Identification and Characterization of Ligninolytic Enzyme by *Serratia marcescens* HY-5 isolated from the Gut of Insect

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Abstract

A lignin degradation bacteria, symbiotic bacteria was isolated from the gut of *Sympetrum depressiusculum* and tested for its lignin degrading activity using lignin model compounds and related aromatic compounds. The strain was identified as *Serratia marcescens* HY-5 based on the 16S rDNA, cellular fatty acid composition, biochemical and physiological characteristics. *S. marcescens* showed 40-50% lignin degrading activity in the media that contained vaillin, guaiacol and dealkaline lignin. *S. marcescens* showed three ligninase activities [laccase, lignin peroxidase(LiP) and Manganase peroxidase(MnP)]. Addition of dealkaline lignin to the basal media increased about 6fold of laccase activity. Vanillic acid or vanillin increase 1.3fold of MnP activity and *p*-coumaric acid increased 12fold of LiP activity which added to the basal medium.

Introduction

Lignin is the second most abundant material on earth after cellulose, and it is a kind of macromolecular compound existing in middle lamella and first cell wall of plant[6]. Lignin takes 15-35% of a total dried wood weight and exists in middle lamella between cell membrane as a high molecular aromatic condensation compound while some of lignin exists in cell membrane[1]. Lignin structure is complexity. therefore many investigation have involved the use of model compound[3]. Various lignin substructure models compounds have been used in biodegradation studies[2]. In this paper we provide a evidence for the biodegradation by bacteria isolated from the gut of symbiotic insect of lignin model compounds and screen bacteria having an ability to degrade phenolic compounds. A lignin degradation bacteria, *Serratia marcescens* HY-5 was isolated from the gut of *Sympetrum depressiusculum* and tested for its lignin degrading activity using lignin model compounds and related aromatic compounds.

Materials and Methods

Media: LB medium(0.5% Yeat extract, 1% Trypton, 1% NaCl)■

Lignin model compounds[2)]

Monomer compounds : Vanillic acid, Vanillin, Guaiacol, ·coumaric acid

Dimer compound : Dealkaline lignin

Active Staining Method[5)] : Ligninase(simple plate test)

Isolation of 16S rDNA : DNA extraction[4)]

Assay of enzyme

Crude extract of horseradish peroxidase(HP) was purchased from SIGMA(P6782). HPpreparation had a specific activity of 1000units·mg of solids. One international unit activity was defined as the amount of enzyme that oxidized 1μmol of ABTS[2,2■-Azino-bis(3-ethylvenzthiazoline-6-sulfonic acid)] per min at 25℃ at pH 5.0. Enzyme activity of ligninase was measured by using culture supernatant of the microorganism as coenzyme source.

Laccase : One unit of enzyme is defined as the amount of enzyme oxidizing 1μmole of ABTS equivalent per min.

Lignin Peroxidase (LiP) : One unit of LiP activity is defined as the amount of enzyme required to oxidize 1μmol of VA per min.

Manganase Peroxidase(MnP) : One unit of MnP activity is defied as the amount of enzyme required to oxidize 1mol of Mn^{2+} per min.

Results and discussion

A lignin degradating bacteria, *Serratia marcescens* HY-5 was isolated from the gut of *Sympetrum depressiusculum* and tested for its lignin degrading activity using lignin model compounds and related aromatic compounds.

For the selection of ligninase-producing strains, selected colonies on LB media were plated to form a clear zone on lignin media(Fig. 1). By the method such as Simple plate test, a selective colony isolated from the gut of *Sympetrum depressiusculum*, was capable of degrading lignin.. *S. marcescens* HY-5 showed 40~50% lignin degrading activity in the media that contained vanillin, guaiacol and dealkaline lignin. However, this strain showed relatively low activities in the vanillic acid, ρ-courmairc acid and phenol added medium(Fig 2,3).

