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Tn5045, 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, Tn5045, was isolated from a permafrost strain of *Pseudomonas* sp. Tn5045 is a compound transposon composed of three distinct genetic elements. The backbone element is a Tn1013-like Tn3 family transposon, termed Tn1013*, 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 housekeeping genes. The second element is class 1 integron, termed InC*, which is inserted into the Tn1013* 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 TnOtChr-like Tn3 family transposon termed TnOtChr*, which is inserted into the transposition module of the integron and contains genes of chromate resistance (*chrB, A, C, F*). Tn5045 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; Tn5045; Tn1013*; InC*; TnOtChr*
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 & 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 \textit{att}I-site under the control of a cassette P\textsubscript{c}\textsuperscript{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 (\textit{int}I1) and the sulfonamide resistance gene (\textit{sul}I), respectively (Stokes & 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 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 \textit{strA-strB}-genes that are associated with transposons related to transposon Tn5393, 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 \textit{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, \textit{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 \textit{Pseudomonas} sp. Tik3 are encoded by a novel compound transposon, designated
Tn\textsubscript{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\textsubscript{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*-str*B* 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 Tn5045 onto a broad-host-range plasmid pRP1.2 (TcR), 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 *E. 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 Tn5045 was named pRP1.45. To obtain a vector plasmid pGEM-7Zf(-) containing Tn5045, we used the conduction method (Mindlin et al., 2001). Briefly, recombinant plasmid pRP1.45 (pRP1.2::Tn5045) was introduced into JF238 cells carrying the vector plasmid pGEM-7Zf(-) by conjugation. After two serial transfers on selective medium containing Sm and Ap, the resulting cultures were mated with *E. coli* C600 rif strain and recombinants that inherited the pGEM-7Zf(-) marker Ap\(^R\) were selected. Plasmid DNA from these recombinants was isolated, used for transformation of JF238 cells, and clones with Sm\(^R\) Tc\(^S\) phenotype were selected among Ap\(^R\) transformants. The resulting plasmid was named pKLH45.1. Two derivative plasmids, pKLH45.2 and pKLH45.3, were obtained from pKLH45.1 by deletion of BamHI and PstI fragments, respectively (Table 1).

2.4. *Southern blot hybridization*

Plasmid DNA was extracted from *Pseudomonas* sp. Tik3 by the alkaline lysis method. Total Tik3 cellular DNA was isolated as described in (Sambrook et al., 1989). Plasmids pKLH45.1 and RSF1010 were used as templates for the synthesis of probes specific for *aadA2* and *strA-strB*-genes, respectively (Table 1).

2.5. *Chromium resistance assays*
Susceptibility of bacterial strains to chromium (VI) was determined by the agar dilution method (Hirai et al., 1986). Bacterial strains were grown in tryptone soya broth (CM129; Oxoid) at 30°C with shaking for 3 h, diluted 100-fold and plated on Adams minimal agar medium supplemented with casamino acids (0.4%) and containing K$_2$Cr$_2$O$_7$ at concentrations ranging from 1 to 10 µg/mL. Plates were incubated at 30°C for 24-30 h and visually inspected.

### 2.6. DNA methodology, sequencing and PCR assays

Basic DNA procedures, including restriction endonuclease digestions, ligation reactions, bacterial cell transformation and agarose gel electrophoresis were carried out as described in (Sambrook et al, 1989). The sequence of Tn5045 was determined from plasmids pKLH45.2 and pKLH45.3 (Table 1) and PCR products using Tn5045-specific primers. DNA sequencing was performed at the Interinstitute “GENOME” Center (Moscow, Russia). Nucleotide sequences were analyzed at the website of the National Center for Biotechnology Information via the Blast network service (Altshul et al, 1997) and using Vector NTI suite 9.0.0 (InforMaz, Inc., Frederick, MD). The sequence of Tn5045 has been deposited in the EMBL database under accession number FN821089.

