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Tn*5045*, a novel integron-containing antibiotic and chromate resistance transposon isolated from a permafrost bacterium

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Abstract

A novel antibiotic and chromate resistance transposon, Tn*5045*, was isolated from a permafrost strain of *Pseudomonas* sp. Tn*5045* is a compound transposon composed of three distinct genetic elements. The backbone element is a Tn*1013*-like Tn*3* family transposon, termed Tn*1013**, that contains the *tnpA* and the *tnpR* genes, encoding the transposase and resolvase, respectively, the *res*-site and four genes (*orfA, B, C, D*) related to different house-keeping genes. The second element is class 1 integron, termed In*C**, which is inserted into the Tn*1013** *res*-region and contains 5′-CS–located integrase, 3′-CS–located *qacEΔ1* and sulfonamide resistance *sulI* genes, and a single cassette encoding the streptomycin resistance *aadA2*-gene. The third element is a Tn*OtChr*-like Tn*3* family transposon termed Tn*OtChr**, which is inserted into the transposition module of the integron and contains genes of chromate resistance (*chrB, A, C, F*). Tn*5045* is the first example of an ancient integron-containing mobile element and also the first characterized compound transposon coding for both antibiotic and chromate, resistance. Our data demonstrate that antibiotic and chromate resistance genes were distributed in environmental bacteria independently of human activities and provide important insights into the origin and evolution of antibiotic resistance integrons.

Keywords: Compound transposon; Integron; Tn*5045*; Tn*1013**; In*C**; Tn*OtChr**

1. Introduction

Many genetic elements of bacteria such as plasmids, transposons and integrons are believed to play a major role in horizontal transfer of antibiotic resistance genes both in the clinic and in the environment (Bennett, 2008; Harbottle et al., 2006; Summers, 2006; Walsh, 2006). Molecular genetics analysis of antibiotic resistance mechanisms has highlighted the key role of integrons, particularly class 1 integrons, in the acquisition of various resistance genes in clinical bacteria (Stokes and Hall, 1989; Partridge et al., 2001). These genetic elements encode a site-specific recombinase (integrase) capable of capturing resistance genes assembled in gene cassettes through their insertion into a specific *attI*-site under the control of a cassette P_c (P_{ant}) promoter. In class 1 integrons, the insertion site of gene cassettes is flanked by two conserved segments (CS), 5′-CS and 3′-CS, encoding the integrase (*intI1*) and the sulfonamide resistance gene (*sul1*), respectively (Stokes and Hall, 1989). While numerous examples of class 1 integron variants carrying various sets of gene cassettes have been found in clinical and environmental bacteria, detailed data on its origin and evolution are still fragmentary. Furthermore, little is known about the role that integrons and mobile elements have played in the spread of drug resistance determinants prior to the introduction of antibiotics into clinical practice.

Recent studies demonstrated that bacteria resistant to the most commonly prescribed antibiotics such as different lactams, aminoglycosides, tetracycline, sulfonamides and others are common in natural bacterial populations (Aminov, 2009; Cantón, 2009; D'Costa et al., 2007; Martinez, 2008). It was therefore proposed that the environmental bacterial communities may act as a natural reservoir of antibiotic resistance

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genes that are horizontally transferred into the clinical bacterial strains (Davies, 1997; D'Costa et al., 2007; Martínez, 2008; Riesenfield et al., 2004). At the same time, many questions remain about the role of natural environments in the maintenance and distribution of clinically relevant antibiotic resistance genes. Thus, it is of great interest to compare the molecular structure of antibiotic resistance transposons and other mobile elements distributed in the environment with those isolated from the clinic. At the same time, studies of modern bacteria isolated from environmental sources can lead to poorly interpretable data, since one cannot rule out the possibility that their antibiotic resistance determinants have, in fact, been acquired from clinical bacteria by horizontal gene transfer (Davies, 1997; Schlüter et al., 2007).

We approached the problem by isolating and studying antibiotic-resistant bacterial strains from permafrost sediments formed long before the discovery of antibiotics and their introduction into medicine and veterinary practice. This analysis identified a plethora of antibiotic-resistant permafrost strains belonging to various bacterial genera (Mindlin et al., 2008, 2009). In particular, we demonstrated that some permafrost strains contain clinically-relevant streptomycin resistance *strA-strB*-genes that are associated with transposons related to transposon Tn*5393*, which is widely distributed among present-day bacteria (Petrova et al., 2008). Furthermore, the remnants of this streptomycin-resistance transposon, together with tetracycline resistance genes, were also found in a pKLH80 plasmid of ancient *Psychrobacter psychrophilus* (*maritimus*) strain (Petrova et al., 2009). Overall, these data suggested that the currently known antibiotic resistance genes were widely distributed among ancient environmental bacteria.

