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RESEARCH ARTICLE

Polycistronic transcription of fused cassettes and identification of translation initiation signals in an unusual gene cassette array from *Pseudomonas aeruginosa* [version 1; peer review: 2 approved with reservations]

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V1 **First published:** 28 Mar 2013, 2:99
<https://doi.org/10.12688/f1000research.2-99.v1>
Second version: 07 Sep 2015, 2:99
<https://doi.org/10.12688/f1000research.2-99.v2>
Latest published: 27 Nov 2015, 2:99
<https://doi.org/10.12688/f1000research.2-99.v3>

Abstract

The gene cassettes found in class 1 integrons are generally promoterless units composed by an open reading frame (ORF), a short 5' untranslated region (UTR) and a 3' recombination site (*attC*). Fused gene cassettes are generated by partial or total loss of the *attC* from the first cassette in an array, creating a fusion with the ORF from the next cassette. These structures are rare and little is known about their mechanisms of mobilization and expression. The aim of this study was to evaluate the dynamic of mobilization and transcription of the *gcu14-bla_{GES-1}/aacA4* gene cassette array, which harbours a fused gene cassette represented by *bla_{GES-1}/aacA4*. The cassette array was analyzed by Northern blot and real-time reverse transcription-polymerase chain reaction (RT-PCR) in order to assess the transcription mechanism of *bla_{GES-1}/aacA4* fused cassette. Also, inverse polymerase chain reactions (PCR) were performed to detect the free circular forms of *gcu14*, *bla_{GES-1}* and *aacA4*. The Northern blot and real time RT-PCR revealed a polycistronic transcription, in which the fused cassette *bla_{GES-1}/aacA4* is transcribed as a unique gene, while *gcu14* (with a canonical *attC* recombination site) has a monocistronic transcription. The *gcu14* cassette, closer to the weak configuration of cassette promoter (Pc), had a higher transcription level than *bla_{GES-1}/aacA4*, indicating that the cassette position impacts the transcript amounts. The presence of ORF-11 at *attI1*, immediately preceding *gcu14*, and of a Shine-Dalgarno sequence upstream *bla_{GES-1}/aacA4* composes a scenario for the occurrence of array translation. Inverse PCR generated amplicons corresponding to *gcu14*, *gcu14-aacA4* and *gcu14-bla_{GES-1}/aacA4* free circular forms, but not to *bla_{GES-1}* and *aacA4* alone, indicating that the GES-1 truncated *attC* is not substrate of integrase activity and that these genes are mobilized

Open Peer Review

Approval Status

	1	2
version 3 (revision) 27 Nov 2015	view

version 2 (revision) 07 Sep 2015	view	view

version 1 28 Mar 2013	view	view

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together as a unique cassette. This study was original in showing the transcription of fused cassettes and in correlating cassette position with transcription.

Keywords

DNA, mRNA, ribosomes

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Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the CNPq and FAPERJ fellowship and Oswaldo Cruz Institute Grant. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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How to cite this article: Fonseca ÉL and Vicente ACP. **Polycistronic transcription of fused cassettes and identification of translation initiation signals in an unusual gene cassette array from *Pseudomonas aeruginosa* [version 1; peer review: 2 approved with reservations]** F1000Research 2013, 2:99 <https://doi.org/10.12688/f1000research.2-99.v1>

First published: 28 Mar 2013, 2:99 <https://doi.org/10.12688/f1000research.2-99.v1>

Introduction

Class 1 integrons are capable of inserting, excising and rearranging gene cassettes by a site-specific recombination mechanism. These assembly platforms can also act as expression systems due to the presence of a promoter region (Pc), which drives the expression of genes captured by integron¹. Moreover, naturally occurring integrons may have a second promoter (P2), which is activated by the insertion of three G residues between -35 and -10 hexamers¹. Gene cassettes are generally promoterless units associated with a recombination site (*attC* or 59-be), which confers the ability of each structure to be mobilized independently². In addition, *attC* sites regulate the translation of downstream cassettes due to their peculiar sequences composed by imperfect inverted repeats. The formation of stem-loop structures by *attC* sites prevents ribosome progression throughout mRNA, reflecting in a decreased expression of more distal genes regarding Pc³. Although rare, fused cassettes may be generated by partial or total loss of the first *attC*, retaining both complete coding regions and, therefore, creating permanent gene arrays comparable to bacterial operons⁴. The functionality of such structures has been indirectly inferred by the resistance profile of transformants carrying the fusion⁵; however, the transcription itself has never been verified.

This study showed the dynamics of fused cassette mobilization, the co-transcription of the *gcu14-bla_{GES-1}/aacA4* cassette array and the effect of cassette position on transcription levels in *Pseudomonas aeruginosa* wild lineages carrying class 1 integrons. Moreover, the presence of translation signals in this gene cassette array was determined.

Material and Methods

An unknown Open Reading Frame (ORF), *gcu14*, followed by the fused cassette *bla_{GES-1}/aacA4*, created by partial loss of GES-1 *attC*, were present in integrons from clinical *P. aeruginosa* isolates (PS1 and PS26)⁶. Total RNA was extracted and purified according to the manufacturers instructions with the SV 96 Total RNA Isolation System (Promega). Northern blot using 7 µg of total RNA from PS1 and PS26 was performed in order to detect the transcript originated from *gcu14-bla_{GES-1}/aacA4* cassette array. After electrophoresis in a denaturing-formaldehyde 1.5% agarose gel, the total RNA was transferred to the Hybond-N⁺ nylon membrane (GE Healthcare) by upward capillary transfer. An amplicon of 519bp corresponding to part of the *bla_{GES-1}* gene was used as a probe (Table 1) in hybridization assay. The GES probe was labeled with the AlkPhos Direct

Table 1 Primers used in conventional, inverse and real-time PCR reactions.

Primers for conventional PCR			
Primer	Primer sequence (5' - 3')	Size (bp)	Target
Ges F Ges R	GCGTTTTGCAATGTGCTC CCAGTTTTCTCTCCAACAACC	519	Internal fragment of <i>bla_{GES}</i> gene
Primers for inverse PCR ^a			
Gcu14 FSQ Gcu14 RSQ	AGCAATCAACACACAGGGG CTCGCGTAAATGCACCGCTT	130	<i>gcu14</i> circular form
GES FSQ GES RSQ	CAAGTTATTACACAACATCAT AGTGCGTGAATGAAGCGCAT	110	<i>bla_{GES-1}</i> circular form
AACA4 FSQ AACA4 RSQ	GCCAGGCATTCGAGCGAACAC ATTAGCCACTCACATAGAGC	188	<i>aacA4</i> circular form
Primers and probes for real time PCR (TaqMan)			
RpsL F RpsL R Probe	GCCTGCGCTGCAAACT TTTCGGCGTGTTGGTGTAT TCGTGGCGTATGCACC	67	<i>rpsL</i> transcripts
Gcu14 F Gcu14 R Probe	CATGCGCTTCTTGTTTCGT ACGCCAGCTTGGATGCAA ATGCCACGAGACCTT	56	<i>gcu14</i> transcripts
Ges F2 Ges R2 Probe	GTGCAGCTTAGCGACAATGG CACAGAGTCGCCAATTTACGAA ATTGCAGCAGGTCCGCC	99	<i>bla_{GES-1}</i> transcripts
AacA4 F AacA4 R Probe	CAAGCGTTTTAGCGCAAGAGT TCGGCTCTCCATTACGATTG CCGTCACCTCCATACATTG	59	<i>aacA4</i> transcripts
Ges F3 AacA4 R2 Probe	TCCTGAGCACGGACAAATAG TCATAGAGCATCGCAAGGTC TTCCGTCACACTGCGCCTCA	134	Polycistronic transcripts

^a Gcu RSQ - AACA4 FSQ and GES RSQ - AACA4 FSQ were used in combination to obtain the full length and the *bla_{GES-1}/aacA4* fused cassette circular forms, respectively.

Labelling kit (GE Healthcare) and hybridized with the target RNA immobilized on the Hybond-N⁺ membrane as recommended. The chemiluminescence was detected with the CDP-Star detection reagent (GE Healthcare) according to manufactures. Immediately after applying the detection reagents, the blot was drained, incubated five minutes at room temperature and exposed to the Hyperfilm ECL (GE Healthcare) for 60 minutes at room temperature.

In order to verify whether the relative position of gene cassettes on the variable region plays a role in transcription level, real-time RT-PCR reactions using the TaqMan System (Applied Biosystems) were performed with primers and probes detailed in Table 1. The single-copy ribosomal *rpsL* gene of the *P. aeruginosa* chromosome was amplified by PCR (Table 1) and used as a reference gene for normalization. The relative quantification (RQ) results were presented as ratios of gene transcription between the target gene (cassettes) and the reference gene (*rpsL*), which were obtained according to the following equation: $RQ = 2^{-\Delta CT}$, where CT is the value corresponding to the crossing point of the amplification curve with the threshold and $\Delta CT = CT \text{ target gene} - CT \text{ reference gene}$. The effect of cassette position on gene transcription was considered significant when the ratios obtained between RQ values (RQ value of cassette 1/RQ value of cassette 2) were ≥ 2.0 , taking into account the standard deviation intervals.

