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An Efficient Method for Isolation of Large Plasmid DNA from *Pseudomonas putida*

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ABSTRACT

The conditions suitable for reproducible recovery of large plasmid DNA from strain of Pseudomonas putida which have degradation properties have been defined. These degradative plasmid could not be isolated by the usual produce where as there are easily isolated by the modified procedure.

Keywords: *Pseudomonas putida , Plasmida, DNA.*

INTRODUCTION

The existence of plasmid DNA in different bacterial species may be established by genetic and physical methods. *Pseudomonas putida* have been shown by genetic means to carry transmissible plasmids, which contain genes for many of the naturally occurring hydrocarbon degradation pathways. Most of the available techniques employed for the isolation of plasmid DNA depend upon the fact that the plasmids exist as covalent closed circular DNA in the cytoplasm of the cell and do not sediment with the cellular membrane and chromosome. Therefore, in all these techniques the cellular membrane and chromosomal DNA are removed by centrifugation or by precipitation in the presence of high NaCl concentration (Clwell & Helinski, Freifelder, & Guery, et al. (1973). The same technique did not work when applied to *Pseudomonas putida* strain containing degradative plasmid. We, there fore, thought two major modifications of our usual procedure. First the harvested cells were not washed with buffer containing EDTA, because there strain showed partial lysis even in the presence of 5 mM. EDTA. Secondly, the cellular membrane and chromosome were not removed by centrifugation by high salt precipitation like usual method. There are some modification introduce in our usual procedure to isolate there degradative plasmids were described in the present communication.

MATERIALS AND METHODS

Bacterial strain and medium

The bacterial strain was originally isolated from the soil and identified by microscopically, physiologically and biochemically as *Pseudomonas putida*.

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The strain was grow approximately $2x10^8$ cells per ml in Luria broth medium consisting 10 gl^{-1} tryptone, 10-gl^{-1} NaCl, 5 gl^{-1} yeast extract with 100 µg ml^{-1} ampicilline as antibiotic resistant at 30 ^{0} C in temperature.

Preparation of clear lysate

40 ml bacterial culture were centrifuge at 10,000 rpm for 5 min at ambient temperature. The pellets were suspended in 1.5 ml. of 25 % sucrose and vortex at maximum speed. Then 0.5 ml of 20 % SDS was added and mix by inversions. This produce a clear viscous solution of lysed cells. At ambient temperature 0.5 ml NaOH (3 N) was added and mix immediately. This rise the pH at 12 and then 1.0 ml Tris (2 M, pH- 7.0) was added for lowing the pH at 8.5.

Removal of membrane – chromosome complexes

The 0.60 ml. of 20% SDS was added in the clear lysate immediately followed by addition of 1.20 ml NaCl (5 M) caused the appearance of white folk – like material. Failure to adding and mixing quickly at ambient temperature give incomplete removal of the chromosomal DNA. After inversion the tubes were chilled in an ice water bath for 1 hr then centrifuge at 12,000 rpm for 15 min at 4^0C caused the salt-precipitated chromosome- membrane complexes to form a large white pellets.

Concentration of Plasmid DNA

The supernatant was poured in to chilled Sorvall cup and equal volume of phenol: chloroform: iso amyl alcohol (25:24:1) was added and centrifuge at 12,000 rpm for 10 min at ambient temperature. Take the upper viscous solution and add 2.5 volume of 100 % ethanol with $1/10^{th}$ of 3 M sodium acetate place it in – 20 ^{0}C for 20 min and then centrifuge at 12,000 rpm for 15 min at 4^0C. The yielded pellets were wash with 70 % ethanol and dry. Dissolve the pellet in to 200 µl of sterile water. This solution contributed a highly enriched crude plasmid preparation that could be subjected to electrophoresis without further purification.

RESULTS AND DISCUSSION

The plasmid DNA was routinely and consistently isolated using a procedure which does not require ultra centrifugation but includes steps designed to separate large plasmid DNA from the bacterial folded chromosome. In this protocol the SDS was added at 20% concentration to give 4% of final concentration in the lysate mixture. Varying the concentration of SDS used in our protocol was found to have a marked effect on plasmid yield. Figure 1 where we prepared the plasmid DNA with final concentration of SDS is 1 to 5%. The yield of plasmid DNA increase as increase the concentration of SDS up to 4% after there is decrease. The 4% final concentration was added in the modified protocol. The addition of NaCl causes the salt precipitation of chromosome – membrane complex to form a large white pellet. Figure 2 indicated that the 5 M concentration of NaCl give high yield of plasmid DNA than 1 to 4 M of NaCl. We added the 5 M of NaCl in our protocol. The failure of addition and mixing of SDS and NaCl quickly at ambient temperature give incomplete removal of chromosomal DNA. There are following factors contribute to the success of the plasmid isolation protocol. The manipulation were all very gentle, consisting only the decanting, inversion, centrifugation, there were no pipetting. The absence of shearing in contrast to some isolation methods for large plasmids (Chakrabarty, Palchaudhuri (1978) & Chakrabarty, & Sharp et al., 1976). At the time of lysis the lysate were kept in the presence of high SDS concentration. These effect the protein denaturation and reduce the degree of enzymatic degradation of the plasmid SDS and alkali both helped for increasing the plasmid yield in another way.

