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An Inducible Plasmid in Corynebacterium
棒状杆菌诱发质粒简报*

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Abstract A review summarised the plasmids of nonpathogenic Corynebacterium. *Corynebacterium pekingense* AS 1.299 had plasmids, which could be induced by treatment of ethidium bromide (EB). Their molecular sizes were 36.2×10^6, 15.2×10^6, 13.0×10^6 and 9.8×10^6 respectively, compared with 8 known plasmids of E. coli v 517. AS 1.299 strain had no plasmids originally. These inducible plasmids disappeared spontaneously from the host after transfered to fresh media by the fifth time.

Key words corynebacterium, plasmid, DNA

摘要 用溴化乙锭诱发质粒,溴化乙锭不同浓度对棒状杆菌基因组起不同作用,低浓度无作用;高浓度消除质粒;适中浓度能诱发质粒。本试验诱发出4种大小不同质粒,转种5代质粒消失。

关键词 棒状杆菌 质粒 DNA
中图法分类号 Q 936; TQ 920.1

Corynebacteria are Gram-positive bacteria which are widely distributed in nature. One group of them, nonpathogenic corynebacteria (NPC), are industrial important organisms. Although they have been used in fermental industry to produce amino acids for about 30 years, the basic genetic knowledge of them is very scanty. Because of the interest both in academic and industrial applications, more attention was paid to the extrachromosomal genetic elements of this group of organioms[1- 6]. Twenty-two plasmids ranging in size from 55 to 4.2 Kb are known to present in 17 strains of NPC[7]. In some strains of NPC, plasmid DNA can not be detected although many efforts have been offered. In the last five years in the other industrial organisms such as streptomyces and micromonospora, an UV induction method was reported to be employed to induce the excision of a chromosomally integrated plasmid to become a free multicopy plasmid[8, 9, 29]. But no report was read in NPC. Yet in our work seeking relationship between bacteriophage resistance and plasmid, we found that a glutamate-producer, *Corynebacterium pekingense* AS 1.299, had plasmids which could be induced by treatment of ethidium bromide (EB). Strain 1.299 is a plasmid-free strain before EB treatment. We have confirmed that it had undetectable plasmid by using at least 7 procedures routinely used for plasmid isolation[10- 16]. Our preliminary work reported here might developed a general method for obtaining plasmid DNA from NPC and should facilitate its development eventually as a vector for gene cloning in NPC. The physical map and electron microscope picture of the inducible plasmid will be reported later.

Strain 1.299 and its sensitive phage A2 were purchased from China Committee for Culture Collections of Microorganisms (CCCCM). E. coli V 517 used as a size reference of plasmid DNA was kindly given by the Department of Biochemistry of Basic Medical Institute of Chinese Academy of Medical Science. Both strains were grown at 28℃ in BPY medium (Perliter: Beef extract 5 g; Yeast extract 5 g; Polypeptide 10 g; Glucose 5 g; NaCl 5 g; pH 7.0 - 7.2; in solid culture, 15 g agar was added). Inducing plate was prepared by adding EB to the BPY

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medium to a final concentration of 0.5μg/mL before used.

Cells to be induced were cultured at 28℃ in liquid BPY. 0.1mL of overnight culture and 0.1mL of dilution of phage A2 were well mixed, then poured onto the inducing-plate by the pour plate procedure. The plates were incubated at 28℃ until single colonies be seen. The colonies were picked up for further screening of plasmid DNA. In this inducing procedure, we used phage A2 as a selection remarker because we had found that the cells in which plasmids were induced became of phage A2 resistance.

The colonies resistant to phage A2 were determined in line with the presence of plasmid DNA by a modification of Bibb's procedure[10]. Cells to be tested were spreaded on the BPY plate and incubated at 28℃. The overnight culture was harvested from 1 cm^2 area of the plate and suspended in 100μL of TE buffer containing lysozyme 10 mg/mL and 34% sucrose then placed at 0℃ overnight. Cells were completely lysed by adding 100μL of 5% SDS and 100 μL of HAS buffer (pH 4.5). After lysis the cell debris and the chromosomal DNA were precipitated by addition of 100μL of 5 mol/L NaCl and centrifugated in Eppendorf centrifuge. The supernatant (containing plasmid DNA) was carefully collected for further gel-electrophoresis analysis. Agarose gel electrophoresis was performed with horizontal agarose gel at 3 V/cm in TAE-buffer at agarose concentration of 0.7%. After electrophorosis, gel was stained with ethidium bromide (0.5μg/mL) for 20 min. The bands of plasmid DNA was visualized under 256 nm UV light.

In our results, four visuable plasmids DNA bands could be reproducible. They were present in the gel in addition to chromosomal DNA while only chromosomal DNA was present without the treatment of EB. Their molecular weight were 36.2×10^6, 15.2×10^6, 13.0×10^6 and 9.8×10^6 respectively, compared with eight known plasmids of E. coli V517. The inducible plasmide disapeared spontaneously from the host after transfered to fresh medium fifth time (Fig. 1). Once the plasmids were induced, the strain 1.299 became of resistance to phage A2 even after the inducible plasmids were cured from the cells. We supposed that the looping out of plasmid DNA from the host genome might have closed a host gene which encoding for a protein responsible for sensitivety to phage A2. This was comfirmed by the fact we observed that strain 1.299 first induced (design as strain 9901) became less sensitive to phage A2 and finally became completely resistant to A2 after transfered in the cells was still high enough to maintain the phage sensitivity although the host gene encoding for the protein had been closed by looping out of plasmid. And as the times of transfer increased, the concentration of the protein reduced to a level unable to keep phage sensitivity and phage resistance occured.