### 3. Results

### 3.1. Initial characterization of Tn5045
Transposon Tn5045 encoding streptomycin/spectinomycin and sulfonamide resistance determinants was recovered from the permafrost strain Pseudomonas sp. Tik3 and transposed into the broad-host-range IncP plasmid pRP1.2 as described in Materials and methods. Preliminary data allowed us to conclude that this transposon differs from other antibiotic resistance transposons studied up to now in several features. In particular, it was revealed that Tn5045 contains an integron with a single cassette gene, aadA2 (M. Petrova, unpublished data, see also below). To analyze the complete Tn5045 structure, we transferred it into cloning vector pGEM-7Zf(-) in an Escherichia coli host strain, resulting in plasmid pKLH45.1, and constructed two derivative plasmids, pKLH45.2 and pKLH45.3, lacking the 5’-region of Tn5045 (Table 1, see Materials and methods for details). The complete Tn5045 sequence was determined from these plasmids using standard sequencing methods. Tn5045 was found to be a novel ~22 kb-long compound transposon consisting of three distinct modules (Fig 1): (i) a backbone transposon Tn1013* (7,791 bp), belonging to the Tn3 family; (ii) a novel class 1 integron InC* (6,983-bp) encoding antibiotic resistance determinants; and (iii) a variant of a recently discovered transposon TnOtChr (Branco et al., 2008), TnOtChr* (7,180-bp), conferring resistance to chromium (VI). In Tn5045, the TnOtChr* transposon is inserted in the InC* integron, which is itself inserted within the Tn1013* transposon (Fig 1).

Detailed analysis of the molecular structure of Tn5045, which is presented in the following sections, demonstrated that component genetic elements and the resistance genes of Tn5045 are closely related to those found in the present-day bacteria.

3.2. The structure of the Tn5045 backbone transposon

The backbone transposon of Tn5045 belongs to the Tn21 subgroup (Liebert et al., 1999) of the Tn3 family, contains 37-bp inverted repeat sequences (IRs) and is flanked by 5-bp
duplications of the target sequence (Fig. 1A). The closest relative of this transposon (with only
a single substitution over the whole transposon length), was found to be Tn1013 (GenBank
accession number AM261760; positions from 21669 to 29468) (Stokes et al., 2007) (Fig. 1B).
We therefore designated it Tn1013*. Another close relative of Tn1013* is a backbone
transposon revealed in the multiple antibiotic resistance transposon Tn1403 (GenBank
accession number AF313472) (Fig. 1C).

Tn1013* is separated into two parts as a result of insertion of a class 1 integron within its
res-region, between the resI and resII-sites. The left 3697-bp part of the Tn1013* transposon
contains IRI, the tnpA and tnpR genes, encoding the TnpA transposase and TnpR resolvase,
respectively, and most of the res region (Fig. 1A). The right 4094-bp part of Tn1013* contains
the remainder of the res region, orfA, orfB, orfC and orfD genes and IRR. The products of
orfA and orfC were previously identified as homologues of a sulfate permease and a DksA-
like protein, respectively (Schnabel and Jones, 1999). The OrfB protein is related to a series
of proteins annotated as universal stress proteins (UspA) (Stokes et al., 2007), while the
product of orfD is an unidentified protein (accession number AM261760). Both Tn1013* and
Tn1013 have a 78 bp deletion in orfC, as compared with the Tn1403 backbone transposon
(Fig. 1).

Close homologues of the right transposon part were found, apart from the Tn1403, in
partially sequenced Tn1404* (Stokes et al., 2007) and in plasmids pND6-1 and pDTG1
(GenBank accession numbers AY208917 and AF491307) which are identical to each other in
this region (not shown).

