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RESEARCH ARTICLE

Novel environmental class 1 integrons and cassette arrays recovered from an on-farm bio-purification plant

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ABSTRACT

Rapid dissemination and emergence of novel antibiotic resistance genes among bacteria are rising problems worldwide. Since their discovery in clinical isolates in the late 1980s, class 1 integrons have been found in a wide range of bacterial genera and have been extensively studied as contributors to dissemination of antibiotic resistance. The present study aimed to investigate the presence and structure of class 1 integrons in plasmid-carrying bacterial isolates obtained from a biopurification system used for decontamination of pesticide-contaminated water as well as their possible role as reservoir of antimicrobial resistance gene cassettes. A total of 35 representative isolates were screened for the presence of class 1 integron integrase encoded by *intI1*. PCR and DNA sequencing revealed the presence of six class 1 integrons with four variable regions: 5’CS-*aadA1b*-3’CS, 5’CS-*aadA2*-3’CS, 5’CS-*aadA11c*Δ-3’CS and 5’CS-*dfrB3*-*aadA1di*-*catB2*-*aadA6k*-3’CS, the last two being unseen arrays of antimicrobial resistance gene cassettes associated with novel environmental alleles of *intI1*. These four class 1 integrons were identified as being present in four different genera, including *Ochrobactrum*, and *Variovorax*, where class 1 integrons have not been previously reported. The results provide evidence of the biopurification systems as a tank of class 1 integron carrying strains and novel environmental class 1 integron integrases associated with antimicrobial resistance gene cassette arrays.

Keywords: integron; antimicrobial resistance; plasmid; biopurification system; lateral gene transfer; agriculture

INTRODUCTION

The overuse of antibiotics in both clinical and farming contexts has increased the spread of resistance determinants not only in clinical settings but also in the environment (Angebault and Andremont 2013; Fletcher 2015). While many studies have focused on the dissemination of antibiotic resistance genes (ARGs) in hospitals, some reports have recently described their spread

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in the environment (Allen *et al.* 2010; Davies and Davies 2010; Nardelli *et al.* 2012; Cantas *et al.* 2013; von Wintersdorff *et al.* 2016). There is evidence suggesting that clinical resistance genes have an environmental origin (Martínez 2012; Berendonk *et al.* 2015; Berglund 2015) and even that some of them can also be captured by active environmental class 1 integron integrases (Chamosa *et al.* 2017). The knowledge about the directionality of the flux of antimicrobial resistance genes between the environment and the clinic, and vice-versa (Gomez-Alvarez *et al.* 2016) and the steps involved in the acquisition of novel ARGs from the open environment are interesting and active subjects of study.

Mobile genetic elements (MGEs) enhance the transmission of ARGs between bacteria via lateral genetic transfer (de la Cruz and Davies 2000). The so called mobile integrons (MIs) and conjugative (i.e. self-transmissible) or mobilizable plasmids that can carry genes conferring resistance to one or more antibiotics are among the most studied and relevant MGEs in bacteria isolated from clinics. Integrons are genetic assembly platforms that allow the capture and expression of exogenous gene cassettes (Stokes *et al.* 2006; Gillings 2014). Among MIs, class 1 integrons play a major role in the flow of ARG cassettes and antiseptic resistance determinants between pathogenic and non-pathogenic bacteria in clinical settings (Perry and Wright 2013; Gillings 2014). Although these integrons were first identified in clinical isolates, there is evidence proving that these platforms originated in natural (i.e. non-clinical) environments (Rowe-Magnus *et al.* 2001; Mazel 2006). Class 1 integrons are linked to some environments with a certain degree of contamination (Gillings *et al.* 2008; Nardelli *et al.* 2012), and their presence has even been proposed as an indicator of anthropogenic pollution (Amos *et al.* 2015; Gillings *et al.* 2015). In the past years, researchers paid particular attention to the study of the presence and dissemination of class 1 integrons in non-antibiotic polluted environments (Rosser and Young 1999; Stokes *et al.* 2006; Hardwick *et al.* 2008; Gillings, Holley and Stokes 2009; Nardelli *et al.* 2012; Koczura *et al.* 2016), since it could help to understand the evolution of these elements as well as their role in the spread of antibiotic and antiseptic resistance determinants in nature. The molecular features of class 1 integrons as a genetic tool for studying the flux of antimicrobial resistance genes are based on the different alleles of *intI1* found in both habitats, the clinic and the open environment, which led to the identification of sources of both 'environmental' and 'clinical' class 1 integrons (Gillings *et al.* 2008). Based on this point of view, the type of allele of *intI1* has been used as a marker to infer the directionality of the strains (Gillings *et al.* 2008; Nardelli *et al.* 2012; Gomez-Alvarez *et al.* 2016).

