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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**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 *gcu*14-*bla*GES-1/*aac*A4 gene cassette array, which harbours a fused gene cassette represented by *bla*GES-1/*aac*A4. 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/*aac*A4 fused cassette. Also, inverse polymerase chain reactions (PCR) were performed to detect the free circular forms of *gcu*14, *bla*GES-1 and *aac*A4. The Northern blot and real time RT-PCR revealed a polycistronic transcription, in which the fused cassette *bla*GES-1/*aac*A4 is transcribed as a unique gene, while *gcu*14 (with a canonical *attC* recombination site) has a monocistronic transcription. The *gcu*14 cassette, closer to the weak configuration of cassette promoter (Pc), had a higher transcription level than *bla*GES-1/*aac*A4, indicating that the cassette position impacts the transcript amounts. The presence of ORF-11 at *attI*1, immediately preceding *gcu*14, and of a Shine-Dalgarno sequence upstream *bla*GES-1/*aac*A4 composes a scenario for the occurrence of array translation. Inverse PCR generated amplicons corresponding to *gcu*14, *gcu*14-*aac*A4 and *gcu*14-*bla*GES-1/*aac*A4 free circular forms, but not to *bla*GES-1 and *aac*A4 alone, indicating that the GES-1 truncated *attC* is not substrate of integrase activity and that these genes are mobilized.
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
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 integron\(^1\). Moreover, naturally occurring integrons may have a second promoter (P2), which is activated by the insertion of three G residues between -35 and -10 hexamers\(^2\). Gene cassettes are generally promoterless units associated with a recombination site (\(\text{attC}\) or 59-be), which confers the ability of each structure to be mobilized independently\(^2\). In addition, \(\text{attC}\) sites regulate the translation of downstream cassettes due to their peculiar sequences composed by imperfect inverted repeats. The formation of stem-loop structures by \(\text{attC}\) sites prevents ribosome progression throughout mRNA, reflecting in a decreased expression of more distal genes regarding Pc\(^3\).

Although rare, fused cassettes may be generated by partial or total loss of the first \(\text{attC}\), retaining both complete coding regions and, therefore, creating permanent gene arrays comparable to bacterial operons\(^4\). The functionality of such structures has been indirectly inferred by the resistance profile of transformants carrying the fusion\(^5\); however, the transcription itself has never been verified. This study showed the dynamics of fused cassette mobilization, the co-transcription of the \(\text{gcu14-bla}\_\text{GES-1}/\text{aacA4}\) cassette array and the effect of cassette position on transcription levels in \textit{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), \textsc{gcu14}, followed by the fused cassette \(\text{bla}\_\text{GES-1}/\text{aacA4}\), created by partial loss of GES-1 \(\text{attC}\), were present in integrons from clinical \(\text{P} \cdot \text{aeruginosa}\) 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 \textsc{gcu14-bla}\_\text{GES-1}/\text{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 \(\text{bla}\_\text{GES-1}\) 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 sequence (5’ - 3’) | Size (bp) | Target |
|-----------------------------|---------------------------|-----------|--------|
| Ges F                       | GCCTGTTTGGCAATGTGCTC      | 519       | Internal fragment of bla\_GES gene |
| Ges R                       | CCAGTTTTTCTCTCCAACAAACC   |           |        |
| Gcu14 FSQ                   | AGCAATCAACACACAGGGG       | 130       | gcu14 circular form |
| Gcu14 RSQ                   | CTGCGGTAATGCGACCGCTT     |           |        |
| GES FSQ                     | CAAGTTATTACACAAACTCAT     | 110       | bla\_GES-1 circular form |
| GES RSQ                     | AGTGGGTAATGGAAGCGCAT     |           |        |
| AACA4 FSQ                   | GCCAGGCATTGCAGGCAAAACAC  | 188       | aacA4 circular form |
| AACA4 RSQ                   | AATTACACCTCATCATAAGGCG   |           |        |