3.3. The structure of the class 1 integron found in Tn5045
The class 1 integron (6983-bp), inserted within the \textit{res}-region of Tn1013*, is related to the In2-In5 family of integrons (Brown et al., 1996). The integron insertion resulted in 5 bp duplications of the target sequence. The integron is bounded by 25-bp long IRs and contains two characteristic conserved segments, 5’-CS and 3’-CSs, and a single gene cassette with the streptomycin/spectinomycin resistance gene \textit{aadA2} inserted at the \textit{attI}-site (Fig. 2). Since this structure is similar to the structure of a hypothetical integron containing a single \textit{aadA2}-cassette, previously named InC (Bissonnette and Roy, 1992) (see Discussion), we designated it as InC*.

The similarity between the InC* integron and In2-In5-family integrons continues through all conserved integron elements, including 5’-CS, 3’-CS and the truncated \textit{tni} region, containing \textit{tniBΔ}1 and \textit{tni}A genes (Fig. 2). The 5’-CS of InC* includes the \textit{intI}1 gene encoding the integrase, the cassette integration site \textit{attI} and the P\textsubscript{c} promoter responsible for expression of the cassette gene (Stokes & Hall, 1989). 3’-CS, as defined by Stokes and Hall (1989), includes the \textit{sulI} sulfonamide resistance gene and two additional open reading frames, \textit{orf}5 and \textit{qacEΔ}1, the second of which was previously identified as a defective version of the quaternary ammonium compound resistance determinant \textit{qacE} (Brown et al., 1996).

In contrast to other members of the In2-In5 family of integrons that contain a \textit{aadA1} gene cassette and belong to the so-called \textit{aadA1} branch of multiresistance integrons (Bissonnette and Roy, 1992), InC* contains the \textit{aadA2} cassette and is therefore a member of the \textit{aadA2} branch (see Discussion). Furthermore, InC* lacks IS-elements IS1326 and IS1353 that are inserted (one within another) in the \textit{tniBΔ}1 gene in In2 but bears an insertion of a Tn3-family transposon within the same gene (Fig. 1 and Fig. 2). InC* and In2 also differ in the organization of the promoter regions responsible for cassette gene expression. Namely, InC* has a P\textsubscript{c} gene cassette promoter variant P\textsubscript{c}H1 and no P2-promoter, while In2 has a much
stronger promoter combination \( P_cW - P_2 \) (Fig. 2B) (GenBank accession number AF071413.3) (Jové et al., 2010).

3.4. The structure of the TnOtChr-like transposon inserted within the Tn5045 integron

The transposon inserted into the \( tniB \)-gene of the \( \text{InC}^* \) integron was identified as a close relative of a recently described Tn3 family chromate-resistance transposon TnOtChr, revealed in the chromosome of a chromate resistance strain of \textit{Ochrobactrum tritici} (Branco et al., 2008). We therefore named the transposon found in Tn5045 TnOtChr*. The TnOtChr and TnOtChr* transposons have lengths of 7,189 and 7,180 nucleotides, respectively, and contain two conserved transposition genes (\( tnpA2 \) and \( tnpR2 \), encoding for resolvase and transposase, respectively), which are divergently transcribed (Fig.1A), a feature typical of the Tn3 subgroup transposons (Branco et al., 2008). The 38-bp IRs of TnOtChr* and TnOtChr were found to be completely identical to each other. All four genes forming the chromate resistance operon (\( \text{chrB, A, C, F} \)) are located, in both cases, on the same DNA strand between the \( tnpR \) and \( tnpA \) genes (Fig.1A). Sequence analysis showed very high similarity of the chromate resistance operons present in the two transposons (Table 2). The only essential difference between the two sequences was a 9-bp deletion in the TnOtChr* \( \text{chrA} \)-gene. Additionally, we revealed another TnOtChr-like transposon with the same 9-bp deletion in a recently published genome of \textit{Comamonas testosteroni} (GenBank accession number GQ281704) (Rosewarne et al., 2010). However, the transposon found in \textit{Comamonas testosteroni} lacks the transposition gene \( tnpR \) (Table 2).

