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Short Communication

Isolation and characterization of plum-seeds degrading aerobic bacteria from plum-grove soil

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The aim of this study was to isolate plum-seeds degrading bacterium from plum-grove soil. Some bacteria utilizing plum-seeds were isolated from the plum-grove soil samples by biodegradation assay. Among them, five bacteria had the activity of cellulase and xylanase. Four of the strains grew in the medium containing xylan instead of glucose. Partial sequencing of 16S rRNA and genome indicated that all the isolates belong to the *Psedomonas* section but two bacteria are unknown. It is proposed that some *Pseudomonas* bacteria could be deployed as inoculant to attain the treatment of plum-seeds.

Key words: Biodegradation, plum-seeds, cellulase, xylanase, microorganism.

INTRODUCTION

Japanese plum (*Prunas mume*) is very popular, and it is processed for a variety of foods containing fruit-brandy. More than 20000 ton of industrial plum -waste are produced a year in Japan. Among them, the plum-seeds are considered as lingo-cellulosic biomass, which may become renewable biological resources.

As micro-organisms are important component of the soil and influence the soil condition through their beneficial or detrimental activities (Batisson et al., 2009; Dastager et al., 2009), we have been screening soil samples for plum-seeds-degrading bacteria that are required for the development of a bioconversion process from cellulose and/or hemi-cellulose to glucose. The bioconversion may be useful for production of bio-fuel and fermentation- foods (Kaparaju et al., 2009; Lo et al., 2009). It is also very important for keeping earth-resources. Though the bacterium has been widely studied for its biological control poperties, very few have been reported for its seeds-degrading activity. In this study, we reported pseudomonas strains with cellulose and xylanase activity as a potential plum-seeds degrading bacterium and can be used as a bioinoculant in agricultural environments.

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MATERIALS AND METHODS

Collection and analysis of samples

The soil used for bacterial isolation was collected from several points of root-free plum-grove soil in Nara Japan. The processed soil sample was serially, spread plated on full strength nutrient agar and incubated at 28°C for 48 h. The culturing basal medium consisted of 7 g of K_2HPO_4, 2 g of KH_2PO_4, 0.1 g of $MgSO_47H_2O$, 1 g of $(NH_4)_2SO_4$ and 10 g of ground plum-seeds per liter of deionized sterile water. Colonies that developed on the plates were subculture repeatedly to obtain pure single colony, which was maintained on agar slants for further characterization and identification. Several white colored bacterial colonies were purified by more repeated culturing on nutrient agar and maintained in 50% glycerol at -80°C.

Enzyme assays

Xylanase activity was assayed at 28°C by measuring the amount of reducing sugars from oat spelt xylan (Petrescu et al., 2000; Li et al., 2009). Cellulase (beta-endoglucanase) activity was defined as the amount of enzyme releasing reducing sugars using 0.5% cellulose as the substrate (Sakamoto and Toyohara, 2000). All the results recorded in this paper are the average outcome of minimum three replications.

Characterization and identification of bacterial isolates

The characterization and identification of the bacterial isolates were based on microscopic cell morphology, biochemical ability and