Primer and probes for real time PCR (TaqMan)

| Primer | Primer sequence (5’ - 3’) | Size (bp) | Target |
|--------|----------------------------|-----------|--------|
| RpsL F | GCCCTGGGCTGCAAAAAACCT  | 67        | rpsL transcripts |
| RpsL R | TTTCCGGCGTGGTGTTAT      |           |        |
| Probe  | TCAGGGCATGATGCCACC      |           |        |
| Gcu14 F | CATCCGCTCTTTGCGGCTT   | 56        | gcu14 transcripts |
| Gcu14 R | AGCAGGGTTTCTTGTTCGT   |           |        |
| Probe  | ATGCCACAGGACCTT         |           |        |
| Ges F2 | GTGCAAGCTTACTGACATTAGG | 99        | bla\_GES-1 transcripts |
| Ges R2 | CACAGAGTGCACATGCAATTTT |           |        |
| Probe  | CCGAAGCCAGGACATTG       |           |        |
| AacA4 F | CAACGCTTTTGCGCGCGAGAGT | 59        | aacA4 transcripts |
| AacA4 R | TCGGCTTACACCTCATTG     |           |        |
| Probe  | ATCCGACCAGGACCTGCGGC   |           |        |
| Ges F3 | TCTTGTGACAGGCAGAACATAG | 134       | Polycistronic transcripts |
| AacA4 R2 | TCTGACACGACCCAAGGTC    |           |        |
| Probe  | TCCGTGACACGACTGCGCTC   |           |        |

\(^a\) Gcu RSQ - AACA4 FSQ and GES RSQ – AACA4 FSQ were used in combination to obtain the full length and the \(\text{bla}\_\text{GES-1}/\text{aacA4}\) fused cassette circular forms, respectively.
Labelling kit (GE Healthcare) and hybridized with the target RNA immobilized on the Hybond-N\(^\text{\textregistered}\) membrane as recommended. The chemiluminescence was detected with the CDP-Star detection reagent (GE Healthcare) according to manufacturer’s instructions. 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\(^{-\Delta\text{C}T}\), where CT is the value corresponding to the crossing point of the amplification curve with the threshold line, and \(\Delta\text{C}T=\text{C}T\) target gene minus CT 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 \(z\) 2.0, taking into account the standard deviation intervals.

In order to induce cassette excision from integrons, PS1 and PS26 strains 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 \(\mu\)L of 10X PCR buffer (1X final concentration); 1 \(\mu\)L of 10mM dNTP mixture (0.2 mM each); 1.5 \(\mu\)L of 50mM MgCl\(_2\) (1.5 mM final concentration); 2 \(\mu\)L of 15 \(\mu\)M of each primer (30 \(\mu\)M each); 100 ng of template DNA; 0.3 \(\mu\)L 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 P\(_c\) configuration (Pc\(_W\)\(_{TGN-10}\)), which presents a C to G mutation 2 bp upstream of the -10 hexamer that causes an abrupt decrease in promoter strength as reported previously\(^5\). Considering that transcription initiates from the P\(_c\) 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 analysis\(^5\). Moreover, this finding gives support to the lack of attC function in terminating transcription of downstream gene cassettes as demonstrated previously\(^6\).

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 mobilization\(^7\). 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 translation\(^8\), 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 linked\(^9\). As demonstrated previously, several stress conditions could evoke the activation of the SOS response resulting in integron-integrase expression\(^10\). Therefore, under...
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 \textit{attC} site, and that these regions are missing in GES-1 \textit{attC}, it is expected that the \textit{bla}\_GES-1/\textit{aac}\_A4 excision occurs only at the \textit{aac}\_A4 \textit{attC} site, and that this structure is excised together as a unique cassette.