The strain of \textit{Ochrobactrum tritici} 5bv11 containing TnOtChr was found to be able to survive in the presence of high concentrations of chromate (up to 10 mM) (Branco et al., 2004). To measure the level of chromate resistance of the \textit{Pseudomonas} sp. Tik3 strain, we
determined minimal inhibitory concentrations (MIC) for K$_2$Cr$_2$O$_7$ (see Materials and methods for details). We found that *Pseudomonas* sp. Tik3 was able to grow only at much lower concentrations of K$_2$Cr$_2$O$_7$ (8-10 µg/ml, or ~30 µM, see supplementary data table). To test whether the chromate resistance operon found in TnOtChr* was functional in chromate-sensitive cells, we compared the resistance to chromium of the isogenic *E. coli* JF238 and its derivatives carrying whole or a portion of Tn5045 on a plasmid to that of the original *Pseudomonas* sp. Tik3 strain. The plasmids carrying the whole Tn5045 or its part with chrB, A, C, F-genes were pRP1.45 (a low copy plasmid), pKLH45.1 (a high copy plasmid) and its shortened derivatives pKLH45.2 and pKLH45.3 (Table 1). It was found that chromate sensitivity of *E. coli* cells bearing the pRP1.45 or pKLH45.1 plasmids was similar to that of cells lacking any plasmid. However, introduction of either of the two other plasmids (pKLH45.2 or pKLH45.3) lacking a 5'-part of Tn5045, into the *E. coli* JF238 cells resulted in two- to threefold higher levels of chromate resistance (see supplementary data table). Thus, the chromate resistance genes of TnOtChr* are not efficiently expressed in *E. coli* in the context of the Tn5045 transposon (at least, under conditions of our experiments), but are somewhat activated when a part of the transposon is deleted. Further studies are required to establish the mechanism of regulation of the chrBACF operon expression in Tn5045.

3.5. Tn5045 is located on the Pseudomonas sp. Tik3 chromosome

As was shown in our previous work, the *Pseudomonas* sp. Tik3 strain contains a medium-size plasmid carrying the streptomycin-resistance genes strA-strB (Mindlin et al., 2009). To determine the genetic location of Tn5045 that contains the determinant of streptomycin resistance of another type (aadA2), we performed Southern blot hybridization with a plasmid and total DNA from this strain (see Materials and methods). While the strA-strB
determinants were clearly detected in plasmid DNA, the Tn5045 aadA2 determinant was only detected from total Pseudomonas sp. Tik3 DNA in Southern blot hybridization (Fig 3). Thus, we concluded that Tn5045 has a chromosomal location.

4. Discussion

Tn5045 described in this work is the first integron-containing multiple antibiotic resistance compound transposon to be found in an ancient bacterial strain. Furthermore, class 1 integron InC* carried by Tn5045 provides the first example of an antibiotic resistance integron that originated in the “pre-antibiotic” era. According to a recently proposed model of integron evolution, the ancestor of the clinical class 1 integrons was likely a typical chromosomal integron which at some time became incorporated into a plasmid-borne Tn402-like transposon (Gillings et al., 2008). The selection of an antibiotic resistance integron ( integrons) was proposed to occur several dozen years ago after antibiotics began to be widely used in the clinic (Gillings et al., 2008). However, the finding of antibiotic resistance class 1 integron InC* in the permafrost bacterial strain suggests that integrons carrying antibiotic resistance cassettes first originated in environmental bacterial populations in the preantibiotic era, whereas the introduction of antibiotics stimulated rapid spread and structural diversification of clinical class 1 integrons. It should be noted that the chromosomal location of Tn5045 is unusual, since essentially all previously studied clinical antibiotic resistance integrons are carried on transposons and/or plasmids (Gillings et al., 2008). Most likely, plasmid location of such transposons facilitated their horizontal transfer among clinical bacteria under strong selective pressure exerted by the widespread use of antibiotics. The most frequent gene cassettes found in class 1 multiple antibiotic resistance integrons isolated from clinical bacteria are either aadA1 or aadA2 streptomycin/spectinomycin.
resistance gene cassettes. Depending on the gene cassette present, two different branches of
aadA-containing integrons were described, aadA1 and aadA2 (Brown et al., 1996; Liebert et
al., 1999; Riccio et al., 2005). Integron In2 that contains a single aadA1 gene cassette was
proposed to be an ancestor integron of the aadA1 family (Brown et al., 1996). On the
contrary, the integron corresponding to a hypothetical In2-like ancestor integron of the aadA2
family, termed InC and containing a single aadA2 cassette (Bissonnette & Roy, 1992), has not
yet been identified. The structure of the InC* integron found in Tn5045 is very similar to the
proposed structure of InC, but InC* lacks the IS1326/IS1353 insertion that is present in other
integrons of the aadA1 and aadA2 branches. Thus, one can speculate that InC* is a direct
descendant of the InC integron that lost the IS1326/IS1353 elements.