In order to induce cassette excision from integrons, PS1 and PS26 strains⁶ were submitted to thermal stress during the log growth phase to induce integrase activity. Cells were grown on Luria-Bertani (LB) broth medium (OXOID) at 37°C for two hours. Subsequently, the bacterial cultures were submitted to a heat shock at 4°C for 30 minutes and immediately incubated at 42°C for another 30 minutes. Briefly, the total DNA from PS1 and PS26 cultured under thermal stress were obtained with the Wizard Genomic DNA purification kit (Promega) following manufacturer recommendations and used as templates in inverse PCR reactions. The inverse PCR was performed with primers facing outwards towards the ends of *gcu14*, *bla*_{GES-1} and *aacA4* so that only circular gene cassette configurations would be amplified. The reactions target the circular forms of *gcu14*, *bla*_{GES-1}, *aacA4* and *bla*_{GES-1}/*aacA4* fusion by using the primers and combinations described in Table 1. The inverse PCR was performed using Platinum Taq DNA Polymerase reagents (Invitrogen), and the following components were added to a sterile 0.2-mL tube: 5 µL of 10X PCR buffer (1X final concentration); 1 µL of 10mM dNTP mixture (0.2 mM each); 1.5 µL of 50mM MgCl₂ (1.5 mM final concentration); 2 µL of 15 µM of each primer (30 µM each); 100 ng of template DNA; 0.3 µL of Platinum Taq DNA Polymerase (1U final concentration). The tubes were incubated in the Eppendorf MasterCycler (Eppendorf) at 94°C for 2 minutes and PCR amplification was performed in 40 cycles consisting of: 94°C for 30 seconds; 55°C for 30 seconds; and 72°C for 3 minutes.

The amplicons generated with the inverse PCR were purified using Wizard SV Gel and PCR Clean-Up system kit (Promega) and directly sequenced on both strands. Sequencing reactions were performed with Big Dye Terminator RR Mix (Applied Biosystems) in an ABI 3730 XL DNA Analyzer (Applied Biosystems). Nucleotide sequences were compared to those available in the GenBank database accessible on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov>). All primers used in PCR, sequencing and real time RT-PCR are described in Table 1.

Analyses *in silico* were performed to search for a potential promoter for *gcu14* gene cassette. The 5'UTR from *gcu14* were submitted to the promoter predictor programs Neural Network for Promoter Prediction version 2.2 (Berkeley Drosophila Genome Project, <http://www.fruitfly.org/index.html>) and BPROM (SoftBerry, <http://linux1.softberry.com/berry.phtml>). Results with the highest scores were selected as candidates for a putative promoter.

Results and Discussion

The integrons analyzed in this study harbored a rare weak Pc configuration (PcW_{TGN-10}), which presents a C to G mutation 2 bp upstream of the -10 hexamer that causes an abrupt decrease in promoter strength as reported previously⁷. Considering that transcription initiates from the Pc promoter placed upstream the *gcu14-bla*_{GES-1}/*aacA4* cassette array, both monocistronic and full length polycistronic transcripts could be identified. In fact, Northern blot and hybridization assays revealed a unique signal of approximately 2,300 bases which corresponds to the co-transcription of the entire array (*gcu14-bla*_{GES-1}/*aacA4*) (Figure 1). This result is in agreement with previous work in which the occurrence of transcripts containing more than one gene cassette was observed by Northern blot analysis¹. Moreover, this finding gives support to the lack of *attC* function in terminating transcription of downstream gene cassettes as demonstrated previously³.

This fusion retained both entire coding regions and suffered partial loss of 91 bp at the GES-1 *attC* site (DQ236170)⁶. Previous studies demonstrated that the *attC* region flanked by Left Hand (LH) and Right Hand (RH) domains, which is an imperfect inverted repeat and is missing in the *bla*_{GES-1} recombination site, is crucial for cassette mobilization⁸. Taking into account that the region responsible for stem-loop formation was missing in GES-1 *attC* and the participation of this site in terminating translation³, our findings indirectly suggested that *bla*_{GES-1} and *aacA4* translation is occurring in a unique step.

Gene cassettes can be found inserted in integrons or in other secondary sites or free in the cytoplasm as a closed circle, in which the 5' end (5' UTR) and the *attC* recombination site are covalently linked⁹. As demonstrated previously, several stress conditions could evoke the activation of the SOS response resulting in integron-integrase expression¹⁰. Therefore, under

Figure 1 Northern blot analysis of *gcu14-bla_{GES-1}/aacA4* gene cassette array from *P. aeruginosa* class 1 integron. The full length transcript (2,300 bases), corresponding to the entire gene cassette array, hybridized with the GES probe (arrow). The fragment sizes of the RNA marker (Promega) used in RNA electrophoresis are indicated.

stress, the integrase activity increases, favoring the occurrence of integration/excision/rearrangements events. Since the excision event depends on the recognition of the LH and RH domains of the *attC* site, and that these regions are missing in GES-1 *attC*, it is expected that the *bla_{GES-1}/aacA4* excision occurs only at the *aacA4 attC* site, and that this structure is excised together as a unique cassette.

Positive results were obtained for the *gcu14*, *gcu14-aacA4* and *gcu14-bla_{GES-1}/aacA4* circular forms, but not for *bla_{GES-1}* and *aacA4* alone, showing that the GES-1 *attC* is not functional and that the fused gene cassette is excised as a unique cassette. Sequencing assessed the recombination point where excision occurred, confirming the occurrence of free circular forms. The lack of activity of a truncated *attC* had also been observed before when associated with *aadA10*¹¹. However, Ramirez and colleagues¹² showed that the integrase was able to recognize and mediate excision of a truncated site associated to *aadA1*, indicating that the genetic context of such truncated sites could influence their role in IntI1 recognition and mobilization.

The relative quantification performed by real time RT-PCR revealed that PS1 and PS26 presented very similar RQ values for *gcu14-bla_{GES-1}/aacA4* transcription (Figure 2). This result

Figure 2 Transcription level of *gcu14/bla_{GES-1}/aacA4* gene cassette array. The relative quantification values obtained by real time RT-PCR are indicated for each gene cassette and for the fusion *bla_{GES-1}/aacA4* when considered as a unique gene.

was expected since integrons from these two strains have the same backbone, including the Pc promoter, and are at the same genetic environment⁶.

gcu14, the first cassette in integrons with the weak Pc_{TGN-10} configuration, presented approximately two-fold higher transcription when compared to *bla_{GES-1}* and *aacA4* separately or when the fused cassette *bla_{GES-1}/aacA4* was considered (Figure 2). The same RQ value obtained for *bla_{GES-1}*, *aacA4* and the fusion reveals these two ORFs are transcribed as a unique gene. The lower transcript amount of *bla_{GES-1}/aacA4* compared to *gcu14* lies on the distance between these gene cassettes and Pc, which is one of the determinants influencing cassette transcription^{1,10}, and it shows the effect of cassette position on expression levels.

A putative promoter for *gcu14* (-35 TTGATG [17 bp] -10 TGTTAC) was found 45 bp upstream from its start codon. Therefore, the increased *gcu14* transcription could have resulted from a synergistic effect of this putative promoter with Pc. Considering that this putative promoter was found at *attI1*, which is highly conserved among class 1 integrons, it can be suggested that such a promoter is able to drive transcription of any cassette placed in the first integron position. Moreover, the ORF-11, which enhances the translation efficiency of downstream TIR (translation initiation region)-deficient cassettes inserted in integrons¹³, was found at the *attI1* region preceding the TIR-deficient *gcu14* gene cassette. This ORF contained its own Shine-Dalgarno (SD) sequence placed 8 bp upstream of the ATG codon. The ribosome at the ORF-11 stop codon could therefore be carried along the mRNA by lateral diffusion, reinitiating translation at the *gcu14* start codon. A potential SD sequence was identified 10 bp upstream of the fused cassette *bla_{GES-1}/aacA4*. In addition, the loss of the GES-1 *attC* region, which is involved in stem-loop formation, may enhance the chances of *aacA4* translation, since this *attC* no longer

constitutes a physical barrier to ribosome progression³. This deletion also then brings the second gene (*aacA4*) closer to Pc¹⁴. Together, these findings create a scenario for the occurrence of *gcu14-bla_{GES-1}/aacA4* expression in PS1 and PS26, which then provides a possible explanation for their resistance profile to β -lactams and aminoglycosides that has been observed elsewhere⁶.

RQ fused cassette raw data for *Pseudomonas aeruginosa* isolates PS1 and PS26. F1000 Research.

2 Data Files

<http://dx.doi.org/10.6084/m9.figshare.651717>

Inverse PCR sequences.