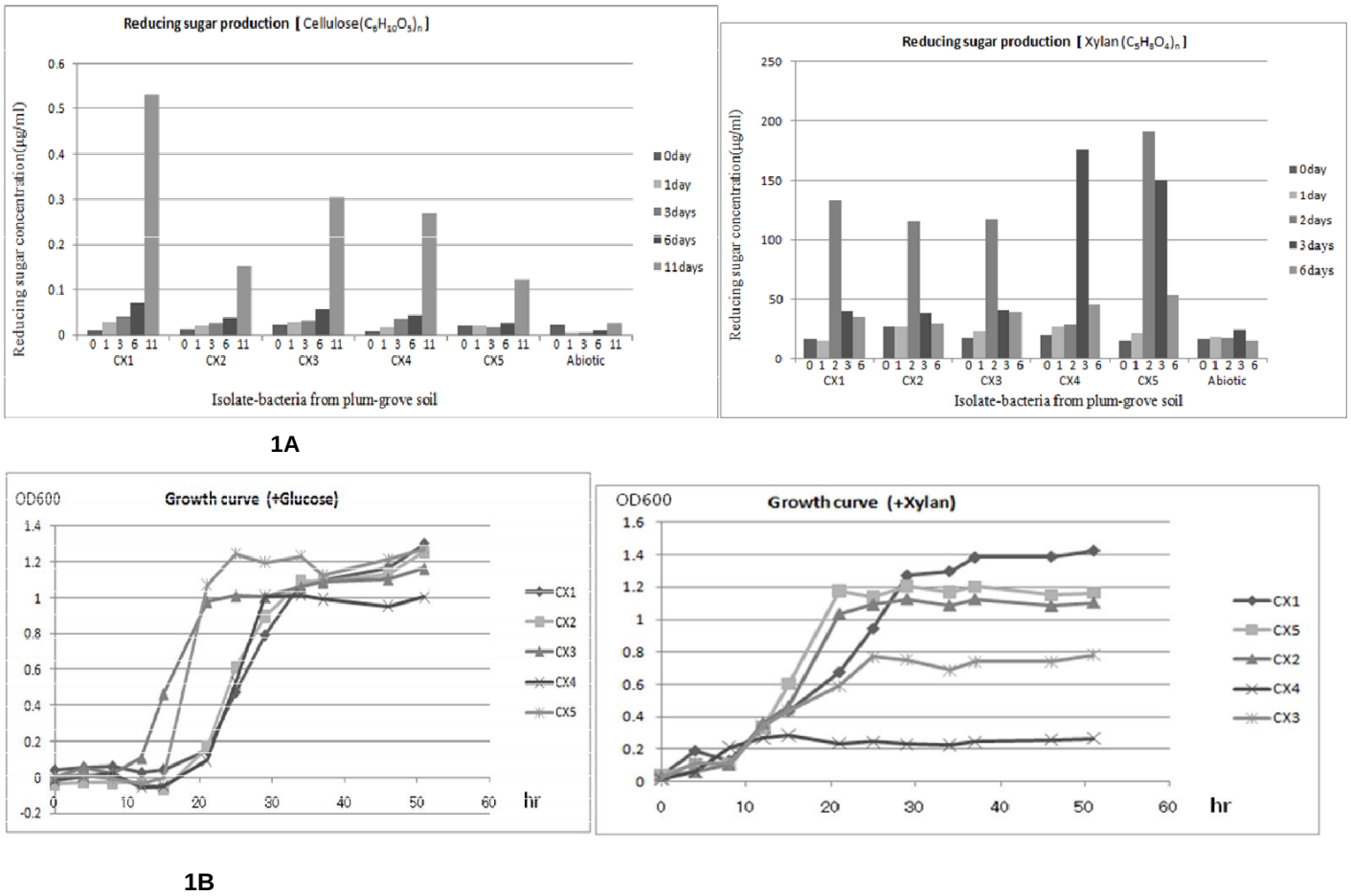

Figure 1. Enzymatic activity (A) and cell growth (B) of the isolates. Cellulase (A upper panel) and Xylanase (A lower panel) in the isolate bacteria are shown. Amounts of reducing sugar produced by each isolates are measured. Time course of growth of the isolates in minimal medium containing glucose (B upper panel) and xylan (B lower panel) are shown. See Materials and Methods. All the results here are representative outcome of minimum three replications.

sequence analysis. For sequencing, genomic DNA was isolated using standard bacterial procedures. The identity of the purified isolates was confirmed by 16S rRNA and genome sequence analysis. We compared the sequences with those in the Genbank, EMBL and DDBJ databases.

RESULTS AND DISCUSSION

Japanese plum-grove soil sample contains several groups of bacteria and fungi when plated on nutrient agar. To check that the microflora of some of the collected soil were able to degrade plum-seeds, aerobic bacteria from plum-grove soil were cultured in minimal medium solution supplemented with ground plum-seeds as sole source of carbon for the isolation of plum -seeds degrading bacteria. The 128 colonies of aerobic bacteria on solid media overlayered with ground plum-seeds always spread. This necessitated further purification by transfers on different media. Incubation longer than 5 days increased the number of colonies. However, almost all the colonies were poorly seeds-lytic. A similar experiment was carried out with autoclaved soil as abiotic control. After all, five different bacterial cultures, designated CX1, CX2, CX3, CX4 and CX5 were successfully isolated and were selected for their ability to degrade plum-seeds. These bacteria had a marked plum-seeds softening activity after incubation at 28°C (data not shown). Little difference among the strains was observed to be activity for plum-seeds degradation (data not shown). Microscopic examination revealed that all the isolates were gram-negative, and the cells appeared rods (data not shown). They were able to grow over a wide range of temperature 10 - 40°C, with optimum at 30°C.

The cellulase and xylanase activities of the isolates were measured and shown (Figure 1A). As a result, all the isolates yielded reducing sugar for both cellulose and xylan, however, the contribution of cellulase activity may be relatively low. Then, we tested the ability of the isolates whether it can grow in the presence of xylan instead of glucose. Growth of the isolates was measured by the increase in turbidity at 600 nm. Figure 1B shows typical growth of the isolates in the presence of glucose

(upper panel) and xylan (lower panel) . Growth was observed with an appreciable lag phase and maximum growth was obtained after 30 h. Relatively, weak growth occurred on xylan medium, unless it contained glucose. Our experiments revealed that CX4 could not grow in the xylan medium, by unknown reason. The CX1, CX2, CX3 and CX5 bacteria were next confirmed by sequencing of 16S rRNA and genomic DNA analysis, which revealed some of the isolates (CX3 and CX5) had 99.8% identity to the sequence of *Pseudomanas aeruginosa*, available in the public domain. On the other hand, the CX1 and CX2 were distinct, and showed no identity to the se-quence of reported bacteria. The most similar sequences of CX1 and CX2 were *Pseudomonas entomophila* (up to 68% identity) and *Pseudomonas fluorescens* (up to 41% identity), respectively. Thus, sequence analysis showed that some of pseudomonas bacteria strains were able to degrade the plum-seeds.

It is demonstrated that these strains had xylanase activity. We thought this activity could be more optimized under suitable conditions. Biotransformation offers an environmentally compatible and efficient route for the production of some kinds of food and energy. Further-more, the isolates may find application as bio-inoculant for remediation of the amount of plum-seeds-waste. More studies need to be undertaken to elucidate the molecular mechanisms of the degradation in the isolates.

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