Positive results were obtained for the \textit{gcu}\_14, \textit{gcu}\_14\_-\textit{aac}\_A4 and \textit{gcu}\_14\_-\textit{bla}\_GES-1/\textit{aac}\_A4 circular forms, but not for \textit{bla}\_GES-1 and \textit{aac}\_A4 alone, showing that the GES-1 \textit{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 \textit{attC} had also been observed before when associated with \textit{aad}\_A1\_11. However, Ramirez and colleagues\_12 showed that the integrase was able to recognize and mediate excision of a truncated site associated to \textit{aad}\_A1, 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 \textit{gcu}\_14\_-\textit{bla}\_GES-1/\textit{aac}\_A4 transcription (Figure 2). This result was expected since integrons from these two strains have the same backbone, including the Pc promoter, and are at the same genetic environment\_6.

\textit{gcu}\_14, the first cassette in integrons with the weak Pc\_W\_\text{TGN-10} configuration, presented approximately two-fold higher transcription when compared to \textit{bla}\_GES-1 and \textit{aac}\_A4 separately or when the fused cassette \textit{bla}\_GES-1/\textit{aac}\_A4 was considered (Figure 2). The same RQ value obtained for \textit{bla}\_GES-1, \textit{aac}\_A4 and the fusion reveals these two ORFs are transcribed as a unique gene. The lower transcript amount of \textit{bla}\_GES-1/\textit{aac}\_A4 compared to \textit{gcu}\_14 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 \textit{gcu}\_14 (-35 TTGATG [17 bp] -10 TGGTAC) was found 45 bp upstream from its start codon. Therefore, the increased \textit{gcu}\_14 transcription could have resulted from a synergistic effect of this putative promoter with Pc. Considering that this putative promoter was found at \textit{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 integrons\_13, was found at the \textit{attI1} region preceding the TIR-deficient \textit{gcu}\_14 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 \textit{gcu}\_14 start codon. A potential SD sequence was identified 10 bp upstream of the fused cassette \textit{bla}\_GES-1/\textit{aac}\_A4. In addition, the loss of the GES-1 \textit{attC} region, which is involved in stem-loop formation, may enhance the chances of \textit{aac}\_A4 translation, since this \textit{attC} no longer
constitutes a physical barrier to ribosome progression\(^3\). This deletion also then brings the second gene (aac(6')-Ib') closer to P\(_c\). Together, these findings create a scenario for the occurrence of bla\(_{v4}\)-bl\(_{a}_{ES}\)/aac(6') expression in PS1 and PS26, which then provides a possible explanation for their resistance profile to \(\beta\)-lactams and aminoglycosides that has been observed elsewhere\(^6\).

**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.

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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
NNGGGTGGTTGGAAAAANACTGGGAANTTGCCTCTCNNCGGTCAGAAGGAANACACAAC
TCATCTCTGACGCGACAAATAGTTGACGCGCTCTTAAAAACAAAGTTAGCCATCAACAAG
TACAGCACTGTGAACAGCAACAGATTTCCGTACACTGCGCTCATAGATGACATGAC
CTTGGGATGCTCTATAGGAGGCTTAAATCGATCTCATATCGTCGAGTGGTGGGGCGGAGAAG
GAAGACGACCGCGACATTCTGCTAGCATACAGAAAGACTACCTGGCAGGCGGAGAAG
GAGTCCCGTCCTCAATACATGCAATGCTGAATGGAGAGCCGATTGGCTGATGCCAGCTCG
TACGTCTCTTTGAGCCGGGACGGATGTGGGGAGAAAGAACCGGTCCAGGAGTACGC
GGAATAAGAGTTACTGCGCAATGATCACTACTGGCAATTTGGGGAACAGCTG
GTTCGAGCTCTGAGGTGCTCTGTTCAATGATCCGAGTCCACCCAGATTCCAAGATCGC
CCCTCCGCGACAGAATTTGAGGAGCAGACTCTGTTCAAGGGGACGTCGTTCAAGTTAGCC
GTTAGGCGAGAAGTCCGTCACTCCATACATTGCAATGCTGAATGGAGAGCCGATTGGCTGATGC

| 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 (67%), Expect = 0.0
Identities = 684/689 (99%), Gaps = 0/689 (0%)
Strand=Plus/Plus

