Genome analysis of drought tolerant rhizobacteria

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Abstract

Three different isolates of drought-tolerant rhizobacteria capable of producing an osmolyte, betaine, have been obtained from different rhizospheres of plants. Genomic DNA preparations from the three isolates have been analyzed by restriction enzyme digestion followed by both conventional and Pulsed Field Gel Electrophoresis (PFGE). Restriction enzyme digestion patterns revealed that the three isolates had significant differences in terms of restriction sites within the genome. The PFGE results confirmed that the three isolates had different genomic structures, despite the fact that they demonstrated similar response to drought, as evidence by the capability of producing betaine. The results thus suggest that different rhizobacterial isolates obtained from different habitats, show polymorphisms in terms of DNA sequences related to drought-tolerance properties.

Keywords: genome — rhizobacteria — PFGE

Introduction

Rhizobacteria is a group of root-colonising bacteria which has been known to evolve special interaction with specific plants resulting in deleterious to neutral and beneficial effects (Kloeper, 1993). Several species of rhizobacteria, e.g. Azotobacter, are known to exert beneficial effects to plant by direct promotion of plant growth through the production and secretion of metabolites, such as indole acetic acid (Arshad and Frankenberger, 1993).

Several studies have demonstrated that certain bacteria, e.g. Escherichia coli and Bacillus subtilis, evolve a mechanism of tolerance towards drought condition. Tolerance towards drought condition is achieved by the synthesis of certain metabolites, such as glycine betaine. Glycine betaine is an osmolyte synthesised by many microorganisms and plants which is used as a protective substance against osmotic stress, such as seawater, as well as drought condition encountered in soil (Munro et al., 1989; Kunin and Rudy, 1991; Peter et al., 1996). It has been demonstrated that staphylococci and E. coli require choline or glycine betaine to achieve maximal salt tolerance (Kunin and Rudy, 1991; Gauthier

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and Rudulier, 1990). Restoration of colony-forming activity of osmotically-stressed E. coli has also been achieved by exposing the cells to betaine (Roth et al., 1988).

Glycine betaine is synthesized through two steps, oxidation of choline to betaine aldehyde, followed by oxidation to glycine betaine. In E. coli, the oxidation of choline to betaine aldehyde is catalyzed by choline dehydrogenase whereas the conversion of betaine aldehyde to glycine betaine is catalyzed by betaine aldehyde dehydrogenase. Complete nucleotide sequences encoding choline dehydrogenase (betA) and betaine aldehyde dehydrogenase (betB) has been characterised (Lamark et al., 1991). Recently, three genes (gbsA, gbsB, and opuD), whose products were essential for glycine betaine biosynthesis, transport and osmoprotection in Bacillus subtilis have also been reported (Boch et al., 1996; Kappen et al., 1996).

Comparison of nucleotide sequences of E. coli bet genes with the similar genes in B. subtilis shows a quite little similarity between the two systems. Recently, several rhizobacterial isolates which exhibit osmotolerance have been obtained. Studies have demonstrated that some of the rhizobacterial isolates were capable of producing glycine betaine (Yuwono et al., 1997a, manuscript in preparation). It is unknown, however, whether these isolates possess similar genetic regulation system of osmotolerance as found in E. coli. It was therefore of interest to study whether the betaine-producing rhizobacteria display similar genome structure. This paper describes the polymorphism of the genomic DNA prepared from three different drought/osmo-tolerant-rhizobacteria using both conventional and Pulsed Field Gel Electrophoresis methods.

Materials and Methods

Microorganisms

The osmotolerant-rhizobacteria were isolated from the rhizosphere of several vegetable growing in dry areas of Yogjakarta Province, Indonesia. The isolation was carried out based upon the ability of the rhizobacteria to grow on mineral media M63 (KH2PO4 100 mM, KOH 75 mM, (NH4)2SO4 15 mM, MgSO4 0.16 mM, FeSO4 3.9 M, D-glucose 10 mM, pH 7.2) supplemented with NaCl at varying concentration. Three isolates, designated as AI 19, A82, and M7B, were found capable of producing glycine betaine and tolerant to NaCl stress, up to 0.75 to 1.0 M.

Genomic DNA Isolation

Genomic DNA was isolated from the rhizobacteria by using Genomic DNA Isolation Kit (Promega) followed by further purification using Wizard DNA Clean-up Kit (Promega).

Electrophoresis

Electrophoresis was carried out both by conventional and by Pulsed Field Gel Electrophoresis (PFGE) methods. Conventional electrophoresis was carried out by using genomic DNA isolated from the rhizobacterial isolates and performed on 0.8% agarose gel essentially as described by Sambrook et al. (1989). The PFGE was carried out by embedding the cells in 0.8% low melting agarose gel, followed by in situ lysis and restriction enzyme digestion. The protocol for PFGE was essentially as described by Brenner and Lai (1993). The PFGE analysis was performed on CHEF DR II Pulsed Field Electrophoresis System (BioRad) at 200 volt, with ramping time of 40 - 60 sec, for 5 - 16 hours.
Restriction enzymes digestion

The isolated genomic DNA isolated from rhizobacterial isolates were digested with several restriction enzymes. Digestion was performed as recommended by the enzyme manufacturer. Digestion of agarose-embedded DNA was carried out essentially as described by Birren and Lai (1993).

