A Tn5051-like mer-containing transposon identified in a heavy metal tolerant strain Achromobacter sp. AO22

Shee Ping Ng¹, Belinda Davis², Enzo A Palombo¹ and Mrinal Bhave*¹

Address: ¹Environment and Biotechnology Centre, Faculty of Life and Social Sciences, Swinburne University of Technology, PO Box 218, Melbourne, Victoria 3122, Australia and ²School of Molecular Sciences, Victoria University, PO Box 14428, Melbourne, Victoria 8001, Australia

Email: Shee Ping Ng - shng@swin.edu.au; Belinda Davis - belinda.davis@ap.csplc.com; Enzo A Palombo - epalombo@swin.edu.au; Mrinal Bhave* - mbhave@swin.edu.au

* Corresponding author

Abstract

Background: Achromobacter sp. AO22 (formerly Alcaligenes sp. AO22), a bacterial strain isolated from a lead-contaminated industrial site in Australia, was previously found to be resistant to moderate to high levels of mercury, copper and other heavy metals. However, the nature and location of the genetic basis for mercuric ion resistance in this strain, had not been previously identified.

Findings: Achromobacter sp. AO22 contains a functional mer operon with all four essential genes (merRTPA) and shows >99% DNA sequence identity to that of Tn501. The mer operon was present on a transposon, designated TnAO22, captured by introducing a broad-host-range IncP plasmid into Achromobacter sp. AO22 and subsequently transferring it to E. coli recipients. The transposition frequency of TnAO22 was 10⁻² to 10⁻³ per target plasmid transferred. Analysis of TnAO22 sequence revealed it belonged to the Tn21 subgroup of the Tn3 superfamily of transposons, with the transposition module having >99% identity with Tn5051 of a Pseudomonas putida strain isolated from a water sample in New York.

Conclusion: TnAO22 is thus a new variant of Tn5051 of the Tn3 superfamily and the transposon and its associated mercury resistance system are among the few such systems reported in a soil bacterium. Achromobacter sp. AO22 can thus be exploited for applications such as in situ mercury bioremediation of contaminated sites, or the mobile unit and mer operon could be mobilized to other bacteria for similar purposes.

Findings

Mercury-resistance encoding mer operons have been reported from many bacterial species isolated from diverse environments including pristine soils, ancient permafrost samples, mercury ores as well as contaminated soil or water samples and enterobacteria [1-6]. These are commonly located on mobile genetic elements such as plasmids, transposons or modules of recombinant structures, although some reside chromosomally [6,7]. mer transposons frequently belong to the Tn3 family where the members are typically flanked by 38 bp inverted repeats (IRs) and contain two genes, trpR and trpA, encoding the enzymes resolvase and transposase, respectively, and a resolution (res) site at which site-specific recombination occurs to resolve the cointegrates formed during transposition [8]. Two archetypal transposons of
this family, Tn21, isolated from plasmid pNR1 from a clinical strain of *Shigella flexneri* from Japan [9], and Tn501, from pVS1 from a *Pseudomonas aeruginosa* isolate from Australia [10], have provided in-depth information on the transposition modules as well as functions of individual *mer* genes and regulation of the operon (reviewed in [7,11]). Many of the *mer*-transposons are closely related and share characteristics of the Tn21 subgroup of Tn3, with genes arranged as *res-tnpR-tnpA*, *tnpR* and *tnpA* separated by only 2 or 3 bp and transcribed in the same direction, away from *res* [12].

A number of variations on the genetic organisation of *mer* operons from Gram negative bacteria have been reported, but most contain the essential genes *merRTPA* with optional accessory genes (*merB, C, D, E, F, G*) but most contain the essential genes

operons from Gram negative bacteria have been reported, A number of variations on the genetic organisation of

to elemental Hg(0) in the cytoplasm which is released

transport system across the cell membrane and

mercury. This work reports the presence and characteriza-

tion, away from

res

entry of 5659-H is 16S rDNA of *A. xylosoxidans* subsp.

*XYLOSOXIDANS*, as pointed out by Wellinghausen et al. [18].
The AO22 sequence was then aligned with all other type

strains of *Achromobacter* spp. and *Achromobacter faecalis* subsp.

