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Detection of IncP replicon-specific regions in DNA from Antarctic microbiota

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Abstract: Plasmids capable of horizontal transfer contribute to the adaptability of bacteria, as they may provide genes that enable their hosts to cope with different selective pressures. Only limited information is available on plasmids from Antarctic habitats, and up until now surveys have only used traditional methods of endogenous plasmid isolation. The method based on primer systems, designed on the basis of published sequences for plasmids from different incompatibility (Inc) groups, is appropriate to detect the replicon-specific regions of corresponding plasmids in cultured bacteria, or in total community DNA, which share sufficient DNA similarity with reference plasmids at the amplified regions. In this study, we applied broad-host-range plasmid-specific primers to DNA from microbial samples collected at six different locations in Northern Victoria Land (Antarctica). DNA preparations were used as targets for PCR (polymerase chain reaction) amplification with primers for the IncP (*trfA2*) and IncQ (*oriV*) groups. PCR products were Southern blotted and hybridized with PCR-derived probes for *trfA2* and *oriV* regions. This approach detected the occurrence of IncP-specific sequences in eight out of fifteen DNA samples, suggesting a gene-mobilizing capacity within the original habitats.

Keywords: broad host range plasmids; IncP; Antarctica; polymerase chain reaction

1 Introduction

Mobile genetic elements (MGE), such as plasmids, promote the evolution and natural adaptation of microbial communities by facilitating the horizontal exchange of genes independently of homologous recombination. Their ability to transfer between bacteria has

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been demonstrated in various abiotic conditions in both simulated and natural environments [1]. Knowledge of the molecular identities and the diversity of plasmids from each specific environment is required in order to understand which ecologically advantageous traits they may disseminate. Despite intensive efforts to exploit various aspects of the vast biodiversity of microorganisms occurring in natural habitats, limited information is available pertaining to the incidence of plasmids on the Antarctic continent, which harbours a range of specialized and sometimes highly localized microbial biotopes imposed by one or more extreme environmental conditions [2].

The first study of plasmid distribution in natural Antarctic microbial assemblages was carried out by Kobori *et al.* [3]. A total of 155 psychrophilic and psychrotrophic bacteria were cultivated from samples collected in McMurdo Sound and investigated for the presence of plasmids. Thirty-one percent of the isolates carried at least one kind of plasmid, but these elements were not investigated further. The distribution of extrachromosomal elements in several collections of Antarctic bacteria was also analyzed by Tutino *et al.* [4] and among those strains were observed a prevalence of high molecular weight genetic elements and a few small, cryptic plasmids which were further isolated and characterized by setting up cloning/expression systems in cold-adapted bacteria [5]. More recently, aromatic hydrocarbon degrading *Pseudomonas* strains isolated from Antarctic soils have been shown to host large self-transmissible plasmids carrying naphthalene dioxygenase genes [6]. All the reported surveys have relied on the traditional method of plasmid isolation from a pure culture of the bacterial host.

The method based on primer systems, designed on the basis of published sequences for plasmids belonging to the incompatibility (Inc) groups, was useful for detecting the replicon-specific regions of corresponding plasmids in cultured bacteria or in total community DNA which share sufficient sequence similarity with reference plasmids at the amplified regions [7]. In this study, we applied the primer systems described for PCR amplification of broad-host-range (BHR) plasmids to DNA from microbial samples collected in coastal and inland ice-free areas of northern Victoria Land (Antarctica).

2 Experimental procedures

2.1 Sampling and culture conditions

Fifteen environmental samples were collected aseptically in February 2002 during the 17^{th} Italian expedition at six different locations (Table 1). Five samples were geothermally heated soils [8] and the remaining samples consisted of soils or salt sediments where the potentially associated microbial community was exposed to low temperatures and hypersalinity. Samples were stored at 4°C and studied upon their arrival in Italy. Samples of soil/sediment (0.5 g) were placed aseptically into a 250 ml flask containing 50 ml of DSMZ medium 259 (0.35% $Na_2HPO_4{\cdot}12H_2O$, 0.1% K_2HPO_4, 0.003% $MgSO_4{\cdot}7H_2O$, 0.05% NH_4Cl, 0.4% yeast extract, pH 6.5). Suspensions were incubated at 4°C, except for those from geothermal soils which were incubated at 55°C. At the appearance of

turbidity, aliquots were distributed into sterile vials and frozen at −20°C. *Escherichia coli* cells, when needed, were grown on Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl) at 37°C.