8 Data Files

<http://dx.doi.org/10.6084/m9.figshare.651718>

Conclusions

Fused cassettes have been found in class 1 integrons^{5,11,12,15–18}; however, the transcription of such structures has never been addressed. This work showed the transcription pattern of a fused

cassette as a polycistronic mRNA and that these unusual structures are excised as a unique cassette.

Nucleotide sequence accession number. The sequence of the cassette array composed by the fusion has been deposited in the GenBank database under accession number [DQ236170](#).

Author contributions

ELF and ACPV conceived the study and designed the experiments. ELF carried out the research and prepared the first draft of the manuscript. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

Competing interests

No relevant competing interests were disclosed.

Grant Information

This work was supported by the CNPq and FAPERJ fellowship and Oswaldo Cruz Institute Grant.

Acknowledgements

We thank those involved in PDTIS platform to enable us to do the sequencing and real-time relative quantification analysis work.

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Appendix 1: This result shows that the entire array *gcu14-GES-1/aacA4* was mobilized together. This is evidence that *GES1* and *aacA4* are excised in the circular form as a unique cassette, showing that the truncated *attC* from *GES-1* is not functional and that recombination occurs in the *aacA4 attC*.

```
>GESFSQ - GESR (REAÇÃO GESFSQ) E sequence exported from chromatogram file
NNGGGTGGTTGGAAAAANACTGGGAANTTGCCTCTCNCGGTCAAGGAGGAANACACAAC
TCATCCTGAGCACGGACAAATAGTTGACGCCCCGTCTAAAAACAAAGTTAGGCATCACAAAG
TACAGCATCGTGACCAACAGCAACGATTCCGTCACACTGCGCCTCATGACTGAGCATGAC
CTTGCGATGCTCTATGAGTGGCTAAATCGATCTCATATCGTCGAGTGGTGGGGCGGAGAA
GAAGCACGCCCCGACACTTGCTGACGTACAGGAACAGTACTTGCCAAGCGTTTTAGCGCAA
GAGTCCGTCACTCCATACATTGCAATGCTGAATGGAGAGCCGATTGGGTATGCCAGTTCG
TACGTTGCTCTTGGAGGCGGGGACGGATGGTGGGAAGAAGAAACCGATCCAGGAGTACGC
GGAATAGACCAGTTACTTGGCAATGCATCACAACTGGGCAAAGGCTTGGGAACCAAGCTG
GTTTCGAGCTCTGGTTGAGTTGCTGTTCAATGATCCCGAGGTCACCAAGATCCAAACGGAC
CCGTCGCCGAGCAACTTGCAGCGATCCGATGCTACGAGAAAGCGGGGTTTGAGAGGCAA
GGTACCGTAACCACCCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTC
GAGCGAACACGCAGTGATGCCTAACCCTTCCATCGAGGGGGACGTCCAAGGGCTGGCGCC
CTTGACGNCCCTGATGTCAAANNTTTNGGCCNC
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[gi|6752459|gb|AF156486.1|AF156486](#) *Klebsiella pneumoniae* integron integrase
INTI1 (intI1), beta-lactamase
GES-1 (ges-1), aminoglycoside 6'-N-acetyltransferase
type II AAC6'Ib (aac(6')-Ib'), dihydrofolate reductase DHFRXVb
(dhfrXVb), chloramphenicol transporter CMLA4 (cmlA4),
and streptomycin 3''-adenylyltransferase AADA2 (aadA2) genes,
complete cds
Length=5863

Score = 1328 bits (670), Expect = 0.0
Identities = 684/689 (99%), Gaps = 0/689 (0%)
Strand=Plus/Plus

Query	54	ACACAACCTCATCCTGAGCACGGACAAATAGTTGACGCCCCGTCTAAAAACAAAGTTAGGCAT	113
Sbjct	2166	ACACAACCTCATCCTGAGCACGGACAAATAGTTGACGCCCCGTCTAAAAACAAAGTTAGGCAT	2225
Query	114	CACAAAGTACAGCATCGTGACCAACAGCAACGATTCCGTCACACTGCGCCTCATGACTGA	173
Sbjct	2226	CACAAAGTACAGCATCGTGACCAACAGCAACGATTCCGTCACACTGCGCCTCATGACTGA	2285
Query	174	GCATGACCTTGCGATGCTCTATGAGTGGCTAAATCGATCTCATATCGTCGAGTGGTGGGG	233
Sbjct	2286	GCATGACCTTGCGATGCTCTATGAGTGGCTAAATCGATCTCATATCGTCGAGTGGTGGGG	2345
Query	234	CGGAGAAGAAGCACGCCCCGACACTTGCTGACGTACAGGAACAGTACTTGCCAAGCGTTTT	293
Sbjct	2346	CGGAGAAGAAGCACGCCCCGACACTTGCTGACGTACAGGAACAGTACTTGCCAAGCGTTTT	2405
Query	294	AGCGCAAGAGTCCGTCACTCCATACATTGCAATGCTGAATGGAGAGCCGATTGGGTATGC	353
Sbjct	2406	AGCGCAAGAGTCCGTCACTCCATACATTGCAATGCTGAATGGAGAGCCGATTGGGTATGC	2465

```

Query   354   CCAGTCGTACGTTGCTCTTGGAGGCGGGGACGGATGGTGGGAAGAAGAAACCGATCCAGG   413
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Sbjct   2466   CCAGTCGTACGTTGCTCTTGGGAAGCGGGGACGGATGGTGGGAAGAAGAAACCGATCCAGG   2525

Query   414   AGTACGCGGAATAGACCAGTTACTGGCGAATGCATCACAACCTGGGCAAAGGCTTGGGAAC   473
          |||
Sbjct   2526   AGTACGCGGAATAGACCAGTCACTGGCGAATGCATCACAACCTGGGCAAAGGCTTGGGAAC   2585

Query   474   CAAGCTGGTTCGAGCTCTGGTTGAGTTGCTGTTCAATGATCCCGAGGTCACCAAGATCCA   533
          |||
Sbjct   2586   CAAGCTGGTTCGAGCTCTGGTTGAGTTGCTGTTCAATGATCCCGAGGTCACCAAGATCCA   2645

Query   534   AACGGACCCGTCGCCGAGCAACTTGCGAGCGATCCGATGCTACGAGAAAGCGGGGTTTGA   593
          |||
Sbjct   2646   AACGGACCCGTCGCCGAGCAACTTGCGAGCGATCCGATGCTACGAGAAAGCGGGGTTTGA   2705

Query   594   GAGGCAAGGTACCGTAACCACCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCA   653
          |||
Sbjct   2706   GAGGCAAGGTACCGTAACCACCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCA   2765

Query   654   GGCATTTCGAGCGAACACGCAGTGATGCCTAACCCCTTCCATCGAGGGGGACGTCCAAGGGC   713
          |||
Sbjct   2766   GGCATTTCGAGCGAACACGCAGTGATGCCTAACCCCTTCCATCGAGGGGGACGTCCAAGGGC   2825

Query   714   TGGCGCCCTTGGACGNCCCTGATGTCAAA   742
          |||
Sbjct   2826   TGGCGCCCTTGGCGCCCTTCATGTCAAA   2854

```

>GESFSQ-GESR (REAÇÃO AACA4 F) sequence exported from chromatogram file

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CGGGNGNCGGTACNCCNNGGANGGTCCAGCTCNACATGGGGAGAANACGCCAGGCATT
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CAAGCGAGCCACCTCCTCGGTGGCTACCAGCAGCCAGGCATGCGCTTCTTGGTTCGTGCA
AGGTCTCGTGGCATGCTTGCATCCAAGCTGGCGTCAGCACAGCAATCAACACACAGGGGT
TTCATGCCAAATCAGCCGCATGCCACCATCGGCCTAACTGTGCGTTGCAGGGGACGCCTC
CACCAGTGCGCGCTTCGGCCACGCCAAGCGCGCGCCCTGAACTTGGGCGTTAGACGGGC
GTACAAAGATAATTTCCATCTCAAGGGATCACCATGCGCTTCATTACGCACTATTACTG
GCAGGGATCGCTCACTCTGCATATGCGTCGGAAAAATTAACCTTCAAGACCGATCTTGAG
AAGCTAGAGCGCGAAAAAGCAGCTCAGATCGGTGTTGCGATCGTCGATCCCCAAGGAGAG
ATCGTCGCGGGCCACCGAAATGGAGCCAGNGTTNNTGCAATGTGCNTCNAACNNTNAAGG
TTTNCNANTANACGNGCTGGGTCTNNTTNAAGAAATGGAANCANGNCACNGGANNGGGGG
GGGATCGAAAAACTTTTCNNTATGGA