Organization of the promoter regions in InC* and In2 integrons is in agreement with the
proposed model of evolution of the In2-In5 integron family from a common ancestor, In0
(Bissonnette and Roy, 1992). Both InC* and In2 promoters likely evolved from a weak In0
promoter variant P_cW through: (i) a single mutation in the -10 element of P_cW, resulting in
generation of a stronger P_cH1-promoter variant in InC*; or (ii) an insertion of three G into
the promoter spacer sequence that generated a second strong functional promoter P2, in
addition to P_cW, in In2 (Fig. 2B) (Jové et al., 2010).

Sequence analysis of known class 1 integron-containing compound transposons encoding
for antibiotic resistance demonstrates that transposons belonging to the Tn3 family have
acquired integrons on at least nine independent occasions (Table 3). In most studied cases, an
integron was inserted into or close to the res sites of a simple backbone mercury resistance
transposon, in accordance with the known insertion specificity of Tn5053-like transposons
(Minakhina et al., 1999). The largest family of such compound transposons is presented by
Tn21-related transposons that originated from a simple Tn5060-like mercury resistance
transposon through insertion of the In2 integron, followed by insertions of additional
antibiotic resistance cassettes (Partridge et al., 2001; Kholodii et al., 2003). Transposons of the Tn21 subgroup are currently broadly distributed in clinical settings as well as in the environment (Toleman et al., 2003; Mindlin et al., 2005). In addition, there are at least five more examples of unrelated compound transposons that independently originated as a result of integron insertion into various mercury resistance transposons (Table 3).

In contrast to these transposons, Tn5045 originated from a backbone transposon (Tn1013*) that does not encode mercury resistance. Two other examples of mercury-sensitive integron-containing transposons include transposon Tn1412, containing a complex integron In32 inserted into a Tn3 subgroup transposon Tn5563 (Minakhina et al., 1999; Partridge et al., 2001) and transposon Tn1403 recovered from a clinical strain of P. aeruginosa (Table 3) (Stokes et al., 2007). Similarly to Tn5045, Tn1403 is based on a Tn1013-like backbone transposon belonging to the Tn21 subgroup, but contains integron In28 instead of InC* and a Tn5393c-like transposon instead of the TnOtChr-like transposon found in Tn5045 (Fig. 1C). Tn1013 may therefore serve as a platform for efficient compound transposon formation.

The apparent prevalence of mercury resistance transposons among integron-containing compound transposons isolated from modern clinical bacteria (Table 3) may be explained by an initial distribution of backbone mercury resistance transposons in the clinic that was stimulated by the use of mercury-containing compounds for cure and disinfection (Porter et al, 1982). Following the introduction of antibiotics, these transposons likely served as a base for formation of complex integron-containing multidrug resistance transposons. In contrast, none of the many mercury resistance transposons recovered from environmental permafrost samples was found to contain an integron (Mindlin et al., 2005).

