medium with and without nitrogen [26]. Aqueous solutions of phenol were added to get 25, 50, 75, and 100 mg $L^{-1}$ final concentrations. The cultures were incubated at the above-mentioned conditions for 7 days. Uninoculated medium with phenol, and medium devoid of phenol inoculated with the organism served as controls.

### Analytical methods

To estimate the residual phenol, and to monitor the phenol degradation by UV-spectroscopy, the cultures were centrifuged at 11 000 × *g* for 15 min. Culture filtrates thus obtained were analysed colorimetrically following the method of Madhav Singh *et al* [21] and by UV spectrophotometry [14] in a Beckman DU 64 Spectrophotometer (Beckman Instruments, Geneva, Switzerland). The results are the average of triplicates.

### *Enzyme assays*

Extracellular polyphenol oxidase was assayed in the culture filtrates by measuring the *dopa* (3,4, dihydroxy phenylalanine) *chrome* formed from *dopa*; laccase activity was assayed by enzymatic oxidation of guiacol. The unit activities of enzymes are expressed as the change in absorbance of 0.001 $cm^{-1}$ $min^{-1}$ at their molar extinction coefficients [23]. For intracellular enzymes, the cultures were washed repeatedly with ASN III medium, and sonicated in a Labsonic 2000 U B. Braun Sonicator (Germany) supplied by M/S B. Braun Singapore PTE Ltd; the contents were centrifuged at 15000 × *g* for 10 min. The supernatants were assayed for polyphenol oxidase and laccase [23]. In both assays heat-killed enzymes served as controls.

### *Biomass estimation*

On the day of estimation the cells were washed thoroughly and repeatedly in ASN III medium to remove residual phenol. Chlorophyll-*a* was measured following the method of MacKinney [20]; protein was estimated following the method of Lowry *et al* [19]. The results expressed are the average of triplicates.

## Results

Of the two media tested, namely phenol with combined nitrogen and phenol devoid of combined nitrogen, the organism in the latter showed neither growth (data not shown) nor degradation of phenol (Figure 1). Phenol in the absence of nitrogen proved inhibitory to growth. Of the four concentrations of phenol tested in the presence of combined nitrogen, only lower concentrations such as 25 and 50 mg $L^{-1}$ supported growth (Figures 2 and 3), while higher concentrations (75 and 100 mg $L^{-1}$) were inhibitory (data not shown). However, at 25 and 50 mg $L^{-1}$ of phenol, growth as measured by chlorophyll of *P. valderianum* BDU 30501 was less than that of the control (Figure 2), but in terms of protein, it was more than the control (Figure 3).

Intracellular and extracellular polyphenol oxidase and laccase activities were analysed under control and experimental conditions. The milieu of the cultures with and without phenol showed neither polyphenol oxidase nor laccase activity. Intracellular polyphenol oxidase activity tested in terms of L-Dopa chrome formed was positive; likewise intracellular laccase also showed considerable activity (Table 1). Compared to 25 mg $L^{-1}$, polyphenol oxidase activity was five-fold higher in cultures grown with 50 mg $L^{-1}$ of phenol. However, laccase activity was only two-fold higher at this concentration compared to the lower concentration (Table 1).

The utilization of phenol by *P. valderianum* BDU 30501

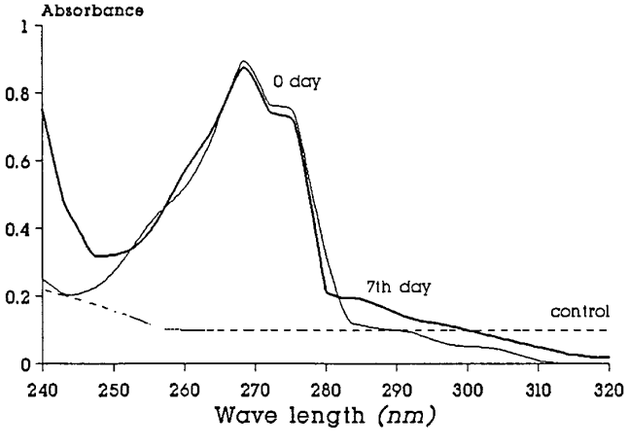


**Figure 1** UV-absorption spectra showing degradation of phenol at a concentration of 25 mg $L^{-1}$ by *P. valderianum* BDU 30501 on different days in the absence of combined nitrogen.

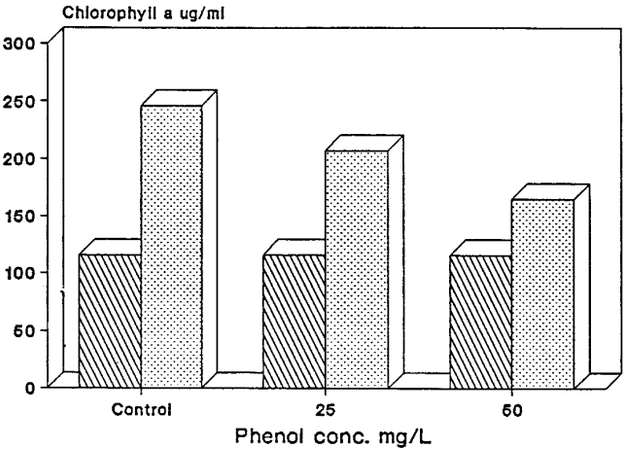


**Figure 2** Effect of phenol on chlorophyll of *P. valderianum* BDU 30501 on the 7th day in the presence of combined nitrogen. ▨ 0 day; ▩ 7th day.