The folded chromosome model for the bacterial genome (Petti john & Hecht, (1973) Worcel & Burgi & Worcel, et al., 1972) suggests that the continuous chromosomal DNA molecule comprises the different loop and RNA is involved in maintaining the loops separate from each other. If DNA in a loop under goes a single strand brack, that loop alone will lose its supercoils ribo-nuclease digestion of folded chromosome will decrease the number of loop, but will not alter the super coiling (Petti john & Hecht, (1973), Worcel &

Burgi & Worcel, et al., 1972). Kline et al. (Zyk, Manis & Otten,) postulated that in *vivo* a super coiled plasmid make a non integrative association with the folded chromosome because the physiochemical structures of the plasmid resemble one of the super coiled domains of the bacterial genome and that such a non integrated association is important for both replication and segregation of the plasmid.

Evidence for such association derives mainly from observation of co-sedimentation of the plasmid with isolated folded – chromosome complexes. Since large plasmids tend to be stringently controlled for replication their complete association with the folded chromosome interfering with plasmid isolation (Clowes, 1972).

SDS are observed to cause the complete unfolding of the condensed chromosome similar to that caused by ribnuclease. SDS acts to dissociate bound protein and is not known to have any direct effect an nucleic acid interactions (Stonington & Pettizohn, 1971). This suggested to us that in our lysis procedure the contribution of the SDS and alkali treatment was help for unfolding the bacterial genome, allowing release of the supercoiled plasmid DNA in to the solution before precipitation of the chromosome-membrane complexes. The successful application of our lysis procedure for recovery of large plasmids DNA from a variety of bacterial genera may apply. Figure 3 showed the high yield of plasmid DNA which have same mobility. These observation suggested that the plasmid DNA have covalent closed circular DNA. The high quality DNA without RNA contamination can be prepared from above procedure. The yield of DNA is quantitative and reproducible and the DNA is sufficient purify for direct use in any application of PCR, restriction digestion, cloning, DNA sequencing, and southern blot analysis etc.

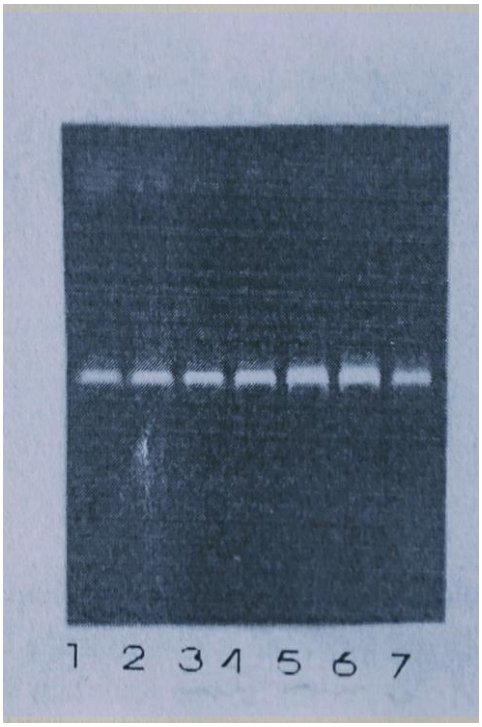

Figure1. Agarose gel electrophosis of plasmid DNA showing the effect of different concentration of SDS. Lane 1 , 2 & 3,4 , 5 and 6 & 7 represented 1%, 2% 3%, 4%, and 5% final concentration of SDS respectively

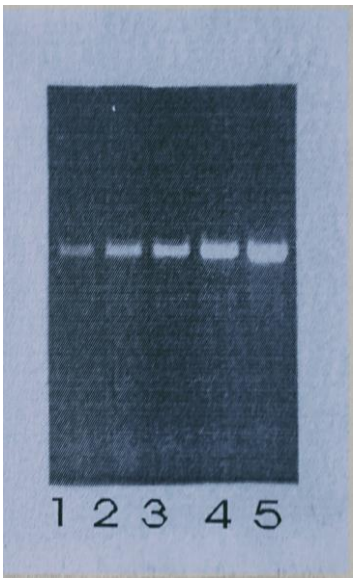

Figure 2. Agarose gel electrophosis of plasmid DNA showing the effect of different concentration of NaCl for the precipitation of membrane chromosome complexes. Lane 1, 2, 3, 4 and 5 represented 1M, 2 M, 3 M, 4 M and 5 M concentration of NaCl respectively.

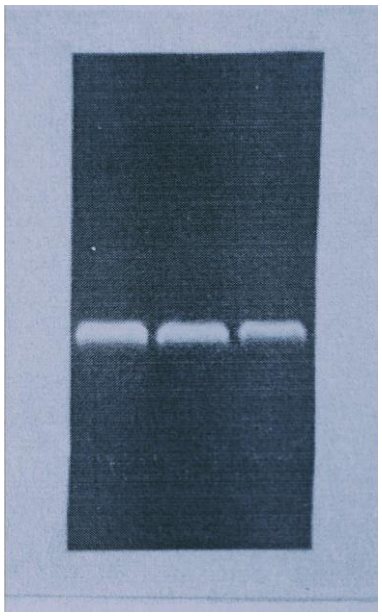

Figure 3. Agarose gel electrophosis of plasmid DNA isolated by modified methods. These band showing the high yield of Plasmid DNA.

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