The association of antibiotic resistance genes with determinants conferring resistance to various heavy metals, besides mercury compounds, has been observed in environmental bacteria (Baker-Austin et al., 2006). It was proposed that heavy metal pollution could provide
the selective pressure sufficient to maintain such gene clusters even in the absence of antibiotic selection (Baker-Austin et al., 2006; Rosewarne et al., 2010). Simultaneous presence of the chromate and antibiotic resistance genes in Tn5045 is indicative of the ancient origin of the gene clusters containing heavy metal and antibiotic resistance determinants and their co-selection.

The antibiotic resistance genes found in Tn5045 are closely related to those found in modern clinical bacteria. Similarly, streptomycin resistance transposons related to clinical transposon Tn5393 and a streptomycin/tetracycln resistance plasmid pKLH80 containing clinically relevant antibiotic resistance genes have been recently found in permafrost bacterial strains (Petrova et al., 2008, 2009). Furthermore, we revealed a plasmid carrying strA-strB genes (Mindlin et al., 2009) in the same permafrost strain Pseudomonas sp.Tik3 that carried Tn5045-encoded aadA2 gene in the chromosome. Although the reasons for having two different types of streptomycin resistance genes in the same bacterium remain unknown, strains harboring both the strA-strB and aadA determinants were also recently detected among streptomycin-resistant Escherichia coli isolated from domestic animals (Sunde and Norström, 2005).

In conclusion, our data strongly support the view that formation of complex genetic elements containing different types of mobile elements and various antibiotic resistance genes is a common event in environmental bacterial populations and occurred long time before the start of commercial use of antibiotics in humans and veterinary.

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References

Altschul, S.F., Madden, T.L., Scaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J.,

Microbiol. 11, 2970-2988.


Bennett, P.M., 2008. Plasmid encoded antibiotic resistance: acquisition and transfer of

Bissonnette, L., Roy, P.H., 1992. Characterization of In0 of Pseudomonas aeruginosa plasmid
pVS1, an ancestor of integrons of multiresistance plasmids and transposons of gram-negative

Branco, R., Alpoim, M.C., Morais, P.V., 2004. Ochrobactrum tritici strain 5bv11 -
characterization of a Cr(VI)-resistant and Cr(VI)-reducing strain. Can. J. Microbiol. 50, 697-
703.

chromate-inducible chrBACF operon from the transposable element TnOtChr confers
resistance to chromium(VI) and superoxide. J. Bacteriol. 190, 6996-7003.


Labbate, M., Chowdhury, P.R., Stokes, H.W., 2008. A class 1 integron present in a human commensal has a hybrid transposition module compared to Tn402: evidence of interaction with mobile DNA from natural environments. J. Bacteriol. 190, 5318-5327.


genes with plasmids and transposons in the present-day bacteria and in the bacterial strains from permafrost. Genetika. 44, 112-116. Russian.


Legends to figures

Fig. 1. Structure of Tn5045 and related transposons. A. The structure of Tn5045 (this work). Numbers below component elements of Tn5045 indicate positions of the first and last nucleotide of each element (according to GenBank accession number FN821089). B. The structure of Tn1013 (GenBank accession no. AM261760). C. The structure of Tn1403 (GenBank accession no. AF313472.2). The location and polarity of genes and ORFs are shown with open arrows; arrowheads indicate terminal inverted repeats (IR).

tnpA and tnpR are the transposition genes of transposons; orfA, orfB, orfC (orfC' – shortened variant of orfC), orfD are the structural genes of Tn1013-like transposons; chrB, chrA, chrC, chrF are the genes of the chromate-resistance operon of TnOtChr*. strA and strB are the streptomycin resistance genes of Tn5393c: intI1 is the integrase gene; qacEΔ1, sul1 and orf5 are genes from the 3'-CS region of integrons; tniA, tniB are the transposition genes found in integron InC*; aadA2 is the InC* cassette gene of streptomycin/spectinomycin resistance; blaP1, cmlA1, aadA1 are the cassette genes of carbenicillin, chloramphenicol and streptomycin/spectinomycin resistance of integron In28, respectively; IS6100 - IS element found in In28.