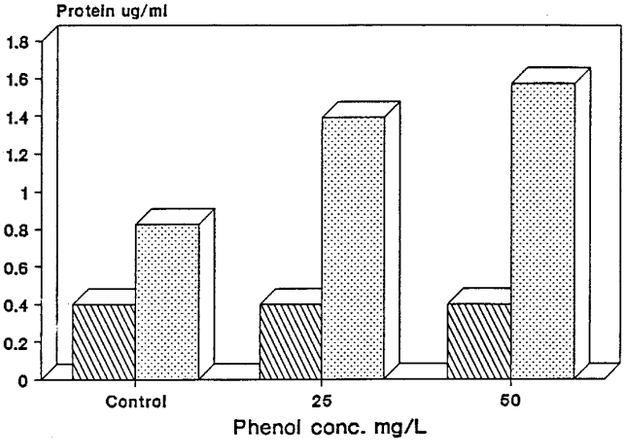


**Figure 3** Effect of phenol on protein of *P. valderianum* BDU 30501 on the 7th day in the presence of combined nitrogen. ▨ 0 day; ▩ 7th day.

**Table 1** Intracellular enzyme activities of *Phormidium valderianum* BDU 30501

| Phenol conc. ($\mu g\ L^{-1}$) | Polyphenol oxidase | Laccase | Residual phenol ($\mu g\ L^{-1}$) |
|---|---|---|---|
| | (Unit activity $mg^{-1}$ protein) | | |
| Control | 0.0 | 0.0 | – |
| 25 | 0.476 | 0.456 | 0 |
| 50 | 2.667 | 0.881 | 11.5 |

is evident from the results of the UV-absorption spectra and residual phenol analysis in the nitrogen-amended condition (Figure 4, Table 1). At 25 mg $L^{-1}$ of phenol, there was complete disappearance of the phenol peak at 270 nm and there was no detectable residual phenol on the 7th day, whereas with 50 mg $L^{-1}$ phenol, 23% remained in the medium after the same retention period (Table 1).

## Discussion

Wastewater treatment with marine cyanobacteria has been successfully demonstrated in outdoor conditions in the Ossein [30] and chloralkali [31] industries. Here we report for the first time the enzymatic degradation of phenol by the marine cyanobacterium *P. valderianum* BDU 30501. It is believed that the mineralization of organic compounds is better under nitrogen-starved conditions [16], but the present study indicates that with *P. valderianum* BDU 30501, the reverse could be true.

With phenol in the medium, a marginal reduction in chlorophyll-*a* and a concentration-dependent increase in protein were noticed (Figures 2 and 3). The increase in protein can be attributed to the *de novo* synthesis of phenol-degrading enzymes and stress-related proteins [5]. At 25 mg $L^{-1}$ of phenol, complete utilization of phenol by *P. valderianum* BDU 30501 was detected both by a colorimetric residual phenol assay (Table 1) and by the spectrophotometric disappearance of the phenol peak at 270 nm in 7 days (Figure 4). At 50 mg $L^{-1}$ a maximal removal of 77% of the phenol from the medium was noticed within 7 days (Table 1).

In earlier studies two freshwater cyanobacteria were shown to metabolize and degrade phenol, but there was no attempt to study the enzymes involved [13]. The present work establishes that a marine cyanobacterium is capable of degrading phenol through the activities of polyphenol oxidase and laccase (Table 1). Enzymes responsible for phenol degradation were found to be intracellular (Table 1). The absence of detectable activity of these enzymes in control cultures establishes the inducible nature of these enzymes in this cyanobacterium.

The fact that a concentration of 50 mg $L^{-1}$ can support the growth of this organism and exhibit maximum activities of phenol-degrading enzymes suggests that this cyanobacterium can be used to treat effluents either containing this concentration or diluted to this concentration of phenol, probably with a retention period slightly longer than 7 days. Further, the ability of *P. valderianum* BDU 30501 to tolerate and grow at various salinity levels, starting from freshwater conditions [25] makes it an ideal candidate for treating phenol-containing effluents from different sources.

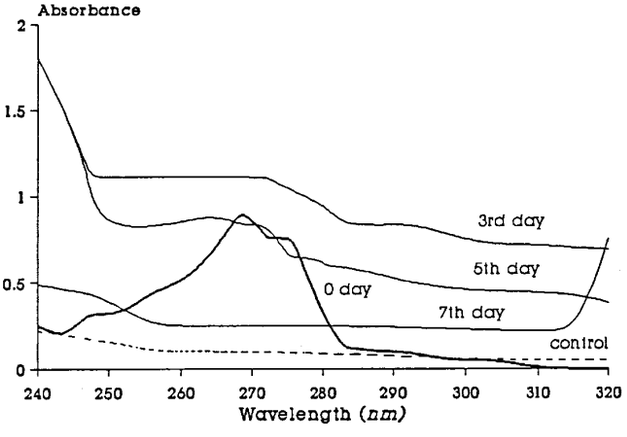


**Figure 4** UV-absorption spectra showing degradation of phenol at a concentration of 25 mg $L^{-1}$ by *P. valderianum* BDU 30501 on different days in the presence of combined nitrogen.

## Acknowledgements

The authors thank the Dept of Biotechnology, Govt of India, for financial support.

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