```

> [gi|66269634|gb|AY920928.1|](#) Pseudomonas aeruginosa transposon Tn5393C TnpA (tnpA) gene, partial

cds; and integron In109 integrase (intI1) gene, complete sequence, GES-9 (bla(GES-9)) gene, complete cds, insertion sequence ISPa21, complete sequence, AAC(6')-Ib (aacA4) and AADB (aadB) genes, complete cds, insertion sequence ISPa21, complete sequence, and unknown gene
Length=8003

Score = 363 bits (183), Expect = 5e-97
 Identities = 186/187 (99%), Gaps = 0/187 (0%)
 Strand=Plus/Plus

```

Query  411  GTTAGACGGGCGTACAAAGATAATTTCCATCTCAAGGGATCACCATGCGCTTCATTCACG  470
          |||
Sbjct  2646  GTTAGACGGGCGTACAAAGATAATTTCCATCTCAAGGGATCACCATGCGCTTCATTCACG  2705

Query  471  CACTATTACTGGGAGGGATCGCTCACTCTGCATATGCGTCGGAAAAATTAACCTTCAAGA  530
          |||
Sbjct  2706  CACTATTACTGGCAGGGATCGCTCACTCTGCATATGCGTCGGAAAAATTAACCTTCAAGA  2765

Query  531  CCGATCTTGAGAAGCTAGAGCGCGAAAAAGCAGCTCAGATCGGTGTTGCGATCGTCGATC  590
          |||
Sbjct  2766  CCGATCTTGAGAAGCTAGAGCGCGAAAAAGCAGCTCAGATCGGTGTTGCGATCGTCGATC  2825

Query  591  CCCAAGG  597
          |||
Sbjct  2826  CCCAAGG  2832

```

Score = 157 bits (79), Expect = 6e-35
 Identities = 82/83 (98%), Gaps = 0/83 (0%)
 Strand=Plus/Plus

```

Query  65  CGAACACGCAGTGATGCCTAACCCTTCCATCGAGGGGGACGTCCAAGGGCTGGCGCCCTT  124
          |||
Sbjct  5599  CGAACATGCAGTGATGCCTAACCCTTCCATCGAGGGGGACGTCCAAGGGCTGGCGCCCTT  5658

Query  125  GGCCGCCCTCATGTCAAACGTT  147
          |||
Sbjct  5659  GGCCGCCCTCATGTCAAACGTT  5681

```

593 NTS INICIAIS DO INTEGRON 1 E 26 (CONTEM A ORF126) x GESFSQ-GESR (REAÇÃO AACA4 F)

Score = 579 bits (301), Expect = e-162
 Identities = 301/301 (100%)
 Strand = Plus / Plus

```

Query: 293 ctcgcgtaaatgcaccgcttgcgagggccaagcgagccacctcctcggtgggtaccagca 352
          |||
Sbjct: 153 ctcgcgtaaatgcaccgcttgcgagggccaagcgagccacctcctcggtgggtaccagca 212

Query: 353 gccaggcatgcgcttcttggttcgtgcaagggtctcgtggcatgcttgcatccaagctggc 412
          |||
Sbjct: 213 gccaggcatgcgcttcttggttcgtgcaagggtctcgtggcatgcttgcatccaagctggc 272

```

Query: 413 gtcagcacagcaatcaacacacaggggtttcatgccaaatcagccgcatgccaccatcgg 472
 |||
 Sbjct: 273 gtcagcacagcaatcaacacacaggggtttcatgccaaatcagccgcatgccaccatcgg 332

Query: 473 cctaactgtgcttgcaggggacgcctccaccagtgcgcgcttcggccacgccaagcgcg 532
 |||
 Sbjct: 333 cctaactgtgcttgcaggggacgcctccaccagtgcgcgcttcggccacgccaagcgcg 392

Query: 533 cgccccctgaacttgggcttagacgggctacaaagataatttccatctcaagggatcac 592
 |||
 Sbjct: 393 cgccccctgaacttgggcttagacgggctacaaagataatttccatctcaagggatcac 452

Query: 593 c 593
 |
 Sbjct: 453 c 453

>GESFSQ-GESR (REAÇÃO AACA4 R) ED sequence exported from chromatogram file
 GCGGTGTGGCATNANCGNAAAGTGACGGTTCNNTCTGCTGGGGAGNCACGATGCTGTACT
 TTGTGATGCCTAACTTTGTTTTAGACGGGCGTCAACTATTTGTCCGTGCTCAGGATGAGT
 TGTGTAATAACTTGACCGACAGAGGCAACTAATTTCGTACGTTCTACGGCCGATAGTTTC
 GGGGCGGTGTATATAANNAATCNATNTCNATATCGNCNAGCGNTGNGACGAAAAACANGN
 ANGCCCGCACACTTGCCNGACGTACTGGTAACAGTACNTGGCAAGCGGTCNAGTGGAAGA
 GTANGCTCGCNTCCATAGCATTTGCNATGCTTNAANGNACCAGCCATATTTGTCCCATNCGC
 AGTNACNCGCTTGCGTCTGTGAGANGCACGGGTACGGANNGATCGGGNAANAACANACC
 NNATCCAGNGTNGTCACATCGCCNNNAGCACCGAGNGNACTGNGCNCNAAATGTCNTCACA
 AACNGGNNCNAAGGNCCTTAGCGGAAACNANANCGTGNCTTCNGAGCTCCNGNTNTGANA
 ATGCTGCNCAATTTNTCCCGGAGNNCACNAAAAACCCNCGGANNNCANTNNCCNAANN
 AACNCGGAGCGNAACNNNTGNTNNCAGCNAAACTGGGGTTNTGAAGAAGCGAANGCTNG
 ANNGNACAACNTNTTCAGNNNCCAAANNNGGAACGNGGGAANCNNCGCCCCAAGNNAATN
 GAGGGNANNACNAGNGANGGNNNACCCCTTNCAGGNAANGNGNACNTTNNAGGNCCGNN
 CAAACNCCCNAANTCAGGGNGNCGCANNTTGGGNGGGGNCNAAANAANNNCNNGGNGGN
 AGG

> [gi|6752459](https://www.ncbi.nlm.nih.gov/nuclot/6752459)|[gb|AF156486.1](https://www.ncbi.nlm.nih.gov/nuclot/AF156486.1)|AF156486 Klebsiella pneumoniae integrase
 INTI1 (intI1), beta-lactamase
 GES-1 (ges-1), aminoglycoside 6'-N-acetyltransferase
 type II AAC6'Ib (aac(6')-Ib'), dihydrofolate reductase DHFRXVb
 (dhfrXVb), chloramphenicol transporter CMLA4 (cmlA4),
 and streptomycin 3''-adenylyltransferase AADA2 (aadA2) genes,
 complete cds
 Length=5863

Score = 293 bits (148), Expect = 4e-76
 Identities = 148/148 (100%), Gaps = 0/148 (0%)
 Strand=Plus/Minus

Query 47 CACGATGCTGTACTTTGTGATGCCTAACTTTGTTTTAGACGGGCGTCAACTATTTGTCCG 106
 |||
 Sbjct 2244 CACGATGCTGTACTTTGTGATGCCTAACTTTGTTTTAGACGGGCGTCAACTATTTGTCCG 2185

```

Query 107      TGCTCAGGATGAGTTGTGTAATAACTTGACCGACAGAGGCAACTAATTCGTCACGTTCTA 166
               |||
Sbjct 2184     TGCTCAGGATGAGTTGTGTAATAACTTGACCGACAGAGGCAACTAATTCGTCACGTTCTA 2125

Query 167      CGGCCGATAGTTTCGGGGCCGTTGTATA 194
               |||
Sbjct 2124     CGGCCGATAGTTTCGGGGCCGTTGTATA 2097

```

>GESFSQ-GESR (REAÇÃO AACA4 FI sequence exported from chromatogram file

```

CGCGTGTGAACACANCTGNAANCNCNTTCNNCGCTNTGGANAAAAANAGAGCCGATTTTC
CCCTGCCCAGNNGAANANTGCTCTTGGAGGCGGGGACGGATGGTGGGAAGAAGAAACCGA
TCCAGGAGTACGCGGAATACACCAGTTACTGGCGAATGCATCACAACCTGGGCAAAGGCTT
GGGAACCAAGCTGGTTCGAGCTCTGGTTGAGTTGCTGTTCAATGATCCCAGGGTCACCAA
GATCCAAACGGACCCGTCGCCGAGCAACTTGCGAGCGATCCGATGCTACGAGAAAGCGGG
GTTTGAGAGGCAAGGTACCGTAACCACCCAGATGGTCCAGCCGTGTACATGGTTCAAAC
ACGCCAGGCATTCGAGCGAACACGCAGTGATGCCTAACCCCTTCATCGAGGGGGACGTCC
AAGGGCTGGCGCCCTTGGCCGCCCTCATGTCAAACGTTAGGCCCTCGCGTAAATGCACC
GCTTGCGCAGGCCAAGCGAGCCACCTCCTCGGTGGCTACCAGCAGCCAGGCATGCGCTTC
TTGGTTTCGTGCAAGGTCTCGTGGCATGCTTGCATCCAAGCTGGCGTCAGCACAGCAATCA
ACACACAGGGGTTTCATGCCAAATCAGCCGNGATGCCACCNCGNCCTAANTGGGGCGT
TNGCAAGGNGACNNCTCCNANCNTTGNGGCNCNTAAGNCCACNNNCAAGCCGCCNCNC
NCCNNGAANCTTGGGGNCTTTGGANNGGGCNGTNACAAANATNATTTNC