*faecalis*, *Bordetella bronchiseptica* and *Cupriavidus necator*

were included for comparison. From the phylogenetic tree

(Additional file 1: Fig. S1), AO22 appears to be most closely related (99.7% identity) to *Achromobacter spanius* and is henceforth designated as *Achromobacter* sp. AO22. As 16S rDNAs of several *Achromobacter* species are >97%

identical, DNA-DNA hybridization may be required to

further test the relatedness.

**Identification and isolation of TnAO22**

*Achromobacter* sp. AO22 was found to carry certain *mer*

gene sequences which were more than 90% similar to those in Tn501[16]. In order to test whether an active transposon was present in this strain, an approach described by Mindlin et al. [19] was used to mobilize it. This involved a two-step conjugation: (i) introduction of a broad-host-range plasmid from an *E. coli* host to AO22; (ii) determining transposition of the mercury transposon (if present) by mating of AO22 containing this plasmid with an *E. coli* recipient and selecting for transconjugants with linkage of the plasmid marker to mercury resistance. In the first step, a tetracycline (Tc) resistant broad-host-

range IncP plasmid pVS520 [20] (Additional file 1: Table S1) was introduced into AO22 by conjugation performed by the spot mating method [21] with modifications. The donor *E. coli* LT104 (pVS520) and recipient (AO22) cultures were grown overnight at 37°C and 30°C respectively in Luria Bertani (LB) broth, with Tc (10 μg ml⁻¹) and Hg (HgCl₂: 0.005 mM) as respective selections. The cultures were diluted 1:100 in fresh LB broth and incubated for a further 5 h with shaking. The donor and recipient cultures were then mixed 1:5, 10 μL aliquots of the mixture spotted on LB agar without selection and incubated for 16–18 h at 37°C. The mixed growth was scraped off the plate, resuspended in 0.85% saline, the suspension serially diluted 10-fold with 0.85% NaCl and 20 μL of each dilution spotted on selective LB agar plates to determine the number of colonies of donor (Tc⁺), recipient (Hg⁺) and transconjugants (Tc⁺Hg⁺), respectively. The conjugation experiment was repeated three times. The transfer frequency of pVS520 was expressed as the number of transconjugants, i.e., AO22 (pVS520) colonies per donor cell, and found to be an average of 1.26 × 10⁴ (SD 7.5 × 10³) from the three independent experiments. The Hg/Tc⁺ transconjugant strain, designated AO22 (pVS520), grew better at 37°C and the plasmid in it remained stable after several transfers on selective media. For the transposition experiment, AO22 (pVS520) was subcultured on LB agar containing Hg and Tc daily for three days to ensure maintenance of pVS520, then mated as above with spontaneous rifampicin-resistant mutants of *E. coli* JIR7062 [22].

Strain AO22 is identified as *Achromobacter sp. AO22*

The strain AO22 had been previously identified as *Alcali-

genus sp. based solely on metabolic tests [16]. In order to

confirm the identity of strain AO22, sequencing of its 16S

ribosomal RNA gene was carried out. Amplification of genomic DNA of AO22 with primers F1 and R2 based on 16S rDNA of *E. coli* [17] led to a 1,500 bp PCR product. DNA sequencing and blastn analysis indicated that this 1463 bp sequence (GenBank number EU696789) exhibited >99% identity to the corresponding regions of 16S rDNAs of *Achromobacter faecalis*, *Achromobacter xylosoxidans* and other *Alcaligenes* spp. and 100% identity with that of *A. faecalis* strain 5659-H (AE509012). A phylogenetic tree based on the alignment of 16S rDNA of AO22 with that of select type strains of *Achromobacter* spp., *Alcaligenes* sp. and several other β-Proteobacteria indicated AO22 and 5659-H belong to the cluster of *Achromobacter* spp. which is relatively distant from *Alcaligenes* spp. (results not shown). Alignment of the AO22 16S sequence with the Ribosomal Database Project [http://rdp.cme.msu.edu/index.jsp](http://rdp.cme.msu.edu/index.jsp) analysis tool also assigned it to the genus *Achromobacter* with 100% confidence. Indeed, the GenBank entry of 5659-H is 16S rDNA of *A. xylosoxidans* subsp.