The success of class 1 integrons is mainly due to their mechanism for gene cassette acquisition and expression as well as to their frequent association with transposons and plasmids, which enhance their dissemination (Stokes *et al.* 2006; Gillings *et al.* 2008; Nardelli *et al.* 2012). Plasmids are one of the most abundant and promiscuous MGEs and have been identified as the main vehicles for lateral gene transfer in bacteria via conjugation (Garcillán-Barcia, Francia and de La Cruz 2009; Harrison and Brockhurst 2012). It has been demonstrated that plasmids carrying diverse genes can be mobilized among bacteria, even those belonging to different phyla (Klümper *et al.* 2015; Shintani, Sanchez, and Kimbara 2015).

Recently, we isolated a collection of 35 plasmid-carrying bacteria from an on-farm biopurification system (BPS) used for the remediation of pesticide-contaminated waters in Kortrijk, Belgium (Martini *et al.* 2015). These environmental isolates belong to 14 genera, including both Gram-negative and Gram-positive bacteria. In particular, this plasmid-carrying bacteria collection harbors more than 50 high molecular weight plasmids. Previous studies on this BPS have demonstrated the presence of class 1 integrons in a total DNA sample of this BPS community (Dealtry *et al.* 2014b), and have described the bacterial community composition and the IncP-1 mobilome changes in response to linuron addition as well (Dealtry *et al.* 2016; Nour *et al.* 2017). BPSs receive pesticides at relatively high concentrations for extended periods of time that in consequence generate a strong and long-term selective pressure for the persistence and growth of pesticide-tolerant or -degrading bacteria (Sniegowski *et al.* 2011). In an attempt to investigate the impact of selective pressures exerted by pesticides on bacteria carrying class 1 integrons in the BPS and their possible role as reservoirs of ARG cassettes, we firstly analyzed the presence of these elements and subsequently characterized the *intI1* ('clinical' or 'environmental'), along with the complete variable region of such integrons.

MATERIALS AND METHODS

Strains used in this study

The bacterial isolates used in this study were previously obtained from a BPS used for pesticide removal from contaminated water located in Kortrijk, Belgium (Martini *et al.* 2015) operational since 2008, and containing a biomix composed of agricultural soil (25 vol%), composted material (25 vol%) and straw mixed with stable manure originating from a nearby horse manege (50 vol%). The BPS was used for water contaminated with different types of pesticides (see Dealtry *et al.* 2014a) from spillage and residue water collected when cleaning the spraying equipment. The following pesticides were found by chemical analysis of the BPS samples: azoxystrobin, bentazone, diflufenican, diuron, epoxiconazole, ethofumesate, fenpropimorph, fluroxypyr, flufenacete, metamitron, metribuzine, propiconazole, S-metolachlor, tebuconazole and terbuthylazine (Dealtry *et al.* 2014a). The measurements of these pesticides were previously performed (Monkiedje *et al.* 2007; Sukul *et al.* 2010). In addition to the pesticides detected in the BPS, a previous study reported the application of several other active pesticide compounds to the BPS (Dealtry *et al.* 2014a).

More than of 1400 isolates obtained from the BPS (Martini *et al.* 2015) were screened by the *in situ* lysis assay (Eckhardt 1978) to investigate their plasmid content. A total of 75 plasmid-containing bacteria were identified. The *in situ* lysis technique allowed the classification of the isolates in 35 representative groups, according to their plasmid profiles (i.e. plasmid number and plasmid size). The 35 representative isolates, that comprised at least 50 high molecular weight plasmids, were further characterized. The isolates presented different tolerance profiles to several antibiotics and metals tested (for more details, see Martini *et al.* 2015). Bacteria were grown on LB medium at 28°C. The strain *Proteus mirabilis* PR9 containing a complex class 1 integron (Arduino *et al.* 2002) was used as a positive control for *intI1*, *qacE*/*qacE*Δ*1*, *sul1* and ISCR1 genes in PCR.

Molecular analysis

Total DNA from each isolate was extracted and used for PCR amplifications. For regular size amplifications (up to 2 Kb), PCR was carried out using 1X PCR buffer (50 mM KCl, 20 mM Tris-HCl, pH 8.0), 200 μM dNTP, 3 mM $MgCl_2$, 2 μl DNA template and 1 U T-free DNA polymerase (Inbio-Highway, Tandil,

Table 1. Primers used in this study.