Query  54    ACACAACTCATCTGGACCGCAGAAATTGTTGACGCGCCCCTCTAAAAACAAAGTTAGCCAT  113
Sbjct  2166  ACACAACTCATCTGGACCGCAGAAATTGTTGACGCGCCCCTCTAAAAACAAAGTTAGCCAT  2225
Query  114   CACAAAGTACAGCATCGTGACCAACAGCAACGATTCCGTCACACTGCGCCTCATGACTGA  173
Sbjct  2226  CACAAAGTACAGCATCGTGACCAACAGCAACGATTCCGTCACACTGCGCCTCATGACTGA  2285
Query  174   GCATGACCTTGGGATGCTCTATAGGAGGCTTAAATCGATCTCATATCGTCGAGTGGTGGGG  233
Sbjct  2286  GCATGACCTTGGGATGCTCTATAGGAGGCTTAAATCGATCTCATATCGTCGAGTGGTGGGG  2345
Query  234   CGGAGAAAGACACGCCCGACACTTTGCTAGCTACAGGAAACAGTACTTGCCAAAGCGTTTT  293
Sbjct  2346  CGGAGAAAGACACGCCCGACACTTTGCTAGCTACAGGAAACAGTACTTGCCAAAGCGTTTT  2405
Query  294   AGCGGAAAGGTCGGTCCTCATTCACTACTTTCCATAGTGGGCAATGGAGGCGAGATTGGGATGC  353
Sbjct  2406  AGCGGAAAGGTCGGTCCTCATTCACTACTTTCCATAGTGGGCAATGGAGGCGAGATTGGGATGC  2465
>GESFSQ-GESR (REAÇÃO AACA4 F) sequence exported from chromatogram file
CGGGNGNCGGTACNCCCNGGANGGTCCAGCTCNNCACATGGGGAGAANACGCCAGGCATT
TTCCCGAACCACCGACTGATGCTCTAACCCTTTCAATCGAGGGGGACGTCCAAGGGCTGGCGC
CCTTGGCCGCCCTCATGTCAAACGTTNNGNNCTCGCGTAAATGCACCGCTTGCGCAGGC
CAAGCGAGCCACCTCCTCGGTGGCTACCAGCAGCCAGGCATGCGCTTCTTGGTTCGTGCA
AGGTCTCGTGGCATGCTTGCATCCAAGCTGGCGTCAGCACAGCAATCAACACACAGGGGT
TTCATGCCAAATCAGCCGCATGCCACCATCGGCCTAACTGTGCGTTGCAGGGGACGCCTC
CACCAGTGCGCGCTTCGGCCACGCCAAGCGCGCGCCCCTGAACTTGGGCGTTAGACGGGC
GTACAAAGATAAATTTCCATCTCAAGGATCATGCCGTCTCATTCATCGCACTATTACTG
GCGAGGGGATGGTCGGCTGCATGGGCATGCGCTTCTTGGTTCGTGCA
AAGCTAGAGCGCGAAAAAGCAGCTCAGATCGGTGTTGCGATCGTCGATCCCCAAGGAGAG
ATCGTCGCGGGCCACCGAAATGGAGCCAGNGTTNNTGCAATGTGCNTNNAANNGG
GGATCGAAAAACTTTTCNNANTNGG

| Query  | 534 | ACGGACCCCCTGCCGCCGAGCAACTTGCGAGCGATCCGATGCTACGAGAAAGCGGGGTTTGA |
| Sbjct  | 2646 | ACGGACCCCCTGCCGCCGAGCAACTTGCGAGCGATCCGATGCTACGAGAAAGCGGGGTTTGA |
| Query  | 594 | GAGGCAAGGTACCGTAACCACACCACAGTGTTCCAGGGCTGACATGTTCTCAAACGAAGCCA |
| Sbjct  | 2706 | GAGGCAAGGTACCGTAACCACACCACAGTGTTCCAGGGCTGACATGTTCTCAAACGAAGCCA |
| Query  | 654 | GCCATTCGCCAGCGAACAGCCAGTATGGCTCCAACCTCCATCGAGGGGACGTCCAAGGGC |
| Sbjct  | 2766 | GCCATTCGCCAGCGAACAGCCAGTATGGCTCCAACCTCCATCGAGGGGACGTCCAAGGGC |
| Query  | 714 | TGCCGCCCTTGGACGCNCCCTGATGTGAAAA |
| Sbjct  | 2826 | TGCCGCCCTTGGACGCNCCCTGATGTGAAAA |