*XYLOSOXIDANS*, as pointed out by Wellinghausen et al. [18].
The AO22 sequence was then aligned with all other type

strains of *Achromobacter* spp. and *Achromobacter faecalis* subsp.

*faecalis*, *Bordetella bronchiseptica* and *Cupriavidus necator* were included for comparison. From the phylogenetic tree (Additional file 1: Fig. S1), AO22 appears to be most closely related (99.7% identity) to *Achromobacter spanius* and is henceforth designated as *Achromobacter* sp. AO22. As 16S rDNAs of several *Achromobacter* species are >97%

identical, DNA-DNA hybridization may be required to

further test the relatedness.
Cloning and sequencing of TnAO22 reveal it has all functionally important features and belongs to the Tn21 subgroup

A 6.7 kb PstI-NcoI fragment of pVS520::TnAO22 was cloned into pGEM®-T Easy vector (Promega Australia) for sequencing purposes. This fragment was sequenced initially using the vector-based primers 17 (5’-GTAATACGACTCAGGGCC-3’) and SP6 (5’-TT TAG GTG ACACA GAATC-3’). As data was generated, a further section of TnAO22 was amplified using pVS520::TnAO22 as template and the primers AO22-F (5’-GACGAATACGACTCAGGGCC-3’) designed 70 bp upstream of the Ncol site and VS520-R (5’-GGCGCGCTGTGGGAAGGCG-3’) designed 100 bp into sequence of pVS520. PCR products were purified and sequenced as above, using the primers used for PCR and additional primers designed based on the emerging sequence data. DNA sequences were assembled and analyzed using the Bioedit Alignment Editor v.7.0.9 [http://www.ncbi.nlm.nih.gov/BLAST](http://www.ncbi.nlm.nih.gov/BLAST). The most closely related sequences were found using the Basic Local Alignment Search Tool (BLAST) program [http://www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov) multiple alignments were performed with CLUSTALW [http://www.ebi.ac.uk/Tools/clustalw2/index.html](http://www.ebi.ac.uk/Tools/clustalw2/index.html) and phylogenetic and evolutionary analyses conducted using MEGA version 4 [http://www.megasoftware.net/](http://www.megasoftware.net/). The sequence data indicated that the 6.7 kb PstI-NcoI fragment of pVS520::TnAO22 contained a 1.1 kb section of pVS520, followed by one end of the putative transposon, a putative mer operon, a tnpR gene, and part of tnpA (Fig. 1A). A primer designed approximately 70 bp upstream of the Ncol site using these data, in combination with a primer designed approximately 100 bp into pVS520, gave a 2.8 kb PCR product from pVS520::TnAO22 templates isolated from E. coli cells. The sequences of the 6.7 kb PstI-NcoI fragment and this PCR product were assembled and showed TnAO22 had a length of 8230 bp (GenBank number EU696790). It was inserted 173 bp downstream of the truncated Tn1 in pVS520 (in pVS520::TnAO22), equivalent to position 10614 of RP1 (BN000925), and had resulted in 5 bp duplications (TCTAT) of target sequence in the flanking region of pVS520 (data not shown), the latter being a characteristic of Tn3 family [12]. TnAO22 was bounded by 38 bp imperfect IRs differing by only 1 bp (Fig. 2), the IR adjacent to mer operon being identical to that of Tn21 at the tnpA end. The IRs were highly similar to those of the ancestral Tn501 except its EcoRI sites and contained conserved sequences recognized by the Tn21 transposase [12]. TnAO22 insertion site in pVS520 (equivalent to a region between Tn1 and oriV in RP1 or its derivatives) appears to be a hot spot for insertions, as reported for several Tn5041-type elements [24]. Nine ORFs were identified within TnAO22, the first seven closest to the IR from insertion point containing sequences homologous to the mer operon, including a merR that terminated within the adjacent IR and merTPADEurf2 transcribed divergently, and the two other ORFs being similar to tnpR and tnpA genes and separated from the mer ORFs by a 131 bp sequence similar to the res site (Fig. 1A).