Primer name	Target	Primer sequence	Amplicon size (bp)	Reference
5′and 3′conserved genes				
int1FP	*intI1*	gggtcaaggatctggatttcg	483	Mazel *et al.* 2000
int1FR		acatgcgtgtaaatcatcgtcg		Mazel *et al.* 2000
Inti1F	*intI1*	cgaggcatagactgtac	925	Quiroga *et al.* (2007)
Inti1R		ttcgaatgtcgtaaccgc		Quiroga *et al.* (2007)
Orf513F	ISCR1	atggtttcatgcgggtt	474	Arduino *et al.* (2003)
Orf513R		ctgagggtgtgagcgag		Orman *et al.* (2002)
Sul1 lower	*sul*1	tttgaaggttcgacagc	580	Barbolla *et al.* (2004)
Sul1 upper		gacggtgttcggcattct		Barbolla *et al.* (2004)
QaceR	*qacE*/ *qacE*Δ1	gcgaagtaatcgcaacatcc	240	Arduino *et al.* (2003)
QaceF		agccccatacctacaaagcc		Arduino *et al.* (2003)
Variable regions				
sulpro3		gcctgacgatgcgtgga	Variable	Lévesque *et al.* (1995)
qacEDelta1B		gcgataacaagaaaaagcc		Quiroga *et al.* (2013)

Argentina). For amplification of large fragments (variable regions of integrons), LongAmp DNA polymerase from New England Biolabs (Massachusetts, United States) was used. PCR was performed as follows: an initial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 20 s, annealing at 50°C –60°C (depending on the primers used; see Table 1 for references) for 30 s and extension at 65°C for LongAmp polymerase or 72°C for Taq polymerase (1 min per 1Kb of DNA). Then, a final extension at 65°C /72°C for 10 min was used.

For the detection of the *intI1* alleles, two strategies were used by amplifying a PCR fragment of 925 bp (representing the whole gene) and another of 483 bp that included the additional motif that it is conserved among integron integrases (Messier and Roy 2001; Gravel *et al.* 1998; Nield *et al.* 2001). Variable regions of class 1 integrons were analyzed in positive *intI1* strains by PCR and sequencing reactions with sulpro3 and qacEDelta1B primers (Table 1). Primers for PCR amplification of genetic determinants ISCR1, *qacE*/*qacE*Δ1 and *sul*1 are also listed in Table 1.

Amplicon sequencing, assembly and analysis

In order to identify the amplified DNA fragments, PCR products were purified using AccuPrep PCR Purification Kit (Bioneer, Daejeaon, Korea) and were then sequenced at Macrogen (Korea) following standard procedures. The resulting sequences were compared with those found in the GenBank database for identity assignment. Contig Express (Invitrogen, Carlsbad, USA) was used for sequence assembly. A function prediction was computed using standard bioinformatic tools such as Blast V2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/). Protein identity values were determined using BLASTp. Based on sequence analysis, the completeness of the integron sequence was established.

Genetic localization of identified integrons

In situ lysis gel electrophoresis was performed to obtain further information on the genomic location of class 1 integrons by analyzing the DNA of the bands corresponding to plasmid(s) or chromosome, as described by Martini *et al.* (2015). The visualized bands were purified using the AccuPrep Gel Purification Kit (Bioneer, Daejeon, Korea). Then, a PCR for the 483 bp *intI1* fragment was carried out using the purified samples. In order to exclude the amplification of contaminating DNA, several controls were included in the PCR with DNA recovered from randomly selected positions of the agarose gel.

Nucleotide sequence accession numbers

Integrons were manually curated and genes were annotated using the best BLASTp hit at National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/).

The nucleotide sequences of the complete class 1 integrons described in this work were deposited at GenBank as accession numbers KY047413- KY047415 and KY047417. The two novel integron arrangements were submitted to the INTEGRALL database as In1368 and In1369 (http://integrall.bio.ua.pt).

RESULTS

Class 1 integrons were found in different genera

To determine if class 1 integrons were present in the plasmid-carrying bacteria studied from the BPS system, the amplification of a 483 bp fragment of *intI1* was performed by PCR (Table 1); in order to analyze the variants of the *intI1* alleles, the 925 bp amplification product obtained with primers Inti1F and Inti1R was sequenced. Six out of the 35 analyzed isolates (see Table 2 for taxonomic classification) were positive for *intI1*, indicating a high frequency (17%) of this element in the BPS bacterial collection in comparison to those obtained in previous studies (Stokes *et al.* 2006; Rosewarne *et al.* 2010; Nardelli *et al.* 2012). Interestingly, besides *Pseudomonas* spp. (two isolates) and *Alcaligenes* sp., the *intI1* was found in genera such as *Ochrobactrum* and *Microbacterium* as well as in *Variovorax*, where they have not been described previously. This finding represents one more piece of evidence of how class 1 integrons successfully disseminate among genomes and habitats.