Fig. 2. Comparative genetic organization of integrons In2 and InC*. A. Structure of integrons. attI1 is the primary recombination site; attC is the 59-base element. Short vertical lines show the positions of IS1326, IS1353, and TnOtChr insertions into the corresponding recipient integrons. Other designations are the same as in Fig.1. B. Promoter variants found in In2 and InC*. Promoter P2 with 14-nt spacer is inactive (nucleotides are depicted in italic); promoter P2 with 17-nt spacer is active. Bases in InC* and In2 that differ from those in In0 are shown in bold.

Fig.3. Southern blot analysis of plasmid and total DNA from Pseudomonas sp. Tik3 A. Gel electrophoresis of NcoI-digested DNAs. B and C: Southern blot hybridization with radioactively labeled probes to aadA2 and strA-strB genes, respectively. 1 – total DNA from strain Tik3; 2 – plasmid DNA from Tik3; 3 – RP1.2::Tn5045 (isolated from E. coli); 4 – pGEM7::Tn5080 containing strA-strB genes [Petrova et al., 2009]; 5 – molecular weight marker (λ phage DNA digested by PstI).
Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. Tik3</td>
<td>prototroph Sm&lt;sup&gt;R&lt;/sup&gt; Sp&lt;sup&gt;R&lt;/sup&gt; Su&lt;sup&gt;R&lt;/sup&gt; Chr&lt;sup&gt;R&lt;/sup&gt;</td>
<td>IMG collection</td>
</tr>
<tr>
<td><em>E.coli</em> K-12 JF238</td>
<td>prototroph <em>recA&lt;sup&gt;+&lt;/sup&gt;</em> Nal&lt;sup&gt;R&lt;/sup&gt;</td>
<td>IMG collection</td>
</tr>
<tr>
<td><em>E.coli</em> K-12 C600 rif</td>
<td><em>thr leu recA&lt;sup&gt;+&lt;/sup&gt;</em> Rif&lt;sup&gt;R&lt;/sup&gt;</td>
<td>IMG collection</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
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<tr>
<td>pRP1.2</td>
<td>IncP Tc&lt;sup&gt;R&lt;/sup&gt;</td>
<td>IMG collection</td>
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<td>RSF1010</td>
<td>IncC Sm&lt;sup&gt;R&lt;/sup&gt; Su&lt;sup&gt;R&lt;/sup&gt;</td>
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<tr>
<td>pGEM-7Zf(-)</td>
<td>pUC19 derivative, Ap&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Promega Corp.</td>
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<td>pRP1.45</td>
<td>pRP1.2::Tn5045</td>
<td>This work</td>
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<td>pKLH45.1</td>
<td>pGEM-7Zf(-)::Tn5045</td>
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<td>pKLH45.1 with deletion of BamHI fragment (removes nucleotides from 1 (<em>IRl</em>) to 7785 (<em>orf5</em>))</td>
<td>This work</td>
</tr>
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<td>pKLH45.3</td>
<td>pKLH45.1 with deletion of PstI fragment (removes nucleotides from 135 (<em>mpAI</em>) to 6600 (<em>sulI</em>))</td>
<td>This work</td>
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Table 2. Comparative analysis of transposon TnOtChr* from Tn5045 and its closest relatives

<table>
<thead>
<tr>
<th>Gene(region)</th>
<th>Coordinates&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Identity at the nucleotide sequence level (bp / %) TnOtChr*/TnOtChr</th>
<th>TnOtChr*/Tn from C. testosteroni</th>
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<tbody>
<tr>
<td>tnpA+IRr</td>
<td>1-3000</td>
<td>2987 / 99.6</td>
<td>2998 / 99.9</td>
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<tr>
<td>chrF+adjacent region</td>
<td>3001-3481</td>
<td>477 / 99.2</td>
<td>480 / 99.8</td>
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<td>ChrC</td>
<td>3482-4088</td>
<td>607 / 100</td>
<td>607 / 100</td>
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<tr>
<td>chrA+adjacent region</td>
<td>4089-5460</td>
<td>1369 / 99.8</td>
<td>1370 / 99.8</td>
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<tr>
<td>(4089-5451)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td>chrB+adjacent region</td>
<td>5461-6569</td>
<td>1102 / 99.4</td>
<td>1109 / 100</td>
</tr>
<tr>
<td>tnpR+IRl</td>
<td>6570-7180</td>
<td>607 / 99.5</td>
<td>- / -&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup> Coordinates of nucleotide regions according to the sequence of TnOtChr (EF469735)

