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Biotechnological Potential of Marine Cyanobacteria in Wastewater Treatment: Disinfection of Raw Sewage by *Oscillatoria willei* BDU130511

UMA, L.[1], K. SELVARAJ[1], R. MANJULA[1], G. SUBRAMANIAN[1], AND SANJAY NAGARKAR[2]*

[1]*National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirapalli-620 024, India*
[2]*Department of Ecology & Biodiversity, The University of Hong Kong, Hong Kong SAR, China*

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Abstract The current study demonstrates the ability of the marine cyanobacterium *Oscillatoria willei* BDU130511 to disinfect raw sewage. Within a holding time of 3 h under laboratory conditions, the organism drastically reduced in the total bacterial and coliform counts at various pH levels, in both unbuffered and buffered sewage, thereby suggesting a potential role for cyanobacteria in wastewater treatment.

Key words: Bacteria, coliforms, cyanobacteria, *E. coli*, *Oscillatoria*, sewage

Arguably the most important, yet least glamorous, practical application of microbiology is the treatment of sewage and wastewater. The ever-increasing world population has resulted in higher stress on water resources worldwide. Despite advances in public health technology, water and food remain important reservoirs of microorganisms that cause diseases in humans and other animals. Many waterborne diseases result from the contamination of drinking water supplies by sewage. One of the major challenges in wastewater treatment is the removal of pathogenic bacteria and viruses, especially coliforms. The survival of total bacteria and coliforms in sewage is controlled by both biotic and abiotic factors [14]. Oxygen, pH, and light have all been shown to have the ability to damage coliforms [6, 8, 11]. Except for a solitary report on algae reducing fecal coliforms in waste stabilization ponds, there is no other report on cyanobacteria, which are also known as blue-green algae [6]. Cyanobacteria can influence the oxygen levels and pH through oxygen evolving photosynthesis [2, 11]. An earlier study by the current authors yielded a detergent-degrading cyanobacterium, *Oscillatoria willei* BDU130511 (unpublished result). Since domestic sewage also contains detergents, this paper highlights the pivotal role of *O. willei* BDU130511 in sewage disinfection.

The current study was carried out using fresh sewage samples collected from the Ponmalai sewage pumping station, Tiruchirapalli, India. The sewage was filtered through cotton wool and then used for the experiment. Based on its faster growth rate even in low light condition of thick sewage, between zero to 150% salinity, and detergent degrading ability, the cyanobacterium *Oscillatoria willei* BDU130511 was selected for study from the germplasm collection of the National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirapalli, India. The organism was maintained in an ASN III medium at 25±2°C under white fluorescent light with an intensity of ~13.7 μE m^{-2} sec^{-1} [12].

The sewage was distributed in 500 ml amounts in 1-l Erlenmeyer flasks. To investigate the effect of pH on the total bacteria and coliforms in both unbuffered and buffered sewage, the pH of the sewage was adjusted to 7, 8, 9, and 10 with 0.1 N NaOH and Tris (0.01 M), and the sewage was divided into two sets, each treatment with three replicates. One set of flasks was inoculated with fixed biomass (3 mg chlorophyll *a* [10]) mid-log cultures of *Oscillatoria willei* BDU130511 into both unbuffered and buffered sewage. The uninoculated sewage set served as controls. All the flasks were incubated at 25±2°C under white fluorescent light with an intensity of ~13.7 μE m^{-2} sec^{-1} [12]. Total bacterial and coliform counts were performed before the incubation and after 3 and 24 h of incubation [8, 9]. The values presented here are the percent reduction in bacterial growth.

The controls did not show any reduction in the total bacteria and coliform counts after a 3 h incubation period under any of the conditions tested (Fig. 1). In contrast, in the sewage inoculated with cyanobacterium *Oscillatoria willei* BDU130511, the number of total viable bacteria and coliforms decreased considerably in both the unbuffered and buffered sewage (Fig. 1). The significant reduction in the total bacteria and coliform counts was initially attributed to the production of toxins, secondary metabolites, and

*Corresponding author
Phone: 852-22990675; Fax: 852-25176082;
E-mail: snagarka@hkusua.hku.hk