The DNA sequence of the res-tnpR region of TnAO22 had highest identity (>99%) to this region of Tn5051 isolated from a P. putida strain in a water sample in New York [19] (Table 1). For optimum alignment with other related transposons, gaps needed to be introduced, the most notable one being a 45 bp gap between the 3’ end of res and start codon of tnpR of TnAO22 compared to Tn501 and other sequences (Fig. 1B). This extra sequence in Tn501 is suggested to be the remainder of a transposon belonging to the Tn5041/κ branch of Tn3 [23]. The putative 186 amino acid TnpA of TnAO22, when aligned with its closest relatives, revealed only one difference with Tn5051 (V48L) and conservation of the invariant serine and the helix-turn-helix DNA-binding motif (Fig. 1C).

The DNA sequence identities of TnAO22 tnpA compared to its close relatives varied between 70.0% and 99.2% (Table 1). The start codon of the putative TnpA was 2 bp after termination of TnpR (data not shown), compared to 3 bp in Tn501, and it terminated within an IR. The putative TnpA is 988 amino acids long and differs from the 459 residues available for TnpA of Tn5051 at 5 positions. Alignment of the amino acid sequence of TnAO22 TnpA with 13 selected Tn3 transposases (Additional file 1 Fig. S2) and the dendrogram (Fig. 3) confirmed that TnAO22 was closest to the Tn21 subgroup of Gram negative trans-
Figure 1
Genetic organisation of TnAO22. (A) Organisation of the mer operon and tnp genes. Select restriction sites are shown (E: EcoRI; N: NcoI; P: PstI). IR: inverted repeats. The solid line between the PstI site and left IR indicates a 1.1 kb section of pSV520 included in the 6.7 kb PstI-NcoI fragment of pVS520::TnAO22 cloned into pGEM-T Easy. (B) Comparison of the res sites: Tn501 from P. aeruginosa pVS1 (Z00027), Tn21 from S. flexneri R100 (NC_002134), Tn4378 from C. metallidurans CH34 pMOL28 (NC_006525) and Tn5051 from Pseudomonas sp. (Y17719). Dots indicate nucleotides identical to those of TnAO22; dashes indicate gaps introduced to optimise identity. (C) Comparison of the putative amino acid sequences of resolvase of TnAO22 with those of Tn501 (CAAA77327), Tn4378 (ABF13038) and Tn21 from S. flexneri (NP_052901) and Tn5051 (CAC14696). Arrow head indicates the presumptive serine involved in recombination. The shaded region indicates the conserved helix-turn-helix motif of resolvases.
posons, separated from Tn3 and the cluster of transposons in Gram positive bacteria.

**TnAO22 houses a mer operon that is very similar to that on Tn501**

The mer operon of TnAO22 had the classical structure mer-RTPADE, with >99% identity to Tn501 at DNA level including the length and sequences of intergenic spacers, and lacked the merC gene noted in Tn21 [11]. As in Tn501, the putative operator/promoter regions and transcription start sites of merR and merTPAD were divergent and the potential binding site of the regulator MerR occurred between the start codons of MerR and MerT. The putative mercuric reductase MerA of TnAO22 was 561 amino acids long, and comparison of its putative N-terminal and C-terminal sequences to MerA of other organisms (Additional file 1 Fig. S3) showed the two conserved pairs of cysteines considered responsible for binding and catalytic reduction of Hg(II) to Hg(0). Downstream of merD were sequences similar to orf1 and orf2 of Tn501. The putative protein encoded by orf1 was homologous to the 78 amino acid protein now known as MerE and suggested to have a role in Hg(II) transport similar to MerT [11], while orf2 encodes a 329 amino acid homologue of the diguanylate phosphodiesterases with the conserved EAL domain thought to be involved in prokaryotic signal transduction pathways [25].