2.2 DNA extraction and PCR-based detection of broad host range plasmids

Two five-millilitre aliquots for each frozen sample were used for total DNA extraction, as described by Yakimov *et al.* [9]. The quality of DNA preparations was examined by agarose gel electrophoresis and concentrations were determined by use of a spectrophometer at 260 nm and 280 nm. Oligonucleotide primers 27F and 1387R were used to amplify 16S rDNA as described by Dang and Lovell [10]. This primer system targets the 16S rDNA of a wide range of members of the domain *Bacteria* at positions 8 through 25 (primer 27F) and positions 1387 through 1404 (primer 1387R) (*E. coli* 16S rRNA numbering) [11]. Identical reaction mixtures that contained no DNA were used as negative controls. Aliquots of extracted DNA were then used as templates with specific primers for amplifying two BHR plasmid backbone regions related to replication: IncP (*trfA2*) and IncQ (*oriV*) [7]. PCR was performed using the conditions described by Götz *et al.* [7]. Plasmid pPR9TT, a derivative of RK2 (IncP) [12], and RSF1010 (IncQ) [13] were included as positive controls by using heat-denatured cells of *E. coli* JM109 (pPR9TT) and *E. coli* C600 (RSF1010) as targets.

2.3 Southern blot analysis and sequence analysis

PCR products obtained with the reference plasmids, pPR9TT and RK2, were extracted from preparative agarose gel slices with Qiagen spin columns according to the instructions of the manufacturer (Qiagen, Hilden, Germany). The DNA fragments were labelled using the random-primed digoxigenin method according to the protocol of the manufacturer (Boehringer, Mannheim, Germany). Southern blotting was performed by the standard protocol described by Sambrook *et al.* [14], followed by hybridization and digoxigenin detection according to the directions of the manufacturer of the kit (Boehringer). Two PCR products derived from sampling stations 2 and 4 (Table 1) were cloned directly into a pGEM-T plasmid using the pGEM-T-Easy Vector System (Promega, Milano, Italy) following the instructions of the manufacturer. The DNA sequence of cloned amplicons was determined directly for each strand by using M13 forward and reverse primers in a CEQ 8000XL automated DNA Analysis System (Beckman Coulter Fullerton, CA, USA). Sequenced data were analyzed with sequencher 4.2.2 (GeneCodes, Ann Arbor, MI, USA) and deposited in the DDBJ/EMBL/GenBank nucleotide sequence database under accession numbers AM504052 and AM502289, respectively.

Sampling station	Latitude S	Longitude E	Sample (n)[a]
1. Mt. Rittman	73° 28'	165° 36'	geothermal heated soil (1)
2. Inexpressible Island	74° 53'	163° 43'	salt sediment from an eutrophic lagoon near a penguin rookery (1)
3. Mt. Melbourne	74° 20'	164° 39'	geothermal heated soil (4)
4. Edmonson Point	74° 20'	165° 08'	volcanic soil covered by salt efflorescences after evaporation of MS (2) and pond sediment covered by salt encrustations after evaporation of SW (3)
5. Point Stocchino	74° 41'	164° 07'	pond sediment covered by salt encrustations after evaporation of SW (1)
6. Cape Russell	74° 52'	163° 53'	granitoid soil (1) and pond sediment covered by salt encrustations after evaporation of MS (2)

[a] number of samples examined
MS, melting snow; SW, sea water

Table 1 Collection sites and sample characteristics.

3 Results and Discussion

For the present study, fifteen DNA preparations from primary cultures of samples (soil/sediment) collected from Northern Victoria Land (Antarctica) were examined in order to investigate the presence of highly promiscuous plasmids. All purified DNA templates were amenable to PCR amplification by using domain *Bacteria*-specific oligonucleotide primers, and amplicons of the expected size (1,360 bp) were generated [10]. Each DNA preparation was then tested with specific primers for BHR plasmids. Four out of fifteen produced PCR positive results with IncQ (*oriV*) and eight with IncP (*trfA2*) primer sets, respectively. To test whether these samples contained the region used for PCR amplification, southern-blotted PCR DNAs were hybridized with probes derived from plasmid RSF1010 (IncQ) and pPR9TT (IncP). No hybridization was observed when the IncQ *oriV* probe was used against all DNA samples, showing that direct PCR with IncQ primers had generated non-specific amplification of non-target genes. However, eight samples (soils and sediments), representative of all collecting stations, with the exception of the geothermal area (Table 1), yielded hybridizing PCR products of the expected size (about 240 bp) with an IncP *trfA2* probe (Figure 1).