<sup>b</sup> 9 Bp deletion in chrA in TnOtChr*

<sup>c</sup> Gene tnpR is absent
<table>
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<tr>
<th>Composite transposon</th>
<th>Size, (bp)</th>
<th>Backbone transposon</th>
<th>Class I integron</th>
<th>Cassette genes</th>
<th>Location of integron</th>
<th>Reference</th>
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<tr>
<td><strong>Mercury-resistance transposons</strong></td>
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<tr>
<td>Tn21</td>
<td>19000</td>
<td>Tn5060-like</td>
<td>In2</td>
<td>aadA1</td>
<td>&gt;350 bp from res region</td>
<td>Liebert et al., 1999</td>
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<tr>
<td>Tn1696</td>
<td>13600</td>
<td>Tn5036-like</td>
<td>In4</td>
<td>aacC1-orfE- aadA2-cmlA1</td>
<td>between resII and resI sites</td>
<td>Partridge et al., 2001</td>
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<tr>
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<td>Tn5036-like</td>
<td>In-t8</td>
<td>oxa30-aadA1</td>
<td>between resII and resI sites, 9 bp from the point of insertion in Tn1696</td>
<td>Villa &amp; Carattoli, 2005</td>
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<tr>
<td>Tn6005</td>
<td>nd</td>
<td>Tn5036-like</td>
<td>In(Tn6006)</td>
<td>a gene cassette with an unknown function</td>
<td>between resII and resI sites, 6 bp from the point of insertion in Tn1696</td>
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<tr>
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<td>unnamed</td>
<td>blaimp13-aacA4</td>
<td>ResI site</td>
<td>Toleman et al., 2003</td>
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<td><strong>Other transposons</strong></td>
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<td>Tn1412</td>
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<td>Tn5563</td>
<td>In32</td>
<td>blaps-1- aphA1a</td>
<td>Res region</td>
<td>Minakhina et al., 1999; Partridge et al., 2001</td>
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<td>19630</td>
<td>Tn1013-like</td>
<td>In28</td>
<td>blaP1-cmlA1-aadA1</td>
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<td>Stokes et al., 2007</td>
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<td>Inc*</td>
<td>aadA2</td>
<td>between resII and resI sites</td>
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3 Nd: not determined
4
**Figures**

**A. Tn5045**

![Diagram of Tn5045]

**B. Tn1013**

![Diagram of Tn1013]

**C. Tn1403**

![Diagram of Tn1403]

---

**Figure 1**
Figure 2
### Supplementary data table

Determination of MIC for chromate resistance of *E.coli* JF238 carrying different plasmids

<table>
<thead>
<tr>
<th>Strain designation</th>
<th>Plasmids with Tn5045 and its derivatives</th>
<th>MIC (mg/kg/ml) of K₂Cr₂O₇</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
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<tbody>
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<td>1</td>
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<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>JF238 (pGEM-7Zf(-))</td>
<td>-</td>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
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<td>JF238 (pKLH45.1)</td>
<td>pGEM-7Zf(-)::Tn5045</td>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>JF238 (pKLH45.2)</td>
<td>pKLH45.1 with BamH1 deletion</td>
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<td>6</td>
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<td>JF238 (pKLH45.3)</td>
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</table>

*Note:* The table represents the MIC values of K₂Cr₂O₇ for various strains and plasmids, indicating the resistance of each strain to chromate.