With some exceptions, many mercury resistance determinants are located on plasmids. However, no plasmid could be isolated from AO22 despite numerous attempts using various volumes of cell culture and alternative protocols, e.g., standard alkaline lysis, the method of Kado and Liu [26] and two commercial kits. This, however, does not rule out the possibility of a megaplasmid in AO22 on which TnAO22 is located, as it is often difficult to detect

**Table 1: DNA sequence identity between TnAO22 and its closest relatives.**

| Genome region | TnAO22 | Tn501 | Tn21 | Tn4378 | Tn5051 |
|---------------|--------|-------|------|--------|--------|
| TnAO22        |        |       |      |        |        |
| **mer**       | 100    | 99.8  | 73.4 | 99.8   | na b   |
| **res-tnpR**  | 100    | 73.1  | 79.6 | 81.3   | 99.5   |
| **tnpA**      | 100    | 70.0  | 90.0 | 92.3   | 99.2   |
| Tn501         |        |       |      |        |        |
| **mer**       | 100    | 100   | 100  | 67.5   | 75.2   |
| **res-tnpR**  | 100    | 100   | 100  | 68.9   | 69.6   |
| **tnpA**      | 100    | 100   | 100  | 68.9   | 69.6   |
| Tn21          |        |       |      |        |        |
| **mer**       | 100    | 100   | 73.4 | na b   | 72.9   |
| **res-tnpR**  | 100    | 100   | 74.3 | na b   | 79.4   |
| **tnpA**      | 100    | 100   | 90.5 | na b   | 89.9   |
| Tn4378        |        |       |      |        |        |
| **mer**       | 100    | 100   | 73.4 | na b   | 81.1   |
| **res-tnpR**  | 100    | 100   | 81.1 | na b   | 91.9   |
| **tnpA**      | 100    | 100   | 90.5 | na b   | 89.9   |
| Tn5051        |        |       |      |        |        |
| **mer**       | 100    | 100   | 100  | na b   | 100    |
| **res-tnpR**  | 100    | 100   | 100  | na b   | 100    |
| **tnpA**      | 100    | 100   | 100  | na b   | 100    |

*mer includes merRTPAD and merE; % identity with Tn5051 was not calculated for this region, as only 339 bp sequence data is available for Tn5051. Accession numbers: Tn501 from Pseudomonas aeruginosa plasmid pVS1 (Z00027), Tn21 from Shigella flexneri plasmid R100 (pNRI) (NC_002134), Tn4378 from Ralstonia metallidurans CH34 plasmid pMOL28 (NC_006525) and Tn5051 from Pseudomonas putida (Y17719).
such plasmids using common methods as well as to totally exclude them from chromosomal DNA preparations. The genomic DNA of AO22 showed positive hybridisation with a ntrPR probe (data not shown). Further work would be required using approaches such as quantitative PCR or Southern hybridisations to test whether there is more than one mercury transposon in AO22, located on its main chromosome and/or plasmid, as in case of Cupriavidus metallidurans CH34 [27]. The fact that AO22 was receptive to introduction of a broad-host-range IncP plasmid indicates any resident plasmid(s) is (are) unlikely to belong to this incompatibility group. The fact that the mer operon of TnAO22 is functional (and not a relic) can be inferred from conferral of mercuric ion resistance to the E. coli host in conjugation experiments. Lack of merB genes suggests narrow spectrum Hg resistance; confirmed on plates containing organomercurials (Davis and Bhave, unpublished). Other mercury-resistant Gram-positive and Gram-negative bacterial genera have been isolated from the same site [16]; it would be interesting to explore whether transposons similar to TnAO22 are also present in these strains.

Based on sequence identities and res-ntrPR-ntrA gene organisation, TnAO22 appears to belong to the Tn21 branch of the Tn3 subgroup of transposable elements [12]. The structures of the mer operon and transposition modules of TnAO22 suggest it is a recombinant transposon, probably a variant of Tn5051. The putative resolvases (TnPR) of both transposons have TG as the possible start codon; though rare, this start codon has been reported among prokaryotes, notably for lacA in the E. coli lac operon [28]. The mer operon of Tn5051 is nearly identical to that of Tn501, and based on the proposed evolution of Tn501 [19], TnAO22 and Tn5051 probably share an ancestor with Tn501 from which the mer operon originated. Very closely related mer transposons are reported from diverse strains and geographical locations, e.g., at least 10 variants of Tn5053 worldwide [6]. TnAO22 appears to be a new variant of Tn5051 and may be involved in horizontal transfer of mercury resistance, possibly giving the host a selective advantage in contaminated sites such as the one Achromobacter sp. AO22 was isolated from. mer-mediated removal of mercury from sewage and industrial effluent has been described [15,29]. Achromobacter sp. AO22 is one of the few soil bacterial species to contain mer genes and is thus well suited for in situ bioremediation or conjugal transfer of mercury resistance to indigenous soil community, as shown for enhanced degradation of organic contaminants [30].