Results and Discussion

Restriction digestion profiles on conventional electrophoresis

Several restriction enzymes used for digestion of genomic DNA were: EcoRI, BamHI, Sau3A, HindIII, Smal, SacI, Kpnl, PstI, and XbaI. The results of digestion (Figure 1) demonstrated that the three rhizobacterial isolates (A1 19, A82, and M7B) showed different DNA profiles. Different degree of digestion between the three isolates suggest that they have quite distinct genomic structure. Digestion of M7B genomic DNA with BamHII, for example, resulted in extensive DNA fragments of varied sizes. Digestion of A1 19 and A82 DNA with the same enzyme, however, gave very minor DNA fragments, if not at all. Similar results were also obtained when other enzymes were used, e.g. HindIII, PstI. The possibility that the DNA fragments were generated through mechanical shearing during the handling, and not due to restriction enzyme digestion, can be excluded as evidence by the fact that the undigested DNA seemed intact on the gel.

PFGE analysis of rhizobacterial genomic DNA

The rhizobacterial genomic DNA were also subjected to PFGE analysis. The PFGE analysis was carried out both for the undigested as well as for the in situ-digested genomic DNA. Figure 2 shows the profile of undigested genomic DNA of rhizobacterial isolates and of E. coli as a compari-

Figure 1. Restriction profiles of three different isolates of drought-tolerant rhizobacteria (A1 19, A82, and M7B). Genomic DNA were isolated from each isolate then digested with several restriction enzymes: E: EcoRI, B: BamHI, H: HindIII, Sau: Sau3A, Sm: Smal, Sac: SacI, S: SstI, K: Kpnl, P: PstI, X: XbaI. M: DNA marker (DNA digested with Hind III and Eco RI). U: undigested. Electrophoresis on 0.8% agarose gel.
son. It is quite clear that the size of the Al 19 genomic DNA is slightly bigger than the genomic DNA of A82 and M78. The result also suggests that the Al 19 isolate may possess additional genetic element as shown by the appearance of a DNA band slightly smaller than the upper band. However, the existence of additional genetic element in this isolate is yet to be confirmed.

**Undigested genomic DNA**

Figure 2. PFGE analysis of undigested genomic DNA of drought tolerant rhizobacteria. A: Al 19, A: A82, M: M78, E: Escherichia coli. Electrophoresis was carried out by using CHEF-DR II (BioRad), ramping time: 40-6 sec, 200 volt, for 16 hours.

The PFGE profiles of digested-genomic DNA (Figure 3) confirm the results of conventional electrophoresis which clearly suggest the difference of the genomic structure of the three rhizobacterial isolates. Both single and double digestions with restriction enzymes resulted in different restriction pattern.

Physiological analysis has shown that the three rhizobacterial isolates had the capability of producing glycine betaine (Yuvono et al., 1997a, manuscript in preparation) despite the fact that morphologically they are of different group of rhizobacteria. Studies have shown (Lamark et al., 1991; Boch et al., 1996; Kappes et al., 1996) that nucleotide sequences similarity of the genes responsible for betaine synthesis in E. coli and B. subtilis is

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Figure 3. PFGE analysis of rhizobacterial genomic DNA digested with several restriction enzymes. U: undigested, E: EcoRI, H: Hind III, B: Bam HI, Sau: Sau 3A, H/B: Hind III and Bam HI, E/H: Eco RI and Hind III, E/B: Eco RI and Bam HI, 1, 4, 7: A1 19; 2, 5, 8: A82; 3, 6, 9: M78. Electrophoresis was carried out by using CHEF-DR II (BioRad), ramping time: 40-6 sec, 200 volt, for 16 hours.
suggest that genes responsible for betaine synthesis in some organisms may comprise of a family of genes. Recently we have been able to amplify, by PCR method, a DNA sequence from rhizobacterial isolate AI 19 using primers for amplifying betA gene of E. coli. The size of the sequence is quite close to the size of betA gene and it has also been found that the sequence gave positive result when hybridised with a probe developed for betA gene of E. coli (Yamane et al., 1997b, manuscript in preparation). We have also determined that the rhizobacterial isolate AI 19 belongs to Klebsiella sp. At present, it is not known yet whether there is any significant similarity between betA sequences of E. coli with that of rhizobacteria sequences.

Conclusion

The results of this study have demonstrated the difference of the genomic DNA structure between three different rhizobacterial isolates capable of producing glycine betaine. It has also been shown that the size of the genome of isolate AI 19 (which belongs to Klebsiella sp.) is bigger than other isolates which, morphologically, are of different group of rhizobacteria. This study also indicates that the Klebsiella sp. isolate may possess an additional genetic element, although it is still yet to be confirmed by further analysis.

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