DEVELOPMENT AND EVALUATION OF A MICROBIAL AGENT CAPABLE OF ORGANIC COMPOUNDS DEGRADATION FOR BIOREMEDIATION OF NEARSHORE WETLAND

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ABSTRACT

A microbial agent was developed for the bioremediation of near shore wetland using a microbial consortium composed of Rhodoccus sp. EBht1 and Pseudomonas sp. EBht3 for the industrial use of the microbial consortium as a bioremediation agent. Marine Broth (MB) medium used for growth of the consortium was modified with addition of new component or deletion of some components of the medium for the purpose of lowering the cost of production, resulting in the discovery of an optimized and modified MB medium. Using the optimized medium, the constructed microbial consortium was cultured in a large scale through batch and fed-batch culture method. A liquid microbial agent was made using the consortium cells produced in a large scale and it was evaluated for the removal of organic compounds. Furthermore, a powered microbial agent was also developed through the formulation process after freeze-drying of the consortium cells. The formulated microbial agent was evaluated for the removal of organic compounds under various environmental conditions and found to work an effective role as a bioremediation agent.

KEY WORDS: Endosulfan, Korean polychaeta, Marine bacteria

INTRODUCTION

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9a-hexahydro-6, 9methano-2,3,4-benzodioxyanthiepin-3-oxide) is widely used cyclodiene insecticide in the world agricultural system [3]. Endosulfan comprises two parent isomers, the α and β endosulfan, respectively. This pesticide was developed in Germany first in 1955. Endosulfan is a chlorinated hydrocarbon and organochlorine compound that is used as an insecticide and acaricide and recently frequently detected in the atmosphere, soils, surface, ground waters, foodstuffs and marine aqueous systems, which is toxic to fish and marine organisms and human health. Endosulfan is subject to long range atmospheric transport. Endosulfan is acutely neurotoxic, kidney, liver, blood chemistry, parathyroid glands and has teratogenic, mutagenic effects to both insects and mammals, including humans [1; 2; 5]. Degradation of endosulfan can occur through both biotic and abiotic processes. When endosulfan enters into soil and water environments, some microorganisms utilize it as an energy and nutrient source. Researchers studied biodegradation of endosulfan by soil microorganisms utilizing it as carbon and sulfur sources [4].

MATERIALS AND METHODS

Isolation of marine bacteria
A marine bacterial species was isolated from Korean polychaeta, *Perinereis aibuhitensis* and wetland were collected from coastal wetland of Suncheon Bay of South Korea. The Korean polychaeta, *Perinereis aibuhitensis* were sliced by a clean knife, washed by 70% ethanol two times, vortexed, and centrifuged by 12000 rpm. The sample was enrichment culture, 100 mL of on BM medium and incubated on a rotary shaker at 160 rpm at 25 °C for 2 weeks. After 2 weeks, for isolation and purification, solid medium was prepared. 0.1 mL of aliquots of the enrichment cultures were transferred onto the solid medium. Each bacterial isolate was grew on a serial marine broth medium (1 MB, 1/2 MB, 1/10 MB) 2216 (MB; Difco) at 30 °C for 2 days under aerobic conditions and stored at -80 °C in marine broth supplemented with 15% (v/v) glycerol.

Enrichment culture and isolation of endosulfan-degrading marine bacteria. Enrichment cultures were performed in a Burk's medium (1M Phosphate buffer 10 mL, $MgSO_4*7H_2O$ 0.25 g, $CaCl_2*2H_2O$ 0.02 g, $FeSO_4*7H_2O$ 0.02 g, $(NH_4)_2SO_4$ 2038 g and sea salt 30 g, and distilled water 990 mL) (Mikesell *et al.*, 1993) containing 1 ppm endosulfan.

Construction of a marine bacterial consortium capable of endosulfan degradation. The three kind of marine bacterial consortium was constructed by strains EBht1 (collection no. EM406), EBht2 (collection no. EM407), EBht3 (collection no. EM408), and EBht4 (collection no. EM409), for the biodegradation of endosulfan. First consortium consist of all four strains, second consortium consist of strains EBht1 (collection no. EM406) and EBht3 (collection no. EM408), and last consortium consist of strains EBht2 (collection no. EM407) and EBht4 (collection no. EM409).

Measurement of the removal of organic compounds by a constructed endosulfan-degrading bacterial consortium under several environmental conditions. The microbial consortium were tested for their ability to remove organic compounds under several pH and temperature conditions. For the purpose of measuring organic compounds, the synthetic wastewater made up of glucose and peptone at the ratio of 1:4 was used. The amount of total organic compounds was measured over the incubation time course for 120 hours at 30 ℃ and pH 3.5, pH 7.0 and pH 9.5 as well as at pH 7.0 and 10 ℃, 30 ℃, and 37 ℃. The concentration of organic nitrogenous compounds used for this study was 10,000 ppm.

Optimization of growth medium for the mass production of a microbial agent. Each microbial members of the consortium, EBht1 (collection no. EM406) and EBht3 (collection no. EM408) were grown on several modified MB media made up of variable components. A total of eight different kinds of modified MB media were used and tested for the growth rate of each bacterial strain, their viability over the time, and the production cost. The medium optimal for the growth of the bacterial strains was selected for further test.