>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  GTTAGACCGGCCGTACAAAGATATAATTTCCATCTCAAGGGATCACTGCGCTTCATTCACG 470
Sbjct 2646  GTTAGACCGGCCGTACAAAGATATAATTTCCATCTCAAGGGATCACTGCGCTTCATTCACG 2705

Query 471  CACTATTACTGGGAGGGATCGCTCACTCTGCATATGCGTCGGAAAAATTAACCTTCAAGA 530
Sbjct 2706  CACTATTACTGGGAGGGATCGCTCACTCTGCATATGCGTCGGAAAAATTAACCTTCAAGA 2765

Query 531  CGATCTTTGAAGCTAAGCGCGGCGGAAGCACTTCAGATCAGTGTTGCTGACGTCGATC 590
Sbjct 2766  CGATCTTTGAAGCTAAGCGCGGCGGAAGCACTTCAGATCAGTGTTGCTGACGTCGATC 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  CGAACACGCAGTGATGCCTAACCCTTCCATCGAGGGGGACGTCCAAGGGCTGGCGCCCTT 5658

Query 125  GGCCGCCCCTCATGTCAAACGTT 147
Sbjct 5659  GGCCGCCCCTCATGTCAAACGTT 5681

593 NTS INICIAIS DO INTEGRON 1 E 26 (CONTEM A ORF126) X GESFSQ-GESR (REAÇÃO AAC4 F)

Score = 579 bits (301), Expect = e-162
Identities = 301/301 (100%)
Strand = Plus / Plus
Query: 413 gtcagcacagcaatcaacacacaggggttttcatgcacacacacacaggggtttcatgccaaatcagccgcatgccaccatcgg 472

Sbjct: 273 gtcagcacagcaatcaacacacaggggttttcatgcacacacacacaggggtttcatgccaaatcagccgcatgccaccatcgg 332

Query: 473 cctaactgtgcgttgcaggggacgcctccaccagtgcgcgcttcggccacgccaagcgcg 532

Sbjct: 333 cctaactgtgcgttgcaggggacgcctccaccagtgcgcgcttcggccacgccaagcgcg 392

Query: 533 cgcccctgtaaccttgggcttagacgggctagcacaagatcataatcctacaagggatcac 592

Sbjct: 393 cgcccctgtaaccttgggcttagacgggctagcacaagatcataatcctacaagggatcac 452

Query: 593 c 593

Sbjct: 453 c 453

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

GCGGTGTGGCATNANCGGNAAGTGACGGTTCNNTCTGCTGGGGAGNCACGATGCTGTACTTTGTGATGCCTAACTTTGTTTTAGACGGGCGTCAACTATTTGTCCGTGCTCAGGATGAGTTGTGTAATAACTTGGACGCAAGACGCAACTAACTTGGTCACGCCCAAGGCGATAGTTTCTGGGGCTTGTTATAANNAATATNCTATCTCGNXNCGTNGNAGGAAAACANGNGNGCCGCACACTTGCCNGACGTACTGGTAACAGTACNGGGCATNNGGAAGAGTANGCTCGNCATTAGCATTGNTTNAANNGNACACGCACTATTGTCCTAACNTNCNGCAGG

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 = 293 bits (148), Expect = 4e-76

Identities = 148/148 (100%), Gaps = 0/148 (0%)