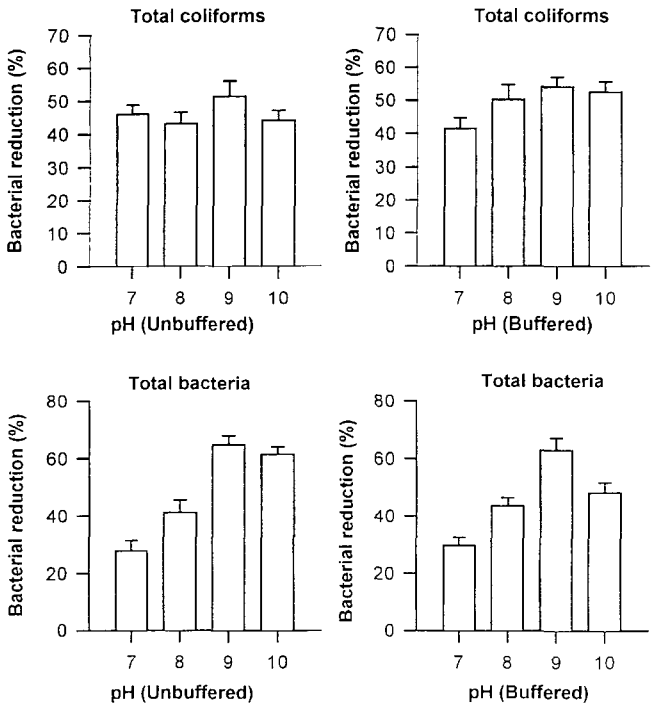

Fig. 1. Effect of marine cyanobacterium *Oscillatoria willei* BDU130511 on total bacteria and coliform counts (percentage reduction) in unbuffered and buffered sewage after 3 h of incubation.

Since there was no reduction in the total bacteria and coliform counts in the control, it was not included in the graph. (n=3; ±Standard Deviation, S.D.)

bioactive compounds by the cyanobacteria [4, 13, 15]. Therefore, based on the possibility that such chemicals released from *O. willei* BDU130511 might have had an inhibitory effect on the total bacteria and coliform counts, *Oscillatoria willei* BDU130511 was screened for various toxins, secondary metabolites, and bioactive compounds produced by the cyanobacteria. Yet, no toxins and bioactive compounds were detected in *O. willei* BDU130511 culture, and organic compounds such as carbohydrate and lipids found in *O. willei* BDU130511 did not show any inhibitory effect on the total bacteria and coliform counts. Next, adsorption of bacteria to the sheath and cell wall of the cyanobacteria was considered as a possibility, however, this was also considered to be unlikely, as *O. willei* BDU130511 does not have a mucilaginous sheath. Moreover, qualitative observation of the cyanobacterium cell wall under scanning electron microscopy did not show any attached bacteria. Finally, as the concentrations of major growth nutrients, i.e. nitrogen and phosphorous, are usually very high in raw sewage, cyanobacterial growth seemed unlikely to reduce nutrients concentration to such low level that might have caused an unfavourable condition for bacterial growth.

Accordingly, the best explanation for the reduction of the total bacteria and coliform counts appeared to be chemical reactions of the photosynthetic products of *O. willei* BDU130511 with the bacteria and coliforms. The results in the present study suggested that, in addition to the apparent light, the oxidative stress by the cyanobacteria apparently reduced the total bacteria and coliform counts in the sewage [2, 6]. With illumination, photoautotrophic oxygen evolving cyanobacteria photolyse water, thereby liberating highly reactive oxygen species [2]. Similar results have also been obtained in a waste stabilization pond [6]. As a sequential reaction, photosynthetic cyanobacteria in autotrophic conditions evolve hydrogen peroxide [5, 7, 11]. Cooper and Zika [5] indicated that hydrogen peroxide gives rise to a number of nonculturable cells and injures the culturable cells of *Escherichia coli*. Similar results have also been obtained in a freshwater environment [1]. These supportive results, therefore, suggest a strong role for hydrogen peroxide in bacterial disinfection.

Another photosynthetic product is hydroxy radicals, which are liberated from hydrogen peroxide, and increase the pH. It has been shown that fecal coliform removal is also higher at higher pH levels [6, 11]. This concept is also supported by the present study, where the cyanobacterium-inoculated sewage exhibited the highest reduction of total bacteria in unbuffered (68%) and buffered (63%) sewage at pH 9.0. A similar trend was found with the total coliforms, where the reduction was 52% and 58% at pH 9.0 in unbuffered and buffered sewage, respectively (Fig. 1).

Consequently, we suggest that the drastic reduction in the total bacteria and coliform counts in the present study was due to reaction of photoproduced hydroxyl ions with hydrogen peroxide liberated by the cyanobacteria, thereby giving rise to perhydroxyl anions [5, 6, 11] which have a strong nucleophilic activity. These anions might have then attacked the bacterial carbonyl groups in the cell wall and membrane and this resulted in a leakage of the cellular contents [1, 5, 6, 7, 11]; however, this hypothesis requires further study. The present study using the single cyanobacterium *O. willei* BDU130511 confirms the earlier findings that cyanobacteria play a role in the disinfection of bacteria, especially coliforms [6]. Therefore, the present study opens an avenue for cyanobacteria as an inexpensive, nonpolluting tool for sewage treatment, as compared to ozone and chlorine treatment methods [3], although further study is still needed. Since the photosynthetic products of cyanobacteria appear to cumulatively reduce the number of bacteria, it is worth investigating other cyanobacterial species for their bacterial reduction abilities. Further studies on large-scale treatment are in need to establish cyanobacteria as a biotechnological tool for disinfecting sewage.

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