**Abbreviations**
IR: inverted repeat; Tn: transposon; Hg2+: mercury resistant; Tc: tetracycline resistant.

**Competing interests**
The authors declare that they have no competing interests.

**Authors’ contributions**
SPN carried out the microbiological and molecular genetic studies, sequence alignments and drafted the manuscript. BD participated in the sequencing. EAP and MB conceived of the study, participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

**Additional material**

**Additional File 1**
Supplementary Table S1 and Figures S1–S3. Table S1. Bacterial strains and plasmids; Figure S1. Neighbour-joining distance dendrogram of the 16S rDNA sequences of the genus Achromobacter and related species and Figure S2: Multiple alignments of the putative transposase of TnAO22 with those of selected transposons from Gram positive and Gram negative bacteria. Figure S3. Comparison of the amino acid sequences of the N-terminal (A) and C-terminal (B) sections of the putative MerA of TnAO22 with MerA of selected bacteria.

Click here for file [http://www.biomedcentral.com/content/supplementary/1756-0500-2-38-S1.doc]

**Acknowledgements**
We thank Dr Dena Lyras (Monash University, Melbourne) for providing JIR7062 and pVS520 and Dr Elena Ivanova (SUT) for advice on bacterial taxonomy.
BMC Research Notes 2009, 2:38

