View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Hemeroteca Científica Catalana

INTERNATL MICROBIOL (1999) 2:115–117
© Springer-Verlag Ibérica 1999

RESEARCH ARTICLE

María A. Ferrús[1] · Jose L. Alonso[2] · Inmaculada Amorós[2] · Manuel Hernández[1] · Javier Hernández[1]

[1]Department of Biotechnology, Polytechnic University of Valencia, Spain
[2]Hydrology and Environmental Institute, Polytechnic University of Valencia, Spain

A rapid procedure for the isolation of plasmid DNA from environmental bacteria

Received 21 December 1998
Accepted 15 April 1999

Correspondence to:
María A. Ferrús. Departamento de Biotecnología. Universidad Politécnica de Valencia. Camino de Vera, 14.
46022 Valencia. Spain.
Tel.: +34-963877423. Fax: +34-963877429.
E-mail: mferrus@btc.upv.es

Summary The INSTA-MINI-PREP™ method, a rapid protocol for plasmid DNA extraction, was originally developed to prepare plasmid DNA from 1 to 3 ml miniprep *Escherichia coli* cultures. Direct extraction of plasmid DNA is achieved by a two-phase solution which is separated by centrifugation in the presence of the INSTA-PREP gel barrier material. This method has been successfully tested on various environmental *Salmonella* strains, although it was not suitable for *Pseudomonas aeruginosa* and enterococci strains. The INSTA-MINI-PREP™ method is a new alternative procedure to screen plasmid contents of *Salmonella* and *E. coli* strains rapidly and easily.

Key words *Pseudomonas aeruginosa* · *Escherichia coli* · *Salmonella* · Plasmid-screening procedure · Plasmid extraction

Introduction

Although there are a number of plasmid extraction procedures which vary in subtle ways, they all have three main stages: lysis of bacterial cells, selective release of plasmid DNA from cell matrices, and removal of such contaminants to recover plasmid DNA [3]. Drawbacks of these procedures include numerous sample manipulations and a significant investment in time [5]. The INSTA-MINI-PREP™ kit (5 Prime-3 Prime Inc, Boulder, CO, USA) has been developed to minimize the time spent preparing miniprep plasmid DNA [7]. Briefly, direct extraction of plasmid DNA is achieved by a two-phase solution consisting of phenol–chloroform–isoamyl alcohol and buffer with separation of the phases by centrifugation in the presence of the INSTA-PREP gel barrier material. This method is considerably faster than the standard miniprep procedures, primarily due to the elimination of ethanol precipitation, washes and drying of DNA pellets [7].

In this article we evaluate the INSTA-MINI-PREP™ method for the isolation and purification of plasmid DNA from environmental bacterial strains.

Materials and methods

Bacterial strains A total of 61 bacterial strains isolated from fresh and marine polluted waters were used in this study. Twenty–eight *Pseudomonas aeruginosa* strains were isolated using Alonso et al. [1] method. Nine enterococci strains were isolated following the technique described by Amorós and Alonso (Proc. IAWQ Congress on Health-Related Water Microbiology, p 105, 1996). Twenty–four *Salmonella* strains were obtained from our stock culture collection. *Escherichia coli* NCTC 50913 was used to provide multiple standard reference plasmids and to develop the optimal conditions for the INSTA-MINI-PREP™ method.

Conventional plasmid DNA isolation methods The plasmid DNA isolation technique for *P. aeruginosa* and *Salmonella* strains was as described by Owen and Hernandez [6], and for enterococci strains as described by Anderson and McKay [2]. Plasmid preparations were stored at 4°C until required.

The INSTA-MINI-PREP™ method Pure strains of *E. coli*, *P. aeruginosa* and *Salmonella* spp. were grown on nutrient broth (Merck), and pure strains of enterococci were grown on Brain Heart Infusion (BHI, Merck), and incubated at 37°C for 24 h. A 4.2 ml (O.D.$_{660}$ = 1.5–1.7) of bacterial cultures was harvested by centrifugation at 14,000 rpm. The cell pellet was suspended in 50 µl of TE buffer (10 mM Tris hydrochloride, 1 mM EDTA, pH 8.0). For enterococci strains, the cell pellet was suspended in TE buffer containing lysozyme (10 mg/l) and then incubated at 37°C for 1 h. 300 µl of PCI reagent (phenol–chloroform–isoamyl alcohol) were added and mixed to release plasmid DNA. The cell–PCI–buffer mixture was then poured into a tube which

contained INSTA-PREP gel. Under centrifugal force, this gel migrates to form a tight barrier between organic and aqueous phases. The organic phase and the interface material are sequestered below the INSTA-MINI-PREP™ gel, and separated from the aqueous upper phase, which contains the isolated plasmid DNA. All the process is performed in two minutes [7]. As plasmid DNA samples contain RNA, 1 μl of 10x RNase PLUS Gel loading buffer (5 Prime-3 Prime Inc) was added to each 10 μl of sample, mixed and incubated for 5 min at 37°C prior to gel loading.

Plasmid DNA was analyzed by 0.7% agarose gel electrophoresis in TBE buffer pH 8.5. Lambda *Hin*dIII digest was used as molecular weight marker, and plasmids sizes were estimated using the computer program HOWBIG, based on the correlation between size and DNA migration distance [4].