Strand=Plus/Minus

Query 47 CACGATGCTGACTTTTGATGCTCCCTAACACTTTTTAGACGGGCGTCAACTATTTTGTCG 106

Sbjct 2244 CACGATGCTGACTTTTGATGCTCCCTAACACTTTTTAGACGGGCGTCAACTATTTTGTCG 2185
>GESFSQ-GESR (REAÇÃO AACA4 FI sequence exported from chromatogram file
CGCGTGTGAACACANCTGNAANCNCCNTTCNNGCCTNTGGAANAAAAAGACCGGATTTTC
CCCTGCCCAGNGAAANANTGCTCTTTGAGGCGGCGGAGCGGTGGGGAGAAGAAACCGGA
TCCAGGAGTAAGCGCGGAAACCAAGCTTGGCCGGACTGCGACAGGCGTAACGCGACAGG
TGTTGTAGCGCAATAGTTTTGCGTTGTACACCAACTCGCGAAGCTGTATCTGAAAAGG
GTGCCGATAGTTTCGGGGCCGTTGTATA

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   TGCTCTTTGGAGCCTGAAATGGGATGTTGGAAGAAGAAACCGGATCCAGGAGTACGCGGAAT  138
Sbjct  5005  TGCTCTTTGGAGCCTGAAATGGGATGTTGGAAGAAGAAACCGGATCCAGGAGTACGCGGAAT  5064

Query  139   ACACCAAGTCTTACGCGGAATGCGCATCAAATGCGGCAAGGCTGGGAAAACAGCTGGTTTCG  198
Sbjct  5065  AGACCAAGTCTTACGCGGAATGCGCATCAAATGCGGCAAGGCTGGGAAAACAGCTGGTTTCG  5124

Query  199   AGCTCTGTGTTGAGGGTTCTGCGGCAATAGTCCGACAAAGGAGVCAAAGGAGTTCTGGTTCG  258
Sbjct  5125  AGCTCTGTGTTGAGGGTTCTGCGGCAATAGTCCGACAAAGGAGVCAAAGGAGTTCTGGTTCG  5184

Query  259   GCCCGACGGACCTGCGGAGCGTCTCGAGGAGAAAGGCGGATTGAGAGGCAAGGTTAC  318
Sbjct  5185  GCCCGACGGACCTGCGGAGCGTCTCGAGGAGAAAGGCGGATTGAGAGGCAAGGTTAC  5244

Query  319   CGTAACCCACCGGATGCTCCAGCGTGTACATGGGTTCCAACACGCCAGCCATTGAGCG  378
Sbjct  5245  CGTAACCCACCGGATGCTCCAGCGTGTACATGGGTTCCAACACGCCAGCCATTGAGCG  5304

[^]  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: 285 gttaggccctcgcgtaaatgcaccgcttgcgcaggccaagcgagccacctcctcggtggc 344
Sbjct: 457 gttaggccctcgcgtaaatgcaccgcttgcgcaggccaagcgagccacctcctcggtggc 516
Score = 335 bits (174), Expect = 1e-88
Identities = 174/174 (100%)
Strand = Plus / Plus

Query: 345 taccagcagccaggcatgcgcttcttggttcgtgcaaggtctcgtggcatgcttgcatcc 404
Sbjct: 517 taccagcagccaggcatgcgcttcttggttcgtgcaaggtctcgtggcatgcttgcatcc 576

Query: 405 aagctggcgtcagcacagcaatcaacacacaggggtttcatgccaaatcagccg 458
Sbjct: 577 aagctggcgtcagcacagcaatcaacacacaggggtttcatgccaaatcagccg 630

Query: 379 AACACGCAGTGATGCCTAACCCTTCCATCGAGGGGGACGTCCAAGGGCTGGCGCCCTTGG 438
Sbjct: 5305 AACACGCAGTGATGCCTAACCCTTCCATCGAGGGGGACGTCCAAGGGCTGGCGCCCTTGG 5364