DNA sequencing of two PCR products from sampling stations 2 (252 bp) and 4 (242 bp) (Table 1) were used to query DNA databases via the BLASTN Network Service [15] and were shown to share 98%-99% base identity with a sequence upstream of the N-terminal end of the *trfA* gene of IncP-1α plasmid pBS228 [16]. Interestingly, the occurrence of conjugation among moderately halophilic bacteria, which are the most abundant prokaryotes that inhabit hypersaline environments, was demonstrated by using the self-transmissible IncP-1α plasmid RK2 [17].

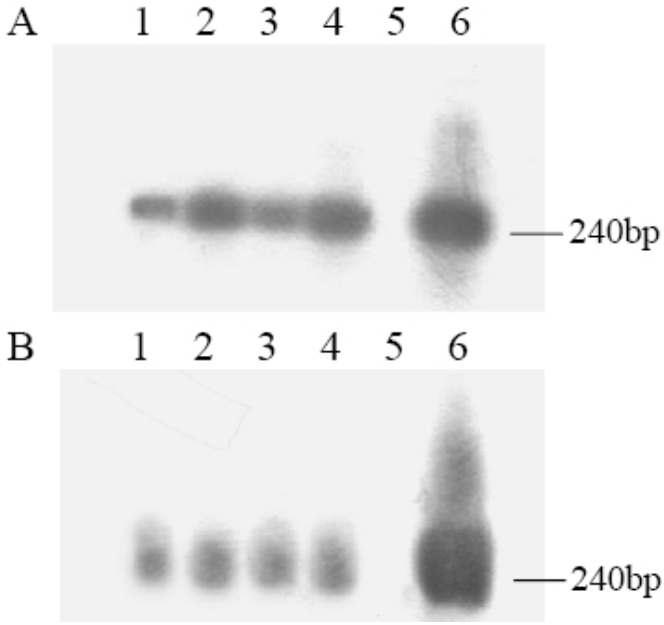

Fig. 1 Southern blot hybridizations of PCR products obtained after amplifications of DNA extracted from cultures of Antarctic samples with IncP *trfA2* primers. (A) Lanes 1-3, samples from station n. 4; lane 4, sample from station n. 2; lane 5, RSF1010 (negative control); lane 6, pPR9TT (positive control). (B) Lane 1, sample from station n. 5; lanes 2-4, samples from station n. 6; lane 5, RSF1010; lane 6, pPR9TT.

The application of replicon-specific primers to genomic DNA extracted from microbial cells collected after incubation of Antarctic soil and/or sediment in yeast extract mineral medium allowed us to assess the presence of IncP plasmid signatures in these samples and it indicated that horizontal gene transfer may occur in an environment that imposes thermodynamic and kinetic limitations on microbial growth. Plasmids classified in the IncP-1 (IncP in *E. coli*) incompatibility group, which has two major evolutionary branches (IncP-1α and IncP-1β), belong to the most stably maintained mobile elements among low copy number plasmids known to date [18]. They can replicate and be stably maintained in almost all Gram-negative bacteria and may be transferred by conjugation to Gram-positive bacteria, yeasts and eukaryotic cell lines [19, 20]. The results reported in this study confirm their transmission and raise interesting questions regarding the origin and evolution of plasmids under the extreme conditions of the South Polar Region.

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References

[1] P.A. Sobecky: "Approaches to investigating the ecology of plasmids in marine bacterial communities", *Plasmid*, Vol. 48, (2002), pp. 213–221.

[2] D.A. Cowan and L.A. Tow: "Endangered Antarctic environments", *Ann. Rev. Microbiol.*, Vol. 58, (2004), pp. 649–690.

[3] H. Kobori, C.W. Sullivan and H. Shizuya: "Bacterial plasmids in Antarctic natural microbial assemblages", *Appl. Environ. Microbiol.*, Vol. 48, (1984), pp. 515–518.