http://www.biomedcentral.com/1756-0500/2/38

References

1. Bogdanova E, Minakhin L, Bass I, Volodin A, Hobman JL, Nikiforov V: Class II broad-spectrum mercury resistance transposons in Gram-positive bacteria from natural environments. Res Microbiol 2001, 152(5):503-514.
2. Essa AM, Julian DJ, Kidd SP, Brown NL, Hobman JL: Mercury resistance determinants related to Tn21, Tn1696, and Tn5053 in enterobacteria from the preantibiotic era. Antimicrob Agents Chemother 2003, 47(1):1115-1119.
3. Holt RJ, Bruce KD, Strike P: Conservation of transposon structures in soil bacteria. FEMS Microbiol Ecool 1999, 30(1):25-37.
4. Huang C-C, Narita M, Yamagata T, Itoh Y, Endo G: Structure analysis of a class II transposon encoding the mercury resistance of the Gram-positive bacterium Bacillus megaterium MB1, a strain isolated from Minamata Bay, Japan. Gene 1999, 234(2):361-369.
5. Khodology G, Mindlin S, Petrova M, Minakhina S: Tn5060 from the Siberian permafrost is most closely related to the ancestor of Tn21 prior to integron acquisition. FEMS Microbiol Lett 2003, 226(2):251-252.
6. Mindlin SZ, Bass IA, Bogdanova ES, Gorlenko ZM, Kalyaeva ES, Petrova MA, Nikiforov VG: Horizontal transfer of mercury resistance genes in environmental bacterial populations. Mol Biol 2002, 36(1):160-170.
7. Barkay T, Miller SM, Summers AO: Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol Rev 2003, 27(2-3):355-384.
8. Sherratt D: Tn3 and related transposable elements: site-specific recombination and transposition. In Mobile DNA Edited by: Berg DE, Howe MM, Washington, D. C.: Am Soc Microbiol; 1989.
9. Nakaya R, Nakamura A, Murata Y: Resistance transfer agents in Shigella. Biochem Biophys Res Commun 1960, 3:654-659.
10. Stanisch VA, Bennett PM, Richmond MF: Characterization of a translocation unit encoding resistance to mercuric ions that occurs on a nonconjugative plasmid in Pseudomonas aeruginosa. J Bacteriol 1977, 129(3):1227-1233.
11. Liebert CA, Hall RM, Summers AO: Transposon Tn21, flagship of the floating genome. Microbial Mol Biol Rev 1999, 63(3):507-522.
12. Grinsted J, de la Cruz F, Schmidt R: The Tn21 subgroup of bacterial transposable elements. Plasmid 1990, 24(3):163-189.
13. Bontidean I, Mortari A, Leth S, Brown NL, Karlson U, Larsen MM, Vangsrød J, Corbisier P, Csoregi E: Biosensors for detection of mercury in contaminated soils. Environ Pollut 2004, 131(2):255-262.
14. Hansen LH, Sørensen SJ: Versatile biosensor vectors for detection and quantification of mercury. FEMS Microbiol Lett 2000, 193(1):123-127.
15. Wagner-Dobler I: Pilot plant for bioremediation of mercury-containing industrial wastewater. Appl MicrobiolBiotechnol 2003, 62(2-3):124-133.
16. Trajanovska S, Britz ML, Bhave M: Detection of heavy metal ion resistance genes in Gram-positive and Gram-negative bacteria isolated from a lead-contaminated site. Biodegradation 1997, 8(2):113-124.
17. Weisburg WG, Barnes SM, Pelletier DA, Lane DJ: 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 1991, 173(2):697-703.
18. Wellingshausen N, Wirths B, Poppert S: Fluorescence in situ hybridization for rapid identification of Achromobacter xylooxidans and Alcaligenes faecalis recovered from cystic fibrosis patients. J Clin Microbiol 2006, 44(9):3415-3417.
19. Mindlin S, Khodology G, Gorlenko Z, Minakhina S, Minakhin L, Kalyaeva Ev, Kopteva AV, Petrova M, Yuivea O, Nikiforov V: Mercury resistance transposons of gram-negative environmental bacteria and their classification. Res Microbiol 2001, 152(9):811-822.
20. Palombo EA, Yusof K, Stanisch VA, Krishnapillai V, Willetts NS: Cloning and genetic analysis of trans-cistrons of the Tra2/Tra3 region of plasmid RPl. Plasmid 1989, 22(1):59-69.
21. Fong ST, Stanisch VA: Location and characterization of two functions on RPl that inhibit the fertility of the IncW plasmid R388. J Gen Microbiol 1989, 135(3):499-502.
22. Schneider K, Beck CF: Promoter-probe vectors for the analysis of divergently arranged promoters. Gene 1985, 42(1):37-48.
23. Khodology G, Yuriieva O, Mindlin S, Gorlenko Z, Rybochkin V, Nikiforov V: Tn5044, a novel Tn3 family transposon coding for temperature-sensitive mercury resistance. Res Microbiol 2000, 151(4):291-302.
24. Khodology G, Yuriieva OV, Gorlenko Z, Mindlin SZ, Bass IA, Lomovskaya OL, Kopteva AV, Nikiforov VG: Tn5041: a chimeric mercury resistance transposon closely related to the toluene degradative transposon Tn4651. Microbiology 1997, 143(8):2549-2556.
25. Galperin MY, Nikolskaya AN, Koonin EV: Novel domains of the prokaryotic two-component signal transduction systems. FEMS Microbiol Lett 2001, 203(1):1-21.
26. Kado CI, Liu ST: Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 1981, 145(3):1365-1373.
27. Mergesy M, Monchy S, Vallaey T, Auquier V, Benotmane A, Bertin P, Taghavi S, Dunn J, Lelie D van der, Watteez R:Ralstonia metalidisurans, a bacterium specifically adapted to toxic metals: towards a catalogue of metal-responsive genes. FEMS Microbiol Rev 2003, 27(2-3):385-410.
28. Hediger MA, Johnson DF, Nierlich DP, Zabin I: DNA sequence of the lactose operon: the lacI gene and the transcriptional termination region. Proc Natl Acad Sci USA 1985, 82(19):6414-6418.
29. Hansen CL, Zwalinski G, Martin D, Williams JW: Bacterial removal of mercury from sewage. Biotechnol and Bioengin 1984, 26(11):1330-1333.
30. Newby DT, Josephson KL, Pepper II: Detection and characterization of plasmid pIP4 transfer to indigenous soil bacteria. Appl Environ Microbiol 2000, 66(1):290-296.

Publish with BioMed Central and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp