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# A HIGH MOLECULAR WEIGHT PLASMID OF ZYMOMONAS MOBILIS HARBOURS GENES FOR $HgCl_2$ RESISTANCE

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## SUMMARY

Plasmids from Z. mobilis could be stably maintained in E. coli HB101 in which the expression of various drug resistance markers could be monitored. A large molecular weight plasmid (5.2 kbp) of Z. mobilis was found to harbour the genes for mercuric chloride degradation and to confer upon E. coli, resistance to a higher mercuric chloride concentration as compared to Z. mobilis. The introduction of this plasmid made E. coli sensitive to concentrations of cadmium acetate which were originally non-inhibitory to it.

## INTRODUCTION

The biology of Zymomonas has been reviewed extensively. This includes its morphology, growth, physiology, biochemistry (Swings, 1977) and alcohol productivity (van Vuuren, 1982; Warr, 1984). With its genetic improvement in mind, the plasmids of Zymomonas have been studied (Tonomura, 1982; Stokes, 1983; Skotnicki, 1984) and the transformation of Z. mobilis has been carried out either with broad host-range plasmids or with new shuttle vectors developed using low molecular weight plasmid of Z. mobilis (Buchholz, 1986; Yanase, 1986; Afendra, 1987; Buchholz, 1987; Conway, 1987). Introduction of desirable properties such as the utilization of substrates like starch for ethanol production, was attempted (Skotnicki, 1984), using the fungal amylase gene, but this foreign DNA was not stably retained in Z. mobilis. Conventional recombinant DNA techniques have been of limited use in the case of Zymomonas due to the incompatibility of the vector DNA with the Z. mobilis restriction-modification system. Naturally occuring plasmids of Z. mobilis, if characterized, could either themselves be used as vectors for transferring desirable genes into Z. mobilis or could be used in the construction of new compatible hybrid vectors. There is no consistency in the observations reported so far regarding characterization of plasmids of Z. mobilis

(Tonomura, 1982; Stokes, 1983; Skotnicki, 1984). Except for the partial characterization of pRUT41 ( 46 Mdal; encoding resistance to gentamicin, kanamycin and Streptomycin) from Z.mobilis CP4 (Walia, 1983), no other plasmid from Z.mobilis has been characterized with respect to the markers located on it.

Z.mobilis exhibits resistance to various drugs and antibiotics. None of the resistances (except gentamicin, kanamycin and streptomycin in Z.mobilis CP4) have been localized on one or more of the naturally occuring plasmids ofZ.mobilis. Therefore, with an intention of characterizing the plasmids of Z.mobilis with respect to drug resistance, we transformed E.coli with purified Z.mobilis plasmids and checked for the expression of such markers. In this paper we report that one of the high molecular weight plasmids of Z.mobilis, which can be stably transferred into E.coli HB101, confers resistance to mercuric chloride and sensitivity to cadmium acetate.

## MATERIALS AND METHODS

Z.mobilis ATCC 10988 and E.coli HB101 were used. Z.mobilis was grown in Yeast extract, Peptone, Dextrose medium (YPD), pH 6.0, at 30°C and E.coli was grown in Luria broth at 37°C, as shake-flask cultures. Total plasmids of Z.mobilis were isolated by the alkaline extraction method of Birnboim and Doly (Birnboim, 1979). A purified preparation of the individual plasmids of Z.mobilis was obtained by elution from a low melting point agarose gel. The transformation of E.coli HB101 with the total plasmid preparation and individual plasmid, pZM1, of Z.mobilis was carried out according to the method of Mandel and Higa (1970). The tranformants obtained using total plasmid preparation were selected on Luria agar containing mercuric chloride (40 µg/ml), or ampicillin (40 µg/ml) or tetracycline (10 µg/ml). The tranformants obtained using the purified preparation of the largest plasmid, pZM1, were selected on Luria agar containing various concentrations of mercuric chloride, and one of the transformants, Z1H37, was further tested for resistance to mercuric chloride and cadmium acetate using the disc assay method.