Results and Discussion

The study involved two different stages. Initially, bacterial plasmid DNA content was estimated by using two different protocols, one by Owen and Hernandez [6] and the other by Anderson and McKay [2]. Although they have shown to be effective for plasmid DNA extraction, the process takes at least 1.5 h to accomplish in both cases.

Plasmid DNA was found in 37.5% (9/24) of the *Salmonella* isolates, 7.6% (2/26) of the *P. aeruginosa* isolates, and 66.6% (6/9) of the enterococci isolates. Molecular sizes of the plasmids ranged from 1.9 to >23 kb (Table 1).

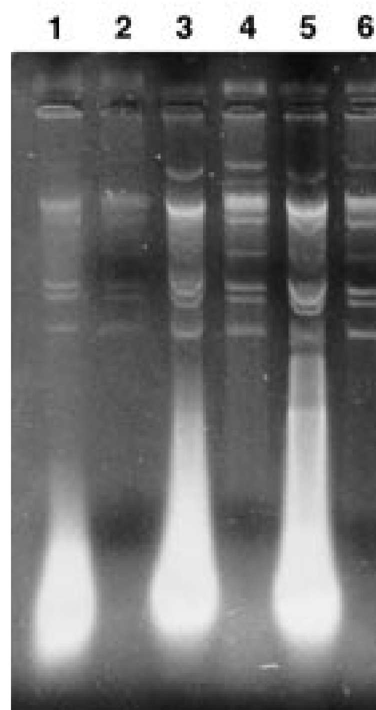

Fig. 1 Agarose gel electrophoresis of plasmid DNA from *Escherichia coli* NCTC 50913: effect of the quantity of bacterial culture, TE, buffer volume, and RNase treatment, respectively. Lanes: 1, 2.8 ml, 75 μl TE, without RNase; 2, 2.8 ml, 75 μl TE, with RNase; 3, 2.8 ml, 50 μl TE, without RNase; 4, 2.8 ml, 50 μl TE, with RNase; 5, 4.2 ml, 50 μl TE, without RNase; 6, 4.2 ml, 50 μl TE, with RNase

Table 1 Bacterial strains harboring plasmids

Strain		No. of plasmids	Estimated plasmid size (kb)
Salmonella	*brandenburg*	1	2.4
	bredeney	6	1.9, 2.3, 2.9, 3.4, 4.0, 5.3
	bredeney	2	1.9, 2.4
	derby	3	2.3, 3.7, 5.4
	derby	3	2.3, 3.7, 5.4
	derby	1	2.4
	derby	1	2.4
	typhimurium	2	2.4, 4.8
	virchow	3	2.3, 3.7, 5.4
Pseudomonas	*aeruginosa* ser O10	1	>23
	aeruginosa ser O16	1	>23
Enterococcus	*casseliflavus*	3	3.5, 6.2, 10.2
	casseliflavus	3	4.1, 5.4, 7.9
	durans	1	15.9
	durans	1	3.5
	faecalis	1	15.9
	faecium	1	15.9

Secondly, the INSTA-MINI-PREP™ kit was applied only to harboring plasmid strains. Direct extraction of plasmid DNA from bacterial cells is achieved by a two-phase solution, which are separated by centrifugation in the presence of the INSTA-PREP gel barrier material. The whole process takes only two minutes and plasmid DNA obtained is suitable for most molecular biology procedures [7].

For optimal results, the amount of cell material is critical (Fig. 1) and should give an O.D.$_{660}$ value of 1.5–1.7. Crude plasmids prepared by this method contain big amounts of RNA, which seems to interfere with the results, as some plasmid bands cannot be observed in gel electrophoresis. As shown in Fig. 1, RNA was eliminated by a RNase treatment. Problems were encountered with the INSTA-MINI-PREP™ method for the isolation of plasmid DNA from bacteria such as *P. aeruginosa* that synthesize large quantities of polysaccharides. Although the enterococci strains were incubated with lysozyme, it was difficult to lyse these Gram-positive bacteria.

The INSTA-MINI-PREP™ procedure was not suitable for *P. aeruginosa* and enterococci strains. This method, however, constitutes a new alternative procedure to screen rapidly (less than 2 min) and easily plasmid contents of *Salmonella* and *E. coli* strains.

References

1. Alonso JL, Garay E, Hernandez E (1989) Membrane filter procedure for enumeration of *Pseudomonas aeruginosa* in water. Water Res 23:1499–1502
2. Anderson DG, McKay LL (1983) Simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl Environ Microbiol 46:549–552
3. Fujita M, Ike M (1994) Wastewater Treatment Using Genetically Engineered Microorganisms. Basel: Technomic Publishing Company
4. Grabner M, Hofbauer R (1991) A computer program for molecular weight determination of DNA fragments (HOWBIG). CABIOS 7:317–319.
5. Grinsted J, Bennett PM (1988) Preparation and electrophoresis of plasmid DNA. In: Grinsted J, Bennett PM (eds) Methods in Microbiology. Vol 21. London: Academic Press, pp 129–142
6. Owen RJ, Hernandez J (1990) Occurrence of plasmids in *Campylobacter upsaliensis* (catalase negative or weak group) from geographically diverse patients with gastroenteritis or bacteraemia. Eur J Epidemiol 6:111–117
7. Tarczynski MC, Meyer WJ, Min JJ, Wood KA, Hellwig RJ (1994) Two minute miniprep method for plasmid DNA isolation. BioTechniques 16:514–519