```

> [gi|45934115|gb|AY507153.1](#) Pseudomonas aeruginosa strain 4122 class I
integron, partial
sequence
Length=7201

Score = 749 bits (378), Expect = 0.0
Identities = 384/386 (99%), Gaps = 0/386 (0%)
Strand=Plus/Plus

```

Query 79      TGCTCTTGAGGCGGGGACGGATGGTGGGAAGAAGAAACCGATCCAGGAGTACGCGGAAT 138
               |||
Sbjct 5005     TGCTCTTGGAAGCGGGGACGGATGGTGGGAAGAAGAAACCGATCCAGGAGTACGCGGAAT 5064

Query 139     ACACCAGTTACTGGCGAATGCATCACAACCTGGGCAAAGGCTTGGGAACCAAGCTGGTTCG 198
               |||
Sbjct 5065     AGACCAGTTACTGGCGAATGCATCACAACCTGGGCAAAGGCTTGGGAACCAAGCTGGTTCG 5124

Query 199     AGCTCTGGTTGAGTTGCTGTTCAATGATCCCAGGGTCACCAAGATCCAAACGGACCCGTC 258
               |||
Sbjct 5125     AGCTCTGGTTGAGTTGCTGTTCAATGATCCCAGGGTCACCAAGATCCAAACGGACCCGTC 5184

Query 259     GCCGAGCAACTTGCGAGCGATCCGATGCTACGAGAAAGCGGGGTTTGAGAGGCAAGGTAC 318
               |||
Sbjct 5185     GCCGAGCAACTTGCGAGCGATCCGATGCTACGAGAAAGCGGGGTTTGAGAGGCAAGGTAC 5244

Query 319     CGTAACCACCCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTCGAGCG 378
               |||
Sbjct 5245     CGTAACCACCCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTCGAGCG 5304

```

```

Query   379   AACACGCAGTGATGCCTAACCTTCCATCGAGGGGACGTCCAAGGGCTGGCGCCCTTGG   438
          |||
Sbjct   5305  AACACGCAGTGATGCCTAACCTTCCATCGAGGGGACGTCCAAGGGCTGGCGCCCTTGG   5364

Query   439   CCGCCCCCATGTGTCAAACGTTAGGCC   464
          |||
Sbjct   5365  CCGCCCCCATGTGTCAAACGTTAGGCC   5390

```

593 NTS INICIAIS DO INTEGRON 1 E 26 (CONTEM A ORF126) x GESFSQ-GESR (REAÇÃO AACA4 FI)

Score = 335 bits (174), Expect = 1e-88
 Identities = 174/174 (100%)
 Strand = Plus / Plus

```

Query: 285 gttaggccctcgcgtaaatgcaccgcttgcgagggccaagcgagccacctcctcggtggc 344
          |||
Sbjct: 457 gttaggccctcgcgtaaatgcaccgcttgcgagggccaagcgagccacctcctcggtggc 516

```

```

Query: 345 taccagcagccaggcatgcgcttcttggttcgtgcaagggtctcgtggcatgcttgcaccc 404
          |||
Sbjct: 517 taccagcagccaggcatgcgcttcttggttcgtgcaagggtctcgtggcatgcttgcaccc 576

```

```

Query: 405 aagctggcgctcagcacagcaatcaacacacagggggtttcatgccaaatcagccg 458
          |||
Sbjct: 577 aagctggcgctcagcacagcaatcaacacacagggggtttcatgccaaatcagccg 630

```

>GESFSQ - GESR (REAÇÃO GES R) ED sequence exported from chromatogram file
 CNGTTTGGGNCGTNCTAAAAGNTGTCGCGTCTCCCGTTTGGTTTCCGATCAGCCACCTCT
 CAATGGTGTGGGTCGAGGTGGACGTCAGTGCGCCGCATAGAGGACTTTAGCCACAGTAC
 GTGCCATAGCAATAGGCGTAGTTGTATCTCTGAGGTCGCCAGGTGTGTTGTCGCCATCT
 CCGGCTCTTTCCGGTCTAGCCGACTCACAGAGTCGCCAATTTTACGAAAATACTGCGTCA
 TTGCAGCAGGTCCGCCAATTTCTCTCAGTAAGAGGTTAGTAGCCCCATTGTCGCTAAGCT
 GCACCGCAGCTTGCGCTGCCTCGAGAACCGTCATGTGTCCCAGTGTAGAAACCGCTCCG
 TGGCAGGAGACCATTCGACGATCATGTCCGGCCCATATGAAAGTTTTCGATCCCCCGCT
 CGGTGCCTGAGTCAATTCTTTCAAAGACCAGCGCGGCTAGCGGAAACTTGAACGTTGAGC
 ACATTGCAAAACGCTGCGCCATTTCGGTGGCCCGGACGATCTCTCCTTGGGGATCGACGA
 TCGCAACACCGATCTGAGCTGCTTTTTCGCGCTCTAGCTTCTCAAGATCGGTCTTGAAGG
 TTAATTTTTCGACGCATATGCAGAGTGAGCGATCCCTGCCAGTAATAGTGCCTGAATGA
 AGCGCATGGTGATCCCTTGAGATGGAAATTATCTTTGTACGCCGCTAACGCCCAAGTT
 CAGGGGCGCGCNGTGGGCGTGGCCGAAACNN

> [gi|18958399|gb|AF355189.1](#) *Pseudomonas aeruginosa* integron In60
 integrase IntI1 (intI1),
 aminoglycoside 3-N-acetyltransferase/aminoglycoside 6'-N-
 acetyltransferase
 fusion protein (aac(3)-Ib/aac(6')-Ib), and beta-lactamase
 GES-1 (blages-1) genes, complete cds
 Length=3525

Score = 1368 bits (690), Expect = 0.0
 Identities = 690/690 (100%), Gaps = 0/690 (0%)
 Strand=Plus/Minus

Query	23	TGTCGCGTCTCCCGTTTGGTTTCCGATCAGCCACCTCTCAATGGTGTGGGTCGAGGTGGA	82
Sbjct	3187	TGTCGCGTCTCCCGTTTGGTTTCCGATCAGCCACCTCTCAATGGTGTGGGTCGAGGTGGA	3128
Query	83	CGTCAGTGCGCCGCCATAGAGGACTTTAGCCACAGTACGTGCCATAGCAATAGGCGTAGT	142
Sbjct	3127	CGTCAGTGCGCCGCCATAGAGGACTTTAGCCACAGTACGTGCCATAGCAATAGGCGTAGT	3068
Query	143	TGTATCTCTGAGGTCGCCAGGTGTGTGTGTCGCCCATCTCCGGCTCTTTCCGGTCTAGCCG	202
Sbjct	3067	TGTATCTCTGAGGTCGCCAGGTGTGTGTGTCGCCCATCTCCGGCTCTTTCCGGTCTAGCCG	3008
Query	203	ACTCACAGAGTCGCCAATTTTACGAAAATACTGCGTCATTGCAGCAGGTCCGCCAATTTTC	262
Sbjct	3007	ACTCACAGAGTCGCCAATTTTACGAAAATACTGCGTCATTGCAGCAGGTCCGCCAATTTTC	2948
Query	263	TCTCAGTAAGAGGTTAGTAGCCCCATTGTTCGCTAAGCTGCACCGCAGCTTGCCTGCCTC	322
Sbjct	2947	TCTCAGTAAGAGGTTAGTAGCCCCATTGTTCGCTAAGCTGCACCGCAGCTTGCCTGCCTC	2888
Query	323	GAGAACCGTCATGTGTCCCGATGCTAGAAACCGCTCCGTGGCAGGAGACCATTTCGACGAT	382
Sbjct	2887	GAGAACCGTCATGTGTCCCGATGCTAGAAACCGCTCCGTGGCAGGAGACCATTTCGACGAT	2828
Query	383	CATGTCCGGCCCATATGAAAGTTTTCGATCCCCCGCTCGGTGCCTGAGTCAATTCTTTTC	442
Sbjct	2827	CATGTCCGGCCCATATGAAAGTTTTCGATCCCCCGCTCGGTGCCTGAGTCAATTCTTTTC	2768
Query	443	AAAGACCAGCGCGGCTAGCGGAAACTTGAACGTTGAGCACATTGCAAAACGCTGCGCCAT	502
Sbjct	2767	AAAGACCAGCGCGGCTAGCGGAAACTTGAACGTTGAGCACATTGCAAAACGCTGCGCCAT	2708
Query	503	TCGGTGGCCCGCGACGATCTCTCCTTGGGGATCGACGATCGCAACACCGATCTGAGCTGC	562
Sbjct	2707	TCGGTGGCCCGCGACGATCTCTCCTTGGGGATCGACGATCGCAACACCGATCTGAGCTGC	2648
Query	563	TTTTTTCGCGCTCTAGCTTCTCAAGATCGGTCTTGAAGGTTAATTTTTTCCGACGCATATGC	622
Sbjct	2647	TTTTTTCGCGCTCTAGCTTCTCAAGATCGGTCTTGAAGGTTAATTTTTTCCGACGCATATGC	2588

```

Query   623   AGAGTGAGCGATCCCTGCCAGTAATAGTGCGTGAATGAAGCGCATGGTGATCCCTTGAGA   682
          |||
Sbjct   2587  AGAGTGAGCGATCCCTGCCAGTAATAGTGCGTGAATGAAGCGCATGGTGATCCCTTGAGA   2528