## RESULTS

The total plasmid preparation of Z.mobilis showed the presence of six plasmids (fig. 1A). E.coli HB101 transformants obtained using the total plasmids of Z.mobilis were screened for resistance to ampicillin (40 µg/ml), tetracycline (10 µg/ml) and mercuric chloride (40 µg/ml). The transformants isolated were found to be resistant to either one or two, but not all three, of these drugs, the majority being resistant to tetracycline (Table 1). These results suggested that Zymomonas plasmids could be stably transferred to E.coli HB101 and pecific drug resistance markers

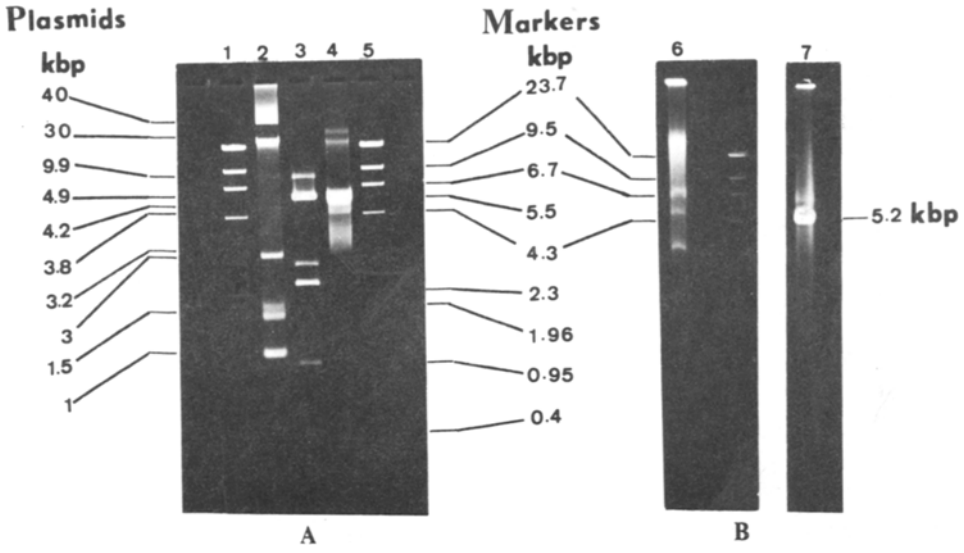


Fig. 1 : Plasmids from Z.mobilis and from the transformant Z1H37. Electrophoretic pattern in 1.0% agarose gels of Hind III digests of lambda DNA (lanes 1,5) and PM2 DNA (lane 3) as markers, total plasmids from Z.mobilis (lane 2) and Z1H37 (lanes 4,6) and Ava II digest of Z1H37 plasmids (lane 7).

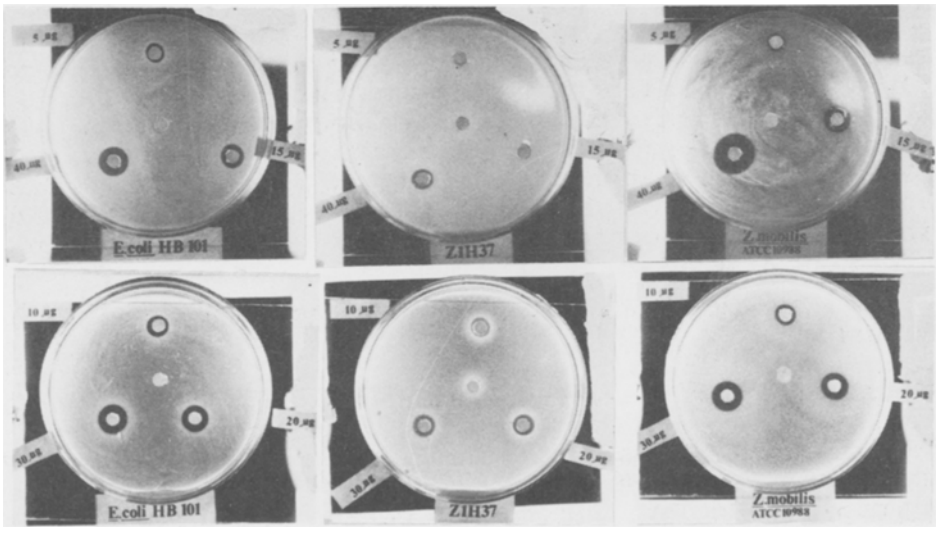


Fig. 2 : Inhibition of growth by mercuric chloride in a disc assay.

Table 1 : Drug resistance of E.coli HB101 transformants obtained using total plasmids of Z.mobilis.

| Growth in the presence of | | | Number of Transformants obtained |
|---|---|---|---|
| Ampicillin (40 µg/ml) | Tetracycline (10 µg/ml) | $HgCl_2$ (40 µg/ml) | |
| + | + | + | 0 |
| + | − | + | 1 |
| + | + | − | 2 |
| − | + | + | 3 |
| + | − | − | 2 |
| − | − | + | 3 |
| − | + | − | 18 |

could be monitored in the transformants. Thus, the purification of individual plasmids of Z.mobilis, followed by their transfer to E.coli HB101, could lead to the localization of specific markers on these plasmids. A purified preparation of a high molecular weight plasmid of Z.mobilis, pZM1, obtained by elution from a low melting point agarose gel, was used to transform E.coli HB101 and the transformants selected on Luria agar plates containing various concentrations of mercuric chloride (4, 10, 20 and 40 µg per ml). One of the transformants, Z1H37, which could grow in liquid medium containing 40 µg/ml mercuric chloride was further analysed for its plasmid content and other properties.

Plasmids harboured by Z1H37 can be seen in both, small-scale preparations (cracking of cells) and purified plasmid preparations, as shown in fig.1. Here, a high molecular weight band can be seen similar to pZM1 of Z.mobilis. In addition, plasmid DNA was also seen at positions corresponding to 30 kbp, 4.9 kbp, 3.8 kbp and 3.2 kbp. These seemed to be due to plasmid DNA in different conformations which linearized to 5.2 kbp size after Ava II treatment (fig. 1B).

In the inhibition zone disc assay (fig.2), the transformant Z1H37 showed more resistance to $HgCl_2$ than either E.coli HB101 or Z.mobilis. The resistant cells of Z1H37 however, seemed to grow after a definite lag, during which probably, the plasmid-based gene was induced. A similar lag was also seen when Z1H37 was grown in a liquid medium, in the presence of 40 µg/ml of $HgCl_2$. Z.mobilis could not grow in liquid medium beyond a concentration of 10 µg/ml of $HgCl_2$; the tolerance level in Z1H37 was raised probably because of a relatively higher copy number of the concerned plasmid as compared to Z.mobilis.

E.coli HB101 was found to be resistant to cadmium acetate (Table 2), whereas Z.mobilis was highly sensitive. The transformant, Z1H37, was inhibited significantly by cadmium acetate (beyond 20 µg), though less than Z.mobilis. Z1H37 exhibited a significant change in both cell and colony morphology as compared to E.coli Hb101. The cells were larger and slightly curved. A number of cells were also arranged in tandom. Unequal division of cells was also observed; the possibility of mini cell production has yet to be confirmed.

**Table 2 : Inhibition zone disc assay**

| Organism \ concentration of cadmium acetate (µg) | 10 | 20 | 30 | 40 | 50 | 60 |
|---|---|---|---|---|---|---|
| | (Inhibition zone in mm) | | | | | |
| E.coli HB101 | 5.5 | 6.5 | 7.5 | 8 | 8.5 | 8.5 |
| Z1H37 | 8.5 | 13 | 13.5 | 14 | 16 | 16 |
| Z.mobilis | 20 | 28 | 28.5 | 36 | 39.5 | 40 |

## DISCUSSION

Z.mobilis would have the potential to convert a variety of raw materials such as cellulose and starch to ethanol after the introduction of the complementary cellulase or amylase genes (Eveleigh, 1987). Engineering of Z.mobilis, however, has not been successfully carried out so far; one of the reasons being, the restriction-modification system in Z.mobilis does not allow the stable transfer and expression of foreign genes.

Z.mobilis also harbours naturally occuring plasmids (Tonomura, 1982; Stokes, 1983; Skotnicki, 1984), which however cannot be eliminated from the cell completely. This may also be due to the possibility that some of the housekeeping genes are localized on these plasmids. Only one of the plasmids of Z.mobilis has been characterized with respect to the markers located on it and no detailed molecular studies of these plasmids have been carried out. Only the large plasmid, pRUT41 (46 Mdal) has been shown to harbour gentamicin, kanamycin and ampicillin resistance genes (Walia, 1983). In addition, the reported number and sizes of these plasmids in Z.mobilis ATCC 10988, vary. A plasmid of Z.mobilis with known markers could be a very useful vector for self-cloning of complementary genes. Due to the possible flux between plasmids and Zymomonas genome, such foreign genes might then be transferred to genomic DNA. On the other hand, plasmids like pZM1, if reintroduced with new desirable gene(s) under the control of the $HgCl_2$ induced promoter, could be used to regulate new gene expression with $HgCl_2$. The transformant, E.coli (Z1H37), with a significant $HgCl_2$ degradation/detoxification activity also could

be used in the treatment of paper-pulp industry effluent which contains $HgCl_2$. Our results demonstrate that the high molecular weight plasmid of Z.mobilis (pZM1), has genes responsible for $HgCl_2$ tolerance. This plasmid also has been found to confer resistance to ampicillin (unpublished results). This plasmid can be stably transferred and expressed in E.coli. The appearance of additional plasmids in E.coli-Z1H37 of sizes similar to plasmids of Z.mobilis could be because of the deletion of that DNA from the large plasmid, a phenomenon which might also be happening in Z.mobilis. Stokes et al. (1982) have already shown that there exists homology among various plasmids of Z.mobilis. The property of resistance to high levels of $HgCl_2$ can be well utilized in constructing new vectors, both for Zymomonas and other bacteria. The presence of other genes on the large plasmid of Z.mobilis can now be easily analysed.

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## REFERENCES

1. Afendra, A.S. and Drainas, C., (1987). J.Gen.Microbiol. **133** : 127-134.
2. Birnboim, H.C. and Doly, J., (1979). Nucleic Acids Res. 7(6) : 1513-1523.
3. Buchholz, S.E. and Eveleigh, D.E., (1986). Appl. Environ.Microbiol. **52** (2) : 366-370.
4. Buchholz, S.E., Dooley, M.M. and Eveleigh, D.E. (1987). TIBTECH **5** : 199-204.
5. Skotnicki, M.L., Goodman, A.E., Warr, R.G. and Rogers, P.L. (1984). Microbios **40** : 53-61.
6. Skotnicki, M.L., Warr, R.G., Goodman, A.E., Lee, K.J. and Rogers, P.L., (1984). Biochem. Soc. Symp. **48** : 53-86.
7. Stokes, H.W., Dally, E.L., Yablonsky, M.D. and Eveleigh, D.E., (1983). Plasmid **9** : 138-141.
8. Swings, J. and De Ley, J., (1977). Bact. Rev. **41** (1) : 1-46.
9. Tonomura, K., Kurose, N., Konishi, S. and Kawasaki, H., (1982). Agric. Biol. Chem. **46** (11) : 2851-2853.
10. Van Vuuren, H.J.J. and Meyer, L., (1982). Biotechnol. Lett. **4** (4) : 253-256.
11. Walia, S.K., Carey, V.C., All III, B.P. and Ingram, L.O., (1983). Appl. Environ. Microbiol. **47** (1) 198-200.
12. Warr, R.G., Goodman, A.E., Rogers, P.L. and Skotnicki, M.L., (1984). Microbios **41** : 71-78.
13. Yanase, H., Kurii, J. and Tonomura, K. (1986). Agric. Biol. Chem. **50** (11) : 2959-2961.
14. Yanase, H., Kotani,T. and Tonomura, K., (1986). Agric. Biol. Chem. **50** (12) : 3139-3144.