[4] M.L. Tutino, A. Duilio, B. Fontanella, M.A. Moretti, G. Sannia and G. Marino: "Plasmids from Antarctic bacteria", In: R. Margesin, F. Schinner (Eds.): *Cold-Adapted Organisms: Ecology, Physiology, Enzymology, and Molecular Biology*, Springer Verlag, 1999, pp. 335–348.

[5] A. Duilio, M.L. Tutino and G. Marino: "Recombinant protein production in Antarctic Gram-negative bacteria", *Methods Mol. Biol.*, Vol. 267, (2004), pp. 225–237.

[6] Y. Ma, L. Wang and Z. Shao: "Pseudomonas, the dominant polycyclic aromatic hydrocarbon-degrading bacteria isolated from Antarctic soils and the role of large plasmids in horizontal gene transfer", *Environ. Microbiol.*, Vol. 8, (2006), pp. 455–465.

[7] A. Götz, R. Pukall, E. Smit, E. Tietze, R. Prager, H. Tschäpe, J.D. Van Elsas and K. Smalla: "Detection and characterization of broad-host-range plasmids in environmental bacteria by PCR", *Appl. Environ. Microbiol.*, Vol. 62, (1996), pp. 2621–2628.

[8] R. Bargagli, M.L. Skotnicki, L. Marri, M. Pepi, A. Mackenzie and C. Agnorelli: "New record of moss and thermophilic bacteria species and physico-chemical properties of geothermal soils on the northwest slope of Mt. Melbourne (Antarctica)", *Polar. Biol.*, Vol. 27, (2004), pp. 423–431.

[9] M.M. Yakimov, G. Gentile, V. Bruni, S. Cappello, G. D'Auria, P.N. Golyshin, L. Giuliano: "Crude oil-induced structural shift of coastal bacterial communities of rod bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria", *FEMS Microbiol. Ecol.*, Vol. 49, (2004), pp. 419–432.

[10] H. Dang and C.R. Lovell: "Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes", *Appl. Envir. Microbiol.*, Vol. 66, (2000), pp. 467–475.

[11] J. Brosius, M.L. Palmer, P.J. Kennedy and H.F. Noller: "Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*", *Proc. Natl. Acad. Sci. USA*, Vol. 75, (1978), pp. 4801–4805.

[12] P.M. Santos, I. Di Bartolo, J.M. Blatny, E. Zennaro and S. Valla: "New broad-host-range promoter probe vectors based on the plasmid RK2 replicon", *FEMS Microbiol. Lett.*, Vol. 195, (2001), pp. 91–96.

[13] P. Scholz, V. Haring, B. Wittmann-Liebold, K. Ashman, M. Bagdasarian: "Complete nucleotide sequence and gene organization of the broad-host-range plasmid RSF1010", *Gene*, Vol. 75, (1989), pp. 271–288.

[14] J.E. Sambrook, E.F. Fritsch and T. Maniatis: *Molecular cloning: a laboratory manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.

[15] S.F. Altschul, W. Gish, W. Miller, E.W. Myers and D.J. Lipman: "Basic local alignment search tool", *J. Mol. Biol.*, Vol. 215, (1990), pp. 403–410.

[16] A.S. Haines, K. Jones, S.M. Batt, I.A. Kosheleva and C.M. Thomas: "Sequence of plasmid pBS228 and reconstruction of the IncP-1α phylogeny", *Plasmid*, doi:10.1016/j.plasmid.2007.01.001.

[17] C. Vargas, M.J. Coronado, A. Ventosa and J.J. Nieto: "Host range, stability and compatibility of broad host-range-plasmids and a shuttle vector in moderately halophilic bacteria. Evidence of intrageneric and intergeneric conjugation in moderate halophiles", *Syst. Appl. Microbiol.*, Vol. 20, (1997), pp. 173–181.

[18] M. Adamczyk and G. Jagura-Burdzy: "Spread and survival of promiscuous IncP-1 plasmids", *Acta Biochim. Pol.*, Vol. 50, (2003), pp. 425–453.

[19] J.A. Heinemann and G.F. Sprague: "Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast", *Nature*, Vol. 340, (1989), pp. 205–209.

[20] V.L. Waters: "Conjugation between bacterial and mammalian cells", *Nat Genet.*, Vol. 29, (2001), pp. 375–376.