Formulation process of the bacterial consortium for the industrial production. For the formulation of a bacterial consortium from coastal wetland of Suncheon Bay, additive mixtures were selected considering the possibility of stabilization and industrialization, along with the possibility of environmental problems occurred when applied to the coastal wetland environment. Freeze-dried bacterial samples were mixed with additives such as orange clay, glucose (dextrose) and vitamin C, followed by wrapping in a plastic bag for the application to the environmental industry.

Evaluation of the formulated microbial agent for the removal of organic compounds under different temperature conditions. The formulated microbial agents which is composed of strains EBht1 (collection no. EM406) and EBht3 (collection no. EM408) were tested based on TOC for the biodegradation of organic compounds using synthetic wastewater over the incubation time course at the room temperature (14-20℃),30℃, and 37℃, and at 100 ppm and 1000 ppm over the incubation time for 14 days at the different temperature (5 ℃, 10 ℃, 15 ℃, 20 ℃, 25 ℃, 30 ℃) conditions.

RESULTS

This study resulted in the isolation and selection of 10 endosulfan- and/or 76 synthetic wastewater-degrading bacterial strains. A total of 86 strains were classified into 5 groups *Alpha*-, *Gamma-proteobacteria*, *Actinobacteria, Bacteroidetes* and *Firmicutes*. They were tentatively placed into 11 orders (or suborder), 17 families and 21 genera. From polychaete habitats, marine bacteria including *Streptomyces* sp., *Halobacillus* sp., *Sphingopyxis granuli, Lysobacter* sp., *Marinobacterium* *rhizophilum, Rhodobacter capsulatus* and *Idiomarina seosinensis* were isolated, which belong to 14 genera. Thirty five bacterial strains were isolated from polychaete, which were placed into 7 genera such as *Bacillus, Micrococcus, Pantoea, Pseudoalteromonas, Rheinheimera, Shewanella, Vibrio*. It is notable that very diverse species of *Bacillus* were found in polychaete (*Perinereis aibuhitensis*). Microbial consortium, consisting of 4 bacterial strains, such as EBht1 (collection no. EM406), EBht2 (collection no. EM407), EBht3 (collection no. EM408), and EBht4 (collection no. EM409) were obtained, which were identified as the *Rhodococcus ruber, Pseudomonas resinovorans, Pseudomonas putida, Pseudomonas oleovorans.* This consortium was revealed a powerful candidate to produce a microbial agent for bioremediation of near shore wetland owing to the high capability to degrade various organic hydrocarbons including endosulfan. Further study on the degradation ability of the consortium members for organic hydrocarbons resulted in the selection of a consortium composed of EBht1 (collection no. EM406) and EBht3 (collection no. EM408).

Test for the removal of organic compounds by the developed liquid microbial agent under several temperature conditions showed that it removed approximately 70% of 10,000 ppm organic compounds in 12 days under the given conditions. For powdered formulation of the consortium microorganisms an excipient for stabilization in near shore wetland and industrialization was used. The final product was made after 10% freeze drying microorganisms, 77% orange clay, 10% glucose and 3% vitamin C were added to the excipient. Test for the removal of organic compounds by the product under several temperature conditions using 100 ppm and 1000 ppm of the synthetic wastewater revealed that over 80% of organic compounds was removed very efficiently under 25-30 °C (Fig. 1). Notably, over 10% of organic compounds were degraded even under 5 °C which most of microorganisms lost their physiological and biochemical activities. These results suggested that the formulated microbial agent can be usefully applied in the environmental industry.

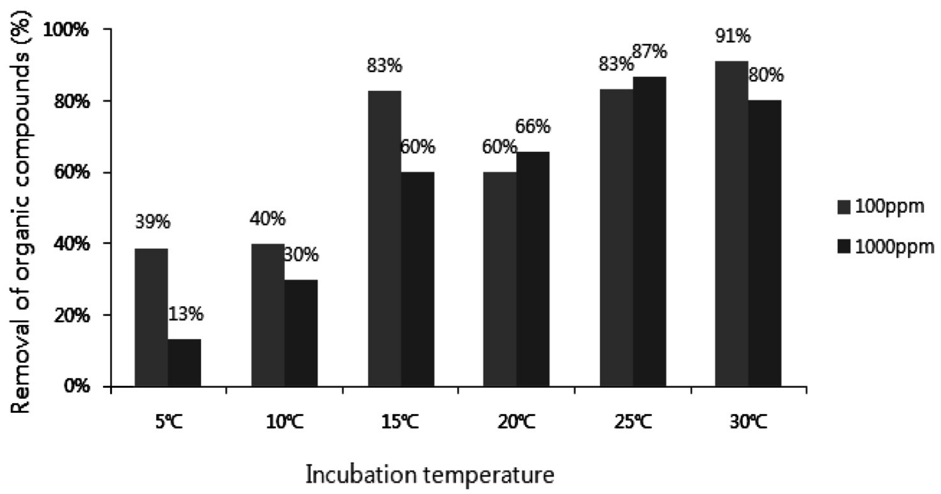

Fig. 1. The removal rate of organic compounds by the formulated microbial agent under different temperature conditions.

CONCLUSION

1. A microbial agent was developed for the bioremediation using a microbial consortium composed of *Rhodoccus* sp. EBht1 and *Pseudomonas* sp. EBht3.
2. The formulated microbial agent was evaluated for the removal of organic compounds under various environmental conditions and found to be eligible to have an effective role as a bioremediation agent.

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