Query: 439 CCGCCCCCTCATGTCAAACGTTAGGCC 464
Sbjct: 5365 CCGCCCCCTCATGTCAAACGTTAGGCC 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
Pseudomonas aeruginosa integron In60
integrase IntI1 (intI1),
aminglycoside 3-N-acetyltransferase/aminoacylcoside 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 | Sbjct |
|-------|-------|
| 23    | 3187  |
| 83    | 3127  |
| 143   | 3067  |
| 203   | 3007  |
| 263   | 2947  |
| 323   | 2887  |
| 383   | 2827  |
| 443   | 2767  |
| 503   | 2707  |
| 563   | 2647  |
>PS1 AACA4 F EDIT 2005 (PAR C sequence exported from chromatogram file
NAANAGTCGGTAACCACCCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTCGAGCGAACACGCAGTGATGCCTAACCCTTCCATCGAGGGGGACGTCCAAGGGCTGGC
GCCCTTGGCCGCCCCTCATGTCAAACGTTAGGCCCTCGCGTAAATGCACCGCTTGCGCAGGCCAAGCGAGCCACCTCCTCGGTGGCTACCAGCAGCCAGGCATGCGCTTCTTGGTTCGTGC
AGGTCTC GTGGCATGCTTGCATCCAAGCTGGCGTCAGCACAGCAATCAACACACAGGGGTTTCATGC
CAGCATC

| Query 623 AGAGTGACGCGATCCTGCCCAGTAATAGTGCGTGAATGAAGCGCATGGTGATCCCTTGAGA 682
| Sbjct 2587 AGAGTGACGCGATCCTGCCCAGTAATAGTGCGTGAATGAAGCGCATGGTGATCCCTTGAGA 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 GTAACCACCCCAAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTCGAGCGA 69
| Sbjct 292 GTAACCACCCCAAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTCGAGCGA 351

Query 70 ACACGCAGTGATGCCTAACCCTTCCATCGAGGGGGACGTCCAAGGGCTGGC
GCCCTTGGC 129
| Sbjct 352 ACACGCAGTGATGCCTAACCCTTCCATCGAGGGGGACGTCCAAGGGCTGGC
GCCCTTGGC 411

Query 130 CGCCCTCTCATGTCAAACGTTAGGCCCTCGCGTAAATGCACCGCTTGCGCAGGCCAAGCGA 189
| Sbjct 412 CGCCCTCTCATGTCAAACGTTAGGCCCTCGCGTAAATGCACCGCTTGCGCAGGCCAAGCGA 471

Query 190 GCCACCTCCTCGGTGGCTACCAGCAGCCAGGCATGCGCTTCTTGGTTCGTGC
AGGTCTC 249
| Sbjct 472 GCCACCTCCTCGGTGGCTACCAGCAGCCAGGCATGCGCTTCTTGGTTCGTGC
AGGTCTC 531

Query 250 GTGGCATGCTTGGC

ACAAGGTCTC 309
| Sbjct 532 GTGGCATGCTTGGC

ACAAGGTCTC 591

Query 310 CAAATCAGCCCGCATGCCACCATCGGCCTAACTGTGCGTTGCAGGGGACGCCTCACCAGT 369
| Sbjct 592 CAAATCAGCCCGCATGCCACCATCGGCCTAACTGTGCGTTGCAGGGGACGCCTCACCAGT 651

Query 370 GCCGCCTTCGGCCACGCCCAAGCGCGCGCCCTGAACCTTGGCGGTAGGCATCACAAGGTA 429
| Sbjct 652 GCCGCCTTCGGCCACGCCCAAGCGCGCGCCCTGAACCTTGGCGGTAGGCATCACAAGGTA 711

Query 430 CAGCATC 436
| Sbjct 712 CAGCATC 718
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 GTAACCACCCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTCGAGCGA 69
Sbjct 1025 GTAACCACCCCAGATGGTCCAGCCGTGTACATGGTTCAAACACGCCAGGCATTCGAGCGA 1084