In this study, we describe the molecular structure of a novel antibiotic resistance transposon which was discovered in a permafrost strain, *Pseudomonas* sp. Tik3. This streptomycin-resistant strain was initially isolated from a 15,000–40,000-year-old permafrost sample (Petrova et al., 2008) and was later shown to exhibit multidrug resistance (Mindlin et al., 2009). Here we demonstrate that the streptomycin/spectinomycin and sulfonamide resistance genes of *Pseudomonas* sp. Tik3 are encoded by a novel compound transposon, designated Tn*5045,* that also contains chromate resistance genes and is composed of three distinct elements, including two transposons and a class 1 integron, all of which are related to corresponding elements of modern bacteria. To our knowledge, Tn*5045* is the first example of an integron-containing antibiotic resistance transposon isolated from ancient bacteria.

2. Materials and methods

2.1. The Siberian permafrost sampling site

The sampling site used for isolation of permafrost bacteria was located near Tiksi, Coast of Laptev Sea. The procedures of sample collection and bacteria isolation were described previously (Petrova et al., 2008; Mindlin et al., 2009). The permafrost sample from which antibiotic-resistant strain *Pseudomonas* sp. Tik3 was isolated was dated to 15,000–40,000 years ago. Previously, *Pseudomonas putida* strain Tik1 carrying plasmid-borne streptomycin resistance genes *strA-strB* together with mercury resistance genes was isolated from the same sample (Petrova et al., 2008; Mindlin et al., 2009).

2.2. Bacterial strains, plasmids and growth conditions

Bacterial strains and plasmids used in this study are listed in Table 1. Unless otherwise stated, bacteria were grown in Luria-Bertani broth (LB) or on LB agar plates at 30 °C. The plates were supplemented with selective agents at the following concentrations (μg/mL): carbenicillin (Cb), 200; streptomycin (Sm), 50; tetracycline (Tc), 15; nalidixic acid (Nal), 20; rifampicin (Rif), 30, sulfathiazole (Su), 800. When sulfathiazole was used, agar plates were prepared from Adams minimal medium supplemented with casamino acids (0.4%) and tryptophan (30 μg/mL).

2.3. Translocation of the Tn5045 transposon onto target plasmids

To translocate Tn*5045* onto a broad-host-range plasmid pRP1.2 (Tc^R), a mating-out assay with modifications was used (Mindlin et al., 2001). Plasmid pRP1.2 was introduced into *Pseudomonas* sp. Tik3 cells by conjugation using *Escherichia coli* K-12 JF238 (pRP1.2) as a donor. After two serial transfers on selective medium (LB agar supplemented with tetracycline and streptomycin), *Pseudomonas* sp. Tik3 transconjugants were mated with *E. coli* JF238 (Nal^R). Sm^R Nal^R transconjugants, expected to carry pRP1.2 containing a transposable Sm^R determinant, were selected on LB plates supplemented with Sm and Nal and then checked for resistance to tetracycline. Genetic linkage between pRP1.2 and the streptomycin-resistance determinant was verified in crosses with *E. coli* K-12 C600 rif. The resulting plasmid containing Tn*5045* was named pRP1.45. To obtain a vector plasmid

Table 1
Bacterial strains and plasmids.

Strain or plasmid	Relevant characteristics	Source or reference
Bacterial strains		
Pseudomonas sp. Tik3	prototroph Sm^R Sp^R Su^R Chr^R	IMG collection
E.coli K-12 JF238	prototroph *recA*$^+$ Nal^R	IMG collection
E.coli K-12 C600 rif	*thr leu recA*$^+$ Rif^R	IMG collection
Plasmids		
pRP1.2	IncP Tc^R	IMG collection
RSF1010	IncC Sm^R Su^R	IMG collection
pGEM-7Zf(−)	pUC19 derivative, Ap^R	Promega Corp.
pRP1.45	pRP1.2::Tn*5045*	This work
pKLH45.1	pGEM-7Zf(−)::Tn*5045*	This work
pKLH45.2	pKLH45.1 with deletion of BamHI fragment (removes nucleotides from 1 (*IRl*) to 7785 (*orf5*))	This work
pKLH45.3	pKLH45.1 with deletion of PstI fragment (removes nucleotides from 135 (*tnpA1*) to 6600 (*sul1*)	This work