```

```

Query   683   TGGAAATTATCTTTGTACGCCCGTCTAACG   712
          |||
Sbjct   2527  TGGAAATTATCTTTGTACGCCCGTCTAACG   2498

```

AACA4 F X AACA4 R

Score = 821 bits (427), Expect = 0.0
 Identities = 427/427 (100%), Gaps = 0/427 (0%)
 Strand=Plus/Plus

```

Query   10   GTAACCACCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTTCGAGCGA   69
          |||
Sbjct   292   GTAACCACCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTTCGAGCGA   351

```

```

Query   70   ACACGCAGTGATGCCTAACCCCTTCCATCGAGGGGGACGTCCAAGGGCTGGCGCCCTTGGC   129
          |||
Sbjct   352   ACACGCAGTGATGCCTAACCCCTTCCATCGAGGGGGACGTCCAAGGGCTGGCGCCCTTGGC   411

```

```

Query   130   CGCCCCCTCATGTCAAACGTTAGGCCCTCGCGTAAATGCACCGCTTGCGCAGGCCAAGCGA   189
          |||
Sbjct   412   CGCCCCCTCATGTCAAACGTTAGGCCCTCGCGTAAATGCACCGCTTGCGCAGGCCAAGCGA   471

```

```

Query   190   GCCACCTCCTCGGTGGCTACCAGCAGCCAGGCATGCGCTTCTTGGTTCTGTGCAAGGTCTC   249
          |||
Sbjct   472   GCCACCTCCTCGGTGGCTACCAGCAGCCAGGCATGCGCTTCTTGGTTCTGTGCAAGGTCTC   531

```

```

Query   250   GTGGCATGCTTGCATCCAAGCTGGCGTCAGCACAGCAATCAACACACAGGGGTTTCATGC   309
          |||
Sbjct   532   GTGGCATGCTTGCATCCAAGCTGGCGTCAGCACAGCAATCAACACACAGGGGTTTCATGC   591

```

```

Query   310   CAAATCAGCCGCATGCCACCATCGGCCCTAACTGTGCGTTGCAGGGGACGCCTCCACCAGT   369
          |||
Sbjct   592   CAAATCAGCCGCATGCCACCATCGGCCCTAACTGTGCGTTGCAGGGGACGCCTCCACCAGT   651

```

```

Query   370   GCGCGCTTCGGCCACGCCAAGCGCGCGCCCTGAACTTGGGCGTTAGGCATCACAAAGTA   429
          |||
Sbjct   652   GCGCGCTTCGGCCACGCCAAGCGCGCGCCCTGAACTTGGGCGTTAGGCATCACAAAGTA   711

```

```

Query   430   CAGCATC   436
          |||
Sbjct   712   CAGCATC   718

```

>PS1 AACA4 F EDIT 2005 (PAR C sequence exported from chromatogram file
 NAANAGTCGGTAACCACCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCA
 TTCGAGCGAACACGCAGTGATGCCTAACCCCTTCCATCGAGGGGGACGTCCAAGGGCTGGC
 GCCCTTGGCCGCCCTCATGTCAAACGTTAGGCCCTCGCGTAAATGCACCGCTTGCGCAG
 GCCAAGCGAGCCACCTCCTCGGTGGCTACCAGCAGCCAGGCATGCGCTTCTTGGTTCTGTG
 CAAGGTCTCGTGGCATGCTTGCATCCAAGCTGGCGTCAGCACAGCAATCAACACACAGGG

GTTTCATGCCAAATCAGCCGCATGCCACCATCGGCCTAACTGTGCGTTGCAGGGGACGCC
 TCCACCAGTGC GCGCTTCGGCCACGCCAAGCGCGCGCCCTGAACTTGGGCGTTAGGCAT
 CACAAAGTACAGCATCGTGACCAACAGCAGCGATTCCGTCACACTGCGCCTCATGACTGA
 GCATGACCTTGCGATGCTCTATGAGTGNANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
 NNN
 NNN
 NNN
 NNN
 NNN

> [gi|34014739|gb|AY348316.1|](#) *Salmonella enterica* subsp. *enterica* serovar
 Typhi class I integron
 DNA integrase (intI1) gene, partial cds; aminoglycoside
 acetyltransferase (aacA6'-Ib) and oxacillinase (blaOXA-1-like)
 genes, complete cds; quaternary ammonium compound (qacEdelta1)
 gene, partial cds; and unknown gene
 Length=2736

Score = 287 bits (145), Expect = 3e-74
 Identities = 145/145 (100%), Gaps = 0/145 (0%)
 Strand=Plus/Plus

Query	10	GTAACCACCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTCGAGCGA	69
Sbjct	1025	GTAACCACCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTCGAGCGA	1084
Query	70	ACACGCAGTGATGCCTAACCCCTTCCATCGAGGGGGACGTCCAAGGGCTGGCGCCCTTGGC	129
Sbjct	1085	ACACGCAGTGATGCCTAACCCCTTCCATCGAGGGGGACGTCCAAGGGCTGGCGCCCTTGGC	1144
Query	130	CGCCCCCTCATGTCAAACGTTAGGCC	154
Sbjct	1145	CGCCCCCTCATGTCAAACGTTAGGCC	1169

Score = 182 bits (92), Expect = 1e-42
 Identities = 95/96 (98%), Gaps = 0/96 (0%)
 Strand=Plus/Plus

Query	412	GTTAGGCATCACAAAGTACAGCATCGTGACCAACAGCAGCGATTCCGTCACACTGCGCCT	471
Sbjct	523	GTTAGGCATCACAAAGTACAGCATCGTGACCAACAGCACCATTCCGTCACACTGCGCCT	582
Query	472	CATGACTGAGCATGACCTTGCGATGCTCTATGAGTG	507
Sbjct	583	CATGACTGAGCATGACCTTGCGATGCTCTATGAGTG	618

>PS1 AAC4 R EDIT 2006 (PAR COM sequence exported from chromatogram file
 CATGCTCAGTCATGAGGCGCAGTGTGACGGAATCGTTGCTGTTGGTCACGATGCTGTACT
 TTGTGATGCCTAACGCCCAAGTTCAGGGGCGCGCTTGGCGTGGCCGAAGCGCGCACTG
 GTGGAGGCGTCCCCTGCAACGCACAGTTAGGCCGATGGTGGCATGCGGCTGATTTGGCAT
 GAAACCCCTGTGTGTTGATTGCTGTGCTGACGCCAGCTTGGATGCAAGCATGCCACGAGA
 CCTTGCACGAACCAAGAAGCGCATGCCTGGCTGCTGGTAGCCACCGAGGAGGTGGCTCGC

TTGGCCTGCGCAAGCGGTGCATTTACGCGAGGGCCTAACGTTTGACATGAGGGGCGGCCA
 AGGGCGCCAGCCCTTGACGTCCCCCTCGATGGAAGGGTTAGGCATCACTGCGTGTTCGC
 TCGAATGCCTGGCGTGTGTTGAACCATGTACACGGCTGGACCATCTGGGGTGGTTACGGTA
 CCTTGCCTCTCAACCCCGCTTTCTAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
 NNN
 NNN
 NNN
 NNN
 NNN
 NNN
 NNN

> [gi|34014739|gb|AY348316.1|](#) *Salmonella enterica* subsp. *enterica* serovar Typhi
 class I integron
 DNA integrase (intI1) gene, partial cds; aminoglycoside
 acetyltransferase (aacA6'-Ib) and oxacillinase (blaOXA-1-like)
 genes, complete cds; quaternary ammonium compound (qacEdelta1)
 gene, partial cds; and unknown gene
 Length=2736

Score = 337 bits (170), Expect = 4e-89
 Identities = 173/174 (99%), Gaps = 0/174 (0%)
 Strand=Plus/Minus

