Note

Three 1.7 Kilobase Pair Plasmids in *Zymomonas mobilis* NRRL B-806Norihiro MISAWA, Katsumi NAKAMURA
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Zymomonas mobilis is a Gram-negative bacterium, which has considerable potential for industrial alcohol fermentation as described by Rogers *et al.*¹⁾ However, it has the disadvantage of having a limited range of fermentable sugars.²⁾ Many studies on genetic improvement of *Z. mobilis* have been performed in attempts to overcome this

disadvantage, though no suitable host-vector system has been developed.^{3~9)} Workers at several laboratories have reported that many strains of *Z. mobilis* contain cryptic plasmids.^{3~6)} A recent study by Walia *et al.*⁷⁾ has shown that a cryptic plasmid (molecular weight, 50 megadaltons) from *Z. mobilis* CP4 carries genes for antibiotic resistances. On the other hand, conjugational transfer experiments have shown that drug-resistant genes of R plasmids are expressed in *Z. mobilis*.^{8,9)} These results suggest that appropriate hybrids between *Z. mobilis* cryptic plasmids and *Escherichia coli* plasmids carrying drug-resistant genes may act as shuttle vectors between *Z. mobilis* and *E. coli*. It is desirable that *Z. mobilis* cryptic plasmids, whose replicative ability is to be exploited, are small enough in size. *Z. mobilis* ATCC 10988 is known to contain several small plasmids of 1.7, 2.7 and 4.2 kilobase pairs (kb) in size.^{3~6)} This paper describes the analysis of native plasmids smaller than 10 kb from *Z. mobilis* NRRL B-806, it was found that 1.7 kb plasmids were composed of three different

FIG. 1. (A) Agarose Gel Electrophoresis of Plasmid DNAs Prepared from *Zymomonas mobilis* NRRL B-806 in a 1.0% Agarose Gel.

a, *Z. mobilis* NRRL B-806 plasmid DNAs; b, λ DNA-*Hind*III/ ϕ $\times$ 174-*Hae*III molecular weight standards (top to bottom): 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb, 2.0 kb, 1.4 kb, 1.1 kb, 0.87 kb and 0.60 kb.

(B) Agarose Gel Electrophoresis of DNAs Recovered from Band A in Lane a and the Digests Obtained with Restriction Endonucleases in a 1.4% Agarose Gel.

c, DNAs recovered from band A in lane a; d, *Eco*RI; e, *Hind*III; f, *Pvu*II; g, *Bgl*II; h, *Hinc*II digests of DNAs recovered from band A in lane a; i, λ DNA-*Hind*III/ ϕ $\times$ 174-*Hae*III molecular weight standards (top to bottom): 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb, 2.0 kb, 1.4 kb, 1.1 kb, 0.87 kb, 0.60 kb, 0.56 kb (faint), 0.31 kb, 0.28 kb (clear), 0.23 kb, 0.19 kb and 0.13 kb.

FIG. 2. Restriction Maps of the Three 1.7 kilobase pair Plasmids.

The numbers show kilobase pairs (kb). These three plasmids have no sites for *KpnI*, *XhoI*, *BamHI*, *SalI*, *MluI*, *SmaI*, *HpaI*, *PstI*, *SacI*, *AvaI* or *HaeII*.

species.

Our strain, *Z. mobilis* NRRL B-806 (ATCC 10988), was obtained from the Northern Regional Research Laboratory. NRRL B-806 was grown at 30°C in RM medium⁸⁾ composed of yeast extract (Oxoid) 10 g/liter, glucose 20 g/liter and KH_2PO_4 2 g/liter (pH6.0). Plasmid DNAs were isolated from NRRL B-806 by the procedure described by Birnboim and Doly,¹⁰⁾ and purified by phenol-chloroform extraction and ethanol precipitation. Plasmid DNAs (0.2~1 µg) were completely digested with 5~10 units of an appropriate restriction endonuclease for 1.5~2.5 hours at 37°C. Plasmid DNAs and the restriction endonuclease digests were analysed by agarose electrophoresis.

Figure 1(A) shows agarose electrophoresis of total plasmid DNAs prepared from NRRL B-806. Many bands were observed in the plasmid DNA preparation of NRRL B-806 (Fig. 1, lane a). Plasmid DNAs in the main bands, A, D, E, F, G and H, were recovered from gels by electroelution and purified with an Elutip[®] column (Schleicher and Shuell). The DNAs recovered were digested with several restriction endonucleases, and then compared with each other as to their restriction banding patterns on the gel. As a result, it became clear that bands D and F were derived from the 2.7 kb plasmid, and bands E, G and H from the 4.2 kb plasmid. It was assumed from their migration distances on the gel that bands D and F corresponded to the ccc (covalently closed circular) and oc (open circular) forms of the 2.7 kb plasmid, respectively, and that bands E, G and H corresponded to the ccc form, oc form and dimer of the 4.2 kb plasmid, respectively. Plasmids 2.7 kb and 4.2 kb in size have already been reported in NRRL B-806.^{3~6)} Figure 1(B) shows agarose electrophoresis of DNAs recovered from band A in Fig. 1 (lane a) and the restriction endonuclease digests. On electrophoresis of DNAs extracted from band A in lane a, bands B and C appeared in addition to band A (Fig. 1, lane c). Only one fragment of 1.7 kb in size appeared, beside bands A, and B and/or C, after digestion with *EcoRI*, *BglII* and *HincII* restriction endonucleases (Fig. 1, lanes d, g and h). From these results, it was assumed that band A corresponded to the ccc form of at least two 1.7 kb plasmids, and that bands B and C corresponded to the oc form of these plasmids.

The fragments cleaved by *EcoRI*, *BglII* and *HincII* were

ligated to the sites for *EcoRI* of pBR322, *BamHI* of pBR322 and *HincII* of pUC9, respectively, and *E. coli* C600 or JM103 was transformed. Hybrid plasmid DNAs were isolated and purified from each transformant. The restriction sites of inserted fragments of hybrid plasmids were analysed by agarose electrophoresis, and the results showed that these three fragments were different as to the sites of restriction endonuclease cleavage. It was therefore clear that there were three different plasmids (ccc form) in band A in Fig. 1 (lane a). These plasmids were designated as pZM81A, pZM81B and pZM81C. Their restriction maps are shown in Fig. 2. The sum of the restriction patterns which was estimated from the restriction maps, agreed well with the results of electrophoresis presented in Fig. 1(B). The 1.7 kb *HindIII* fragment appearing in Fig. 1 (lane e) was considered to be a partial digest of pZM81B or pZM81C.

This is the first report of three 1.7 kb plasmids in NRRL B-806. These three plasmids are of low molecular weight, and have appropriate restriction cleavage sites. Such small plasmids in NRRL B-806 may act as agents of shuttle vectors between *Z. mobilis* and *E. coli*.

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