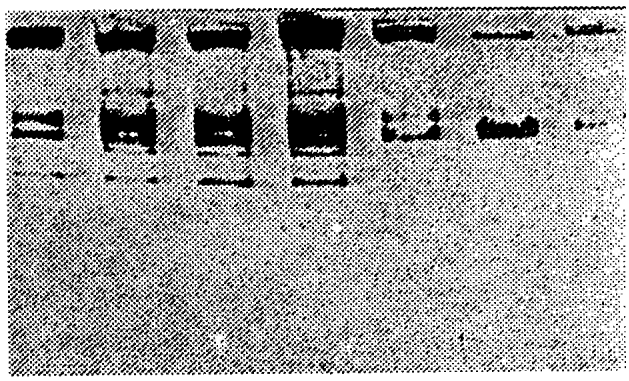

Fig. 1

Plasmids, in some case, have a direct or indirect influence on the sensitivity of host to bacteriophage. It was reported that bacteriophage W31 could replicate in Hfr strains but not in other F-containing cells[17]. This system is interesting, but has not been studied extensively. Duckworth postulated that integration of the F factor had inactivated a host gene required for the abortive infection[18]. Obviously, the mechanism is different from that in corynebactetia we mentioned above. Addition to these two theories, the third was described by Anderson that a salmonellae strain which had been sensitive to 30 of the typing phase was sensitive to only 9 after introduction of a R factor[19]. The question is still open to know whether there is another way for plasmid to influence the host on phage-sensitivity.

Plasmids are subcellar microorganisms. They are found in virtually all bacterial species; they are probably present in most individual bacterial cells[20]. In our opinion, there is no bacterium which has no plasmid; there is bacterium which has no plasmid; there is bacterium which has plasmid undetectable. Due to the development of new methods in the last 10 years[8~26,21], more and more plasmids

were found to present in the species that had no plasmids were detected before. Most of the new methods, however, seem most likely suitable for isolation plasmids presenting in cytoplasm. It is the most important thing that the attention was drawn to the induction of excision of chromosomally integrated plasmid[8,9]. Many substances can be used as inducing reagent. In our experiments, we used EB as plasmid-inducing reagent rather than plasmid-curing reagent. We postulate that the plasmids integrated can be eliminated by supplementing culture medium with reagents. The elimination must have been carried out via (1) plasmids are induced from the genome of host cells into cytoplasm; (2) plasmids are cured from the cytoplasm. Plasmid, therefore, is able to be detected if the first step goes slowly enough. Inuzuka once reported that the sex factor in Hfr strain was cured from the host genome by sodium dodecyl sulface (SDS) and he reasoned that before the final elimination of F-factor, there must have been a F period present[22]. However, he did not confirmed by experiment. Ultraviolet light (UV) could also be used as plasmid inducing reagent. Cohen and Paray had successfully performed induction method to induce the excision of chronosomally integrated plasmids from streptomyces and micromonospora respectively[8,9].

Since EB, SDS and UV are plasmid-curing reagents, it seems most likely that the plasmid-curing reagents can play a role of inducing plasmid if the condition control is suitable. In our work, inducible plasmid was not found if the supplement of EB was high beyond concentration of 1.0μg/mL. Low concentration probably functions in plasmid induction while the high concentration in plasmid elimination.

Antibiotics, which were also used to cure plasmid in some bacteria[23,24] have not been reported to be a plasmid-inducing reagent yet. However, the widespread use of a greater variety of antibiotics in the last 30 years has been accompanied by an increase in multiple drug-resistance most of which is plasmid-encoded[25-27]. The rapid increase in the proportion of resistant bacteria, in people who have been treated with antibiotics is due mainly to the selection of R bacteria. The view is often expressed that R plasmids have in some way been selected by clones of cells so that most of the clone can dispense with the plasmid when it is not needed. (That is, when there are no antibiotics in the environment.) When the selection pressure is reapplied, the few cells in the clone which still have a copy of the plasmid will be selected. Recently, this general view of R Plasmids as dispensable cell elements was denied by some authors because of being unreasonable and plasmid undue emphasis on cells rather than genes as the fundamental elements of evolution[28]. It seems to us that antibiotics probably have a function to induce chromosomally integrated plasmid looping out of host genome. We also assume that this induction function is nonspecific. If the fregnents induced happen to have a resistant gone, the drug resistance occurs. The view, however, lacks experimental support.

In summary, although the mechanism of induction free plasmid in the plasmid deficient bacteria is not known in detail, and we do not have already the glimmering of solution to obtaining plasmid from industrial bacteria, induction may be a general method to sreen valuable plasmid for academic and practical porpose.

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