Query	332	GGCCTAACGTTTGACATGAGGGGCGGCCAAGGGCGCCAGCCCTTGACGTCCCCCTCGAT	391
Sbjct	1169	GGCCTAACGTTTGACATGAGGGGCGGCCAAGGGCGCCAGCCCTTGACGTCCCCCTCGAT	1110
Query	392	GGAAGGGTTAGGCATCACTGCGTGTTCGCTCGAATGCCTGGCGTGTGTTGAACCATGTACA	451
Sbjct	1109	GGAAGGGTTAGGCATCACTGCGTGTTCGCTCGAATGCCTGGCGTGTGTTGAACCATGTACA	1050
Query	452	CGGCTGGACCATCTGGGGTGGTTACGGTACCTTGCCTCTCAACCCCGCTTTCT	505
Sbjct	1049	CGGCTGGACCATCTGGGGTGGTTACGGTACCTTGCCTCTCAAACCCCGCTTTCT	996

Score = 139 bits (70), Expect = 2e-29
 Identities = 73/74 (98%), Gaps = 0/74 (0%)
 Strand=Plus/Minus

Query	1	CATGCTCAGTCATGAGGCGCAGTGTGACGGAATCGTTGCTGTTGGTCACGATGCTGTACT	60
Sbjct	596	CATGCTCAGTCATGAGGCGCAGTGTGACGGAATCGTTGCTGTTGGTCACGATGCTGTACT	537
Query	61	TTGTGATGCCTAAC	74
Sbjct	536	TTGTGATGCCTAAC	523

ORF126 X AACA4 F (PCR INVERSO PAR COM AACA4 R)

Score = 519 bits (270), Expect = 1e-144
 Identities = 270/270 (100%), Gaps = 0/270 (0%)
 Strand=Plus/Plus

Query	1	GTTAGGCCCTCGCGTAAATGCACCGCTTGCGCAGGCCAAGCGAGCCACCTCCTCGGTGGC	60
Sbjct	147	GTTAGGCCCTCGCGTAAATGCACCGCTTGCGCAGGCCAAGCGAGCCACCTCCTCGGTGGC	206
Query	61	TACCAGCAGCCAGGCATGCGCTTCTTGGTTCGTGCAAGGTCTCGTGGCATGCTTGCATCC	120
Sbjct	207	TACCAGCAGCCAGGCATGCGCTTCTTGGTTCGTGCAAGGTCTCGTGGCATGCTTGCATCC	266
Query	121	AAGCTGGCGTCAGCACAGCAATCAACACACAGGGGTTTCATGCCAAATCAGCCGCATGCC	180
Sbjct	267	AAGCTGGCGTCAGCACAGCAATCAACACACAGGGGTTTCATGCCAAATCAGCCGCATGCC	326
Query	181	ACCATCGGCCTAACTGTGCGTTGCAGGGGACGCCTCCACCAGTGCGCGCTTCGGCCACGC	240
Sbjct	327	ACCATCGGCCTAACTGTGCGTTGCAGGGGACGCCTCCACCAGTGCGCGCTTCGGCCACGC	386
Query	241	CAAGCGCGCGCCCCTGAACTTGGGCGTTAG	270
Sbjct	387	CAAGCGCGCGCCCCTGAACTTGGGCGTTAG	416

Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 21 May 2013

<https://doi.org/10.5256/f1000research.903.r961>

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Béatrice Berçot

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Paris, France

This work is conducted to address the transcription of the fused gene cassette *bla*_{GES-1}/*aacA4*. This arrangement is rare but not exceptional in a class 1 integron. In this work, the experimentation confirmed that the three gene cassettes harbored in this class 1 integron were expressed together from a unique polycistronic transcript.

It seems that the authors have observed a circular form containing the *gcu14* gene cassette associated with the *aacA4* gene cassette. Could they explain this arrangement? Is it possible that the *Pseudomonas* isolates harbored another class 1 integron containing the *gcu14* gene followed by the *aacA4* gene?

The authors have to provide in the paper the classical length of the *bla*_{GES-1} *attC* site, which is 110bp and contained the 1L, 2L, 2R and 1R sequences-shown in bold.

TTGACGCCC **GTCTAAC** AATTC **GTTCAAGC**
CGACGTTGCTTCGTGGCGGCGCTTGCGTGCTACGCTAAGCTTCGCACGCCGCTTGCCACTGCGCACC GCG
GCTTAAC TCAGGC **GTTAGNC**

In the *Pseudomonas* SP26 and PS1 isolates, the *bla*_{GES-1} *attC* site is reduced to the 6 bp of 1 L box.

TTGACGCCC **GTCTAA** AACAAAGTTAGGCATCACAAAGTACAGCATC **GTG**

So, the distance from of the stop of *bla*_{GES-1} to the start codon of the *aacA4* gene is 46bp. The translational start codon of *aacA4* gene is erroneous in the Genbank data base ([DC236170](#)). Indeed, it has been determined by N-terminal amino acid sequencing to be a GTG (in italic in the sequence above) and the beginning of the cassette was 24 bp before [[Hanau-Bercot B. et al., 2002](#)].

-Could the Genbank annotation be modified as explained above?

-The Appendix is not necessary and should be deleted.

-In the sentence page 4, line 13, "The single copy of *rpsL* gene of *Pseudomonas*...", should be

replaced by “the *rpsL* gene of *Pseudomonas* (see Bodilis et al., 2012)

-In the conclusion, page 6, change “never” to “rarely”.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Aug 2015

Erica Fonseca, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

It seems that the authors have observed a circular form containing the *gcu14* gene cassette associated with the *aacA4* gene cassette. Could they explain this arrangement? Is it possible that the *Pseudomonas* isolates harbored another class 1 integron containing the *gcu14* gene followed by the *aacA4* gene?

Response: Yes, the presence of the *gcu14*-*aacA4* circular form indicates that this strain harbours a second integron composed by the *gcu14*-*aacA4* arrangement. However, this result does not invalidate our main conclusion, which is that the truncated GES-1 *attC* site is not functional and this gene is only mobilized when recombination occurs in *aacA4 attC*. A brief explanation was included in the text (Results section).

The authors have to provide in the paper the classical length of the *bla*_{GES-1} *attC* site, which is 110bp and contained the 1L, 2L, 2R and 1R sequences-shown in bold.

Response: A figure showing the truncated and the complete canonical *attC* from GES1 was included in the new version.

So, the distance from of the stop of *bla*_{GES-1} to the start codon of the *aacA4* gene is 46bp. The translational start codon of *aacA4* gene is erroneous in the Genbank data base (DC236170). Indeed, it has been determined by N-terminal amino acid sequencing to be a GTG (in italic in the sequence above) and the beginning of the cassette was 24 bp before [Hanau-Bercot B. et al., 2002].

-Could the Genbank annotation be modified as explained above?

Response: We performed this modification and the updated sequence regarding the beginning of *aacA4* gene is already accessible on GenBank database under the same accession number (DQ236170).

-The Appendix is not necessary and should be deleted.

Response: The appendix is only for helping referees in their evaluation. It will not be published.

-In the sentence page 4, line 13, “The single copy of *rpsL* gene of *Pseudomonas*...”, should be replaced by “the *rpsL* gene of *Pseudomonas* (see Bodilis et al., 2012).

Response: this was modified in the new version of the manuscript.

In the conclusion, page 6, change “never” to “rarely”.

Response: this was modified in the new version of the manuscript.

Competing Interests: There is no competing interests

Reviewer Report 02 May 2013

<https://doi.org/10.5256/f1000research.903.r920>

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Sally Partridge

Sydney West Area Health Service, Sydney, NSW, Australia

This paper looks at expression of genes in a gene cassette array that includes a fused cassette and at the excision of cassette from this array. Polycistronic transcripts from cassette arrays and the excision of other fused cassettes have previously been noted by others, as cited in this paper. The sequences in Appendix 1 need to be annotated properly, rather than just the results of searches given, and the point illustrated by each sequence needs to be explained to make it possible to assess whether they support the conclusions drawn in the paper.

Some reorganisation (moving some information in the Results and Discussion to the Introduction) would help to make the paper easier to follow and the Conclusions section is very short and could be expanded. The English could also be improved and the manuscript checked for typos etc.

A few specific points also need correcting or clarifying:

- Abstract Line 5- fused cassettes do not always have fused orfs (e.g. *aacA1/orfG*)
- Abstract 6th Line from end and Results and Discussion p. 5 1st Line of 2nd paragraph – what is in the *gcu14-aacA4* circular form? Should this be *blaGES-1/aacA4*?
- Materials and Methods Line 1 – this needs rewording to explain that *gcu* is a gene cassette of unknown function.
- Results and Discussion p. 4 – the fusion here is the type where part of the *blaGES-14 attC* site is replaced by part of the *attI1* site (see ref. 14). The LH and RH domains are part of the *attC* site, rather than flanking it. The 5' UTR contains 6 bp of the *attC* site.
- Results and Discussion p. 5 – most of the 1L core site and the 1R core site of the *blaGES attC* site are present.
- Results and Discussion p. 6 – the deletion of part of the *attC* site doesn't bring the *aacA4* gene much closer to Pc and ref. 14 makes slightly different point (that expression of the downstream cassette may be enhanced).