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

Query 412 GTTAGGCATCACAAAGTACAGCATCGTGACCAACAGCAGCGATTCCGTCACACTGCGCCT 471
Sbjct 523 GTTAGGCATCACAAAGTACAGCATCGTGACCAACAGCAGCGATTCCGTCACACTGCGCCT 582

Query 472 CATGACTGAGCATGACCTTGCGATGCTCTATGAGTG 507
Sbjct 583 CATGACTGAGCATGACCTTGCGATGCTCTATGAGTG 618
TTGGCCTGCGCAAGCGGTGCATTTACGCGAGGGCCTAACGTTTGACATGAGGGGCGGCCAAGGGCGCCAGCCCTTGGACGTCCCCCTCGATGGAAGGGTTAGGCATCACTGCGTGTTCGCCTTGCCCTCTCAACCCCCGCTTTCTAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...
Query 1  GTTAGGCCCTCGGTAAATGCACCGCTTGCGCAGGCCAAGCGAGCCACCTCCTCGGTGGC  60
  |________________________________________________________________________|
Sbjct 147 GTTAGGCCCTCGGTAAATGCACCGCTTGCGCAGGCCAAGCGAGCCACCTCCTCGGTGGC  206
Query 61  TACCAGCAGCCAGGCATGCAGCTTCTTTAGTCTTGCAAGGTCCTCGTGCGAGCTGGCATGCTTGCATCC  120
  |________________________________________________________________________|
Sbjct 207 TACCAGCAGCCAGGCATGCAGCTTCTTTAGTCTTGCAAGGTCCTCGTGCGAGCTGGCATGCTTGCATCC  266
Query 121  AAGCTGGCGTCAGCACAGCAATCAACACACAGGGGTTTCATGCCAAATCAGCCGCATGCC  180
  |________________________________________________________________________|
Sbjct 267 AAGCTGGCGTCAGCACAGCAATCAACACACAGGGGTTTCATGCCAAATCAGCCGCATGCC  326
Query 181  ACCATCGGCCTAAGTGCGTTGCGAGGGACGCCCTCCACCAGTGCGCTTCGGCCACGC  240
  |________________________________________________________________________|
Sbjct 327 ACCATCGGCCTAAGTGCGTTGCGAGGGACGCCCTCCACCAGTGCGCTTCGGCCACGC  386
Query 241  CAAGCGCGCGCCCCTGAAGCTTGCCGCTTAG  270
  |________________________________________________________________________|
Sbjct 387 CAAGCGCGCGCCCCTGAAGCTTGCCGCTTAG  416
Béatrice Berçot  
Service de Bactériologie-Virologie, Hôpital de Bicêtre, Paris, France

This work is conducted to address the transcription of the fused gene cassette \textit{bla}_{GES-1}/\textit{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 \textit{gcu14} gene cassette associated with the \textit{aacA4} gene cassette. Could they explain this arrangement? Is it possible that the \textit{Pseudomonas} isolates harbored another class 1 integron containing the \textit{gcu14} gene followed by the \textit{aacA4} gene?

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

\begin{verbatim}
TTGACGCCC GTCTAAC AATT CGAC GTTCAAGC
CGAC GTTCAAGC GCTG CTAGCTATAAGCTTCCGACGCGCTTGCACTGCGCACCGCG
GCTTAACTCAG GCAGAGTC
\end{verbatim}

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

\begin{verbatim}
TTGACGCCC GTCTAA AACAAGTTAGGCACTACAAGGTACAGCATC GTG
\end{verbatim}

So, the distance from of the stop of \textit{bla}_{GES-1} to the start codon of the \textit{aacA4} gene is 46bp. The translational start codon of \textit{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 \cite{Hanau-Bercot, 2002}.

-Could the Genbank annotation be modified as explained above?
-The Appendex is not necessary and should be deleted.
-In the sentence page 4, line 13, “The single copy of \textit{rpsL} gene of \textit{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 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.
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

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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 (Int1R32_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