S. marcescens HY-5 showed three ligninase activities [laccase, lignin peroxidase(LiP) and Manganase peroxidase(MnP)]. Addition of dealkaline lignin to the basal

media increased about 6fold of laccase activity. Vanillic acid or vanillin increase 1.3fold of MnP activity and p-coumaric acid increased 12fold of LiP activity which added to the basal medium.

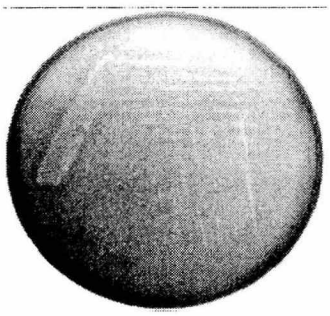

Figure 1. Simple plate test for lignin staining.

Ligninase staining on 0.02% lignosulfonate medium containing 0.5% glucose, 0.5% NH_4-tartrate, 0.1% malt extract, 0.001% CaCl · $2H_2O$, 0.01% NaCl, 0.001% $FeCl_3$,(pH 5.8) and 1%(w/v) $FeCl_3$, $K_3[Fe(CN)_6]$ destaining.

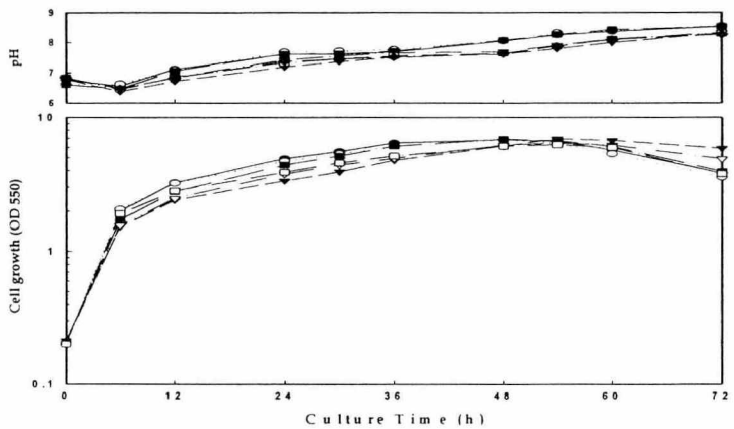

Fig. 2. Time profiles of cell growth and pH of lignin model compounds on LB medium at 37℃.

Symbols are : (●) dealkaline lignin ; (○) vanillic acid ; (▼) vanillin ; (▽) guaiacol ; (■) p-coumaric acid ; (□) phenol

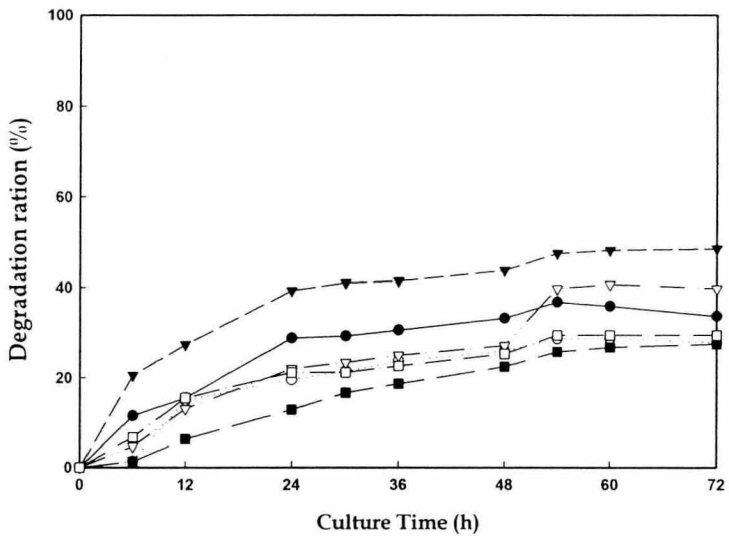

Fig. 3. Time profiles of lignin model compounds degradation on LB medium at 37℃. Symbols are the same as in Fig. 2

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