Formatting “14” of *gcu14* and “A4” of *aacA4* should be in italics. Transposon, gene and species names etc are not correctly formatted in the references.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Aug 2015

Erica Fonseca, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

The sequences in Appendix 1 need to be annotated properly, rather than just the results of searches given, and the point illustrated by each sequence needs to be explained to make it possible to assess whether they support the conclusions drawn in the paper.

Response: The sequence resulted from inverse PCR product, showing the circular form of the *gcu14-blaGES-1-aacA4* array, was submitted to GenBank under accession number KT336477. This information was included in the text. Moreover, we provided a new figure showing a schematic representation of primer targeting sites used in inverse PCR in order to make easier for the reviewers comprehend our strategy and results.

Some reorganisation (moving some information in the Results and Discussion to the Introduction) would help to make the paper easier to follow and the Conclusions section is very short and could be expanded. The English could also be improved and the manuscript checked for typos etc.

Response: We agree that some points from results and discussion section would be more adequate in the introduction section, and they were moved in the new version of the manuscript (Page 3, lines 59-60 and 65-70; page 4, lines 71-76 in the new version). The English was reviewed by a native spoken-English, and the conclusion was expanded.

A few specific points also need correcting or clarifying:

Abstract Line 5- fused cassettes do not always have fused orfs (e.g. *aacA1/orfG*)

Response: We agree and it was modified: We affirmed that only in some cases fused orfs can be created.

Abstract 6th Line from end and Results and Discussion p. 5 1st Line of 2nd paragraph – what is in the *gcu14-aacA4* circular form? Should this be *blaGES-1/aacA4*?

Response: The presence of the *gcu14-aacA4* circular form indicates that this strain harbours a second integron composed by the *gcu14-aacA4* arrangement. However, this result does not invalidate our main conclusion, which is that the truncated *GES-1 attC* site is not functional and this gene is only mobilized when recombination occurs in *aacA4 attC*. A brief explanation was included in the text (Results section).

Materials and Methods Line 1 – this needs rewording to explain that *gcu* is a gene cassette of unknown function.

Response: It was included in the text.

Results and Discussion p. 4 – the fusion here is the type where part of the *blaGES-1 attC* site is replaced by part of the *attI1* site (see ref. 14). The LH and RH domains are part of the *attC* site, rather than flanking it. The 5' UTR contains 6 bp of the *attC* site.

Response: We change the text emphasizing that the attC was replaced by part of attI1. The referee is right; LH and RH domains are part of the attC site, rather than flanking it. This general idea concerning attC site and its domains was modified throughout the text.

Results and Discussion p. 5 – most of the 1L core site and the 1R core site of the blaGES attC site are present.

Response: In fact, only the 1L core site is present in the truncated attC. We included a figure (figure 2) in this new version showing this.

Results and Discussion p. 6 – the deletion of part of the attC site doesn't bring the aacA4 gene much closer to Pc and ref. 14 makes slightly different point (that expression of the downstream cassette may be enhanced).

Response: The referee is right. This statement was removed.

Formatting "14" of *gcu14* and "A4" of *aacA4* should be in italics. Transposon, gene and species names etc are not correctly formatted in the references.

Response: All formatting errors were properly corrected.

Competing Interests: There is no competing interests

Comments on this article

Version 1

Author Response 05 Aug 2015

Erica Fonseca, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

Dear Authors,

Besides the comments already made by the reviewers, I would like to specify some points:

- The C to G mutations that converts the usual weak PcW variant into PcWTGN-10 has been shown to increase (instead of 'decrease', first part of the Results section).

Response: This sentence was removed.

- I have not checked which IntI1 had been used for the excision assay, usually these are made with the most efficient "PcW" IntI1 (IntI1R32_H39); note that in our PLoS Genetics paper ([Jove et al. 2010](#)) we evidenced the IntI1 "PcWTGN-10" (IntIP32_H39) were much less efficient for excision of gene cassettes, which could had been taken into account in your discussion.

Response: As properly noticed by you, our Pc configuration is the weak one, and this was changed

in the text. However, we agree that including this discussion we will improve our manuscript (lines 182 and 184).

- The hypothesis of a synergistic effect between the Pc and P(gcu14) tandem promoters does not fit the observed phenotypes: since the resulting level of transcription observed for the downstream genes is lower I suggest that some transcriptional interferences occur rather than synergistic interactions. By the way in absence of any experimental evidence for the functionality of the gcu14 promoter, such hypothesis is overstated. It would have been interesting to check the level of transcription of the blaGES-1,aacA4 gene cassette when gcu14 is deleted.

Response: we totally agree with this observation, in fact our conclusion is overstated since no experimental assay was performed to test the promoter synergy. Therefore, we remove this part from the text. We also agree that would be interesting to verify the expression of the fused cassette in the absence of gcu14, however it was not the focus of this paper.

- First, the class 1 integron sequence of the PS1 strain in Genbank DQ236170 does not display the rare C to G mutation that converts a weak "PcW" promoter into a stronger "PcWTGN-10" as stated in the paper. Consequently you should write "the weak PcW configuration" instead of "weak PcWTGN-10". Also it means that its own IntI1 integrase is likely to be optimally efficient.

Response: Dr. Jové is absolutely right, the DQ236170 accession number presented the PcW configuration and not the PcWTGN-10 as stated in the manuscript, we apologize about this mistake. This was corrected in the text.

- Then, the sequence of the class 1 integron as deposited in the Genbank does not display the complete array of gene cassette (the end of the attCaacA4 is not available) which means that it is ambiguous whether there is a downstream third gene cassette or not. Consistently this array of gene cassette, although a novel one, has not been numbered in the INTEGRALL database (<http://integrall.bio.ua.pt/?acc=DQ236170>)

Response: We know that aacA4 was the last cassette in the array because this variable region was obtained using primers annealing in the attI1 and in the beginning of qacEΔ1 from 3'CS. It is true that the aacA4 attC is not completed in the GenBank accession number DQ236170. Therefore, spite of the incomplete attC sequence we are sure that the aacA4 is the last cassette from this array.

- Lastly, as an element of reply to the reviewer Dr Bercot, I would like to notify that the so-called gcu14 GC has not been reported in other reports except in an environmental strain of Citrobacter (and the sequence is not 100% identical, Genbank FM998050).

Response: thanks for this observation. It was included in the text.

Competing Interests: There is no competing of interests

Reader Comment 23 May 2013

Thomas Jové, LGPB, Belgium

Dear authors,

I would like to add some further comments:

- First, the class 1 integron sequence of the PS1 strain in Genbank DQ236170 does not display the rare C to G mutation that converts a weak "PcW" promoter into a stronger "PcWTGN-10" as stated in the paper. Consequently you should write "the weak PcW configuration" instead of "weak PcWTGN-10". Also it means that its own IntI1 integrase is likely to be optimally efficient.

- Then, the sequence of the class 1 integron as deposited in the Genbank does not display the complete array of gene cassette (the end of the attCaacA4 is not available) which means that it is ambiguous whether there is a downstream third gene cassette or not. Consistently this array of gene cassette, although a novel one, has not been numbered in the INTEGRALL database (<http://integrall.bio.ua.pt/?acc=DQ236170>)

- Lastly, as an element of reply to the reviewer Dr Bercot, I would like to notify that the so-called gcu14 GC has not been reported in other reports except in an environmental strain of *Citrobacter* (and the sequence is not 100% identical, Genbank FM998050).

Competing Interests: None

Reader Comment 17 May 2013

Thomas Jové, LGPB, Belgium

Dear Authors,

Besides the comments already made by the reviewers, I would like to specify some points:

- The C to G mutations that converts the usual weak PcW variant into PcWTGN-10 has been shown to increase (instead of 'decrease', first part of the Results section).
- I have not checked which IntI1 had been used for the excision assay, usually these are made with the most efficient "PcW" IntI1 (IntI1R32_H39); note that in our PLoS Genetics paper ([Jove et al. 2010](#)) we evidenced the IntI1 "PcWTGN-10" (IntIP32_H39) were much less efficient for excision of gene cassettes, which could had been taken into account in your discussion.
- The hypothesis of a synergistic effect between the Pc and P(gcu14) tandem promoters does not fit the observed phenotypes: since the resulting level of transcription observed for the downstream genes is lower I suggest that some transcriptional interferences occur rather than synergistic interactions. By the way in absence of any experimental evidence for the functionality of the gcu14 promoter, such hypothesis is overstated. It would have been interesting to check the level of transcription of the blaGES-1,aacA4 gene cassette when gcu14 is deleted.
- Some years ago [Jacquier et al. \(2009\)](#) published data suggesting that attC sites rather influence the level of translation between each gene cassette while the level of transcription

was unaffected.

Competing Interests: None

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