'Clinical' and novel 'environmental' alleles of *intI1* were detected in the plasmid-carrying bacteria

A 925 bp fragment of *intI1* (Table 1) was found by PCR in all isolates with the exception of *Variovorax* sp. BF19, where no PCR product was obtained with these primers but it was positive the amplification of 483 bp. This result suggests the presence of a truncated *intI1* in this isolate as previously described in other environmental samples (Nardelli *et al.* 2012). Complete sequence of

Table 2. Presence of the *intI1* and class 1 integron-related genetic determinants in the studied isolates. Since *qacE* and *sul1* can occur independently of integrons, the amplicons obtained for Sulpro3/QaceF and QaceR/Sul1 lower were sequenced, confirming that both genetic determinants are linked to the class 1 integrons in the studied isolates. [a]Truncated *intI1*. NC: not corresponds.

Isolate		Taxonomic class	*intI1*	*qacE/qacE*Δ*1*	*sul1*	Sulpro3/QaceF	QaceR/Sul1 lower	ISCR1
BF02	*Ochrobactrum* sp.	α-proteobacteria	+	+	+	+	+	–
BF13	*Ochrobactrum* sp.	α-proteobacteria	–	–	–	NC	NC	–
BF14	*Cellulosimicrobium cellulans*	Actinobacteria	–	–	–	NC	NC	–
BF15	*Ochrobactrum* sp.	α-proteobacteria	–	–	–	NC	NC	–
BF19	*Variovorax* sp.	β-proteobacteria	+[(a)]	+	+	+	+	–
BF21	*Paenibacillus* sp.	Bacilli	–	–	+	NC	NC	–
BF22	*Paenibacillus tundrae*	Bacilli	–	–	+	NC	NC	–
BF25	*Pseudomonas putida*	γ-proteobacteria	+	+	+	+	+	–
BF27	*Pseudomonas* sp.	γ-proteobacteria	+	+	+	+	+	–
BF28	*Paenibacillus xylanexedens*	Bacilli	–	–	+	NC	NC	–
BF30	*Bordetella* sp.	β-proteobacteria	–	–	–	NC	NC	–
BF31	*Cellulosimicrobium* sp.	Actinobacteria	–	+	–	NC	NC	–
BF33	*Pseudomonas putida*	γ-proteobacteria	–	–	–	NC	NC	–
BF36	*Microbacterium* sp.	Actinobacteria	–	–	–	NC	NC	–
BF37	*Serratia* sp.	γ-proteobacteria	–	–	–	NC	NC	–
BF42	*Alcaligenes* sp.	β-proteobacteria	+	+	+	+	+	–
BF43	*Serratia* sp.	γ-proteobacteria	–	–	–	NC	NC	–
BF44	*Microbacterium oxydans*	Actinobacteria	+	+	+	+	+	–
BF46	*Serratia* sp.	γ-proteobacteria	–	–	–	NC	NC	–
BF48	*Microbacterium* sp.	Actinobacteria	–	+	+	NC	NC	–
BF52	*Brevibacterium* sp.	Actinobacteria	–	–	–	NC	NC	–
BF53	*Agromyces cerinus*	Actinobacteria	–	–	–	NC	NC	–
BF55	*Pseudomonas putida*	γ-proteobacteria	–	+	–	NC	NC	–
BF56	*Acinetobacter* sp.	γ-proteobacteria	–	–	–	NC	NC	–
BF58	*Pseudomonas* sp.	γ-proteobacteria	–	–	+	NC	NC	–
BF59	*Sphingobacterium* sp.	Sphingobacteria	–	–	–	NC	NC	–
BF60	*Sphingobacterium* sp.	Sphingobacteria	–	–	+	NC	NC	–
BF61	*Pseudomonas* sp.	γ-proteobacteria	–	–	–	NC	NC	–
BF62	*Pseudomonas* sp.	γ-proteobacteria	–	–	–	NC	NC	–
BF67	*Pseudomonas* sp.	γ-proteobacteria	–	–	–	NC	NC	–
BF68	*Pseudomonas* sp.	γ-proteobacteria	–	–	–	NC	NC	–
BF70	*Bacillus pumilus*	Bacilli	–	–	–	NC	NC	–
BF71	*Bacillus* sp.	Bacilli	–	–	–	NC	NC	–
BF73	*Bacillus megaterium*	Bacilli	–	–	–	NC	NC	–
BF75	*Pseudomonas* sp.	γ-proteobacteria	–	–	–	NC	NC	–

the *intI1* was identified in Alphaproteobacteria, Betaproteobacteria and Actinobacteria. Five sequenced *intI1* belonged to two different alleles, a 'clinical' one and a novel 'environmental' allele