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The Polyvinyl Alcohol Oxidase from *Xanthomonas* sp. J2Y

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Introduction

Polyvinyl alcohol (PVA) is water-soluble polymer widely used as a temporary protective coating for metals, plastics, and ceramics. Because this xenobiotic compound is usually removed slowly by microorganisms, removal of PVA from treatment of wastewater is difficult. PVA-degrading bacteria have been recommended for development of process of wastewater treatment. The present paper describes the isolation of PVA-degrading bacteria and some properties of the PVA oxidase involved in cleavage of PVA molecules.

Results and Discussion

Isolation and Characterization of Polyvinyl Alcohol Utilizing Bacteria

Two strains of polyvinyl alcohol (PVA) utilizing bacteria were isolated from the soil. These strains, J2Y and J2W, were identified *as Xanthomonas campestris and Pseudomonas pseudpmallei*, respectively, based on morphological and biological characteristics(1).

These strains, J2Y and J2W, were able to utilize PVA symbiotically as a carbon source, but could not utilize PVA separately. In the mixed culture of these strains, 0.5 percent of PVA was almost completely degraded in 3 days. Strain J2Y was able to grow in pure culture of PVA medium supplemented with PQQ but Strain J2W not. These bacteria were able to utilize PVA in the desizing wastewater of factory as well as enrichment PVA medium.

Purification and Properties of the Polyvinyl Alcohol Oxidase

The Polyvinyl alcohol (PVA) oxidase has been purified to homogeneity from culture broth of *Xanthomonas campestris* J2Y grown in a minimal medium containing PVA as a sole carbon source. The cells of *Xanthomonas* sp. J2Y was harvested by centrifugation and was disrupted with a French pressure cell. The PVA oxidase was purified from cell pellet of *Xanthomonas* sp. J2Y by the procedure of protamine sulfate precipitation, ammonium sulfate precipitation, DEAE-cellulose ion-exchange chromatography, Sephadex G-200 gel chromatography, and Continuous elution electrophoresis with Miniprep-cell. The purified PVA oxidase was electrophoretically homogeneous both in the absence and presence of SDS(2).

The molecular weight of the enzyme was estimated to be about 55,000 daltons by SDS-polyacrylamide gel electrophoresis and Sephadex G-150 gel filtration. The native enzyme existed as a monomer. The optimal pH and temperature was shown to be pH 7 and 30℃ respectively. The activity of enzyme was stable below 55 ℃ and between pH 5 and pH 11. Metal compounds such as Ag^{2+} or Hg^{2+} significantly inhibited the activity of enzyme. While metal ions, such as Mn^{2+} or Cu^{2+} stimulated the activity. PQQ, as a coenzyme for PVA dehydrogenase, did not affect its activity(3).

Cloning of PVA Oxidize Gene

The N-terminal sequence of amino acid of the purified PVA oxidize was determined and the forward primer based on the amino acid sequences was designed for use in polymerize chain reaction.

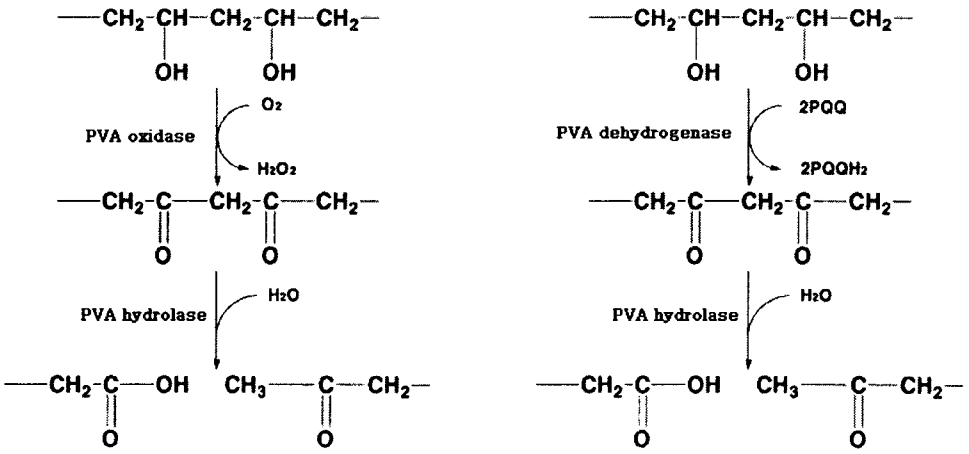
About 1.7kbp DNA of PCR product was obtained through PCR using chromosomal DNA of *Xanthomonas* sp. J2Y as template.

The PCR product was inserted into pGEM T-Easy plasmid and then the nucleotide sequence of the insert of the plasmid was determined by automatic DNA sequencer. The insert DNA fragment was subcloned to expression vector pET28a(+) and PVA oxidase gene was expressed in *E. coli* BL21 clone. The PVA oxidase in the in *E. coli* BL21 clone was localized in the cytoplasm.

Cloned PVA oxidase gene was coded 552 amino acids and molecular weight was 61,030 daltons.

Conclusion

1) *Xanthomonas campestris* J2Y and *Pseudomonas pseudpmallei* J2W, two strains of Polyvinyl alcohol (PVA) utilizing bacteria were isolated from the soil.
2) The molecular weight of the PVA oxidase was estimated to be about 55,000 daltons. The optimal pH and temperature was shown to be pH 7 and 30℃ respectively.
   The activity of enzyme was stable below 55 ℃ and between pH 5 and pH 11
3) PVA oxidase gene was cloned by PCR and its nucleotides sequence was determined.



Proposed mechanism of the PVA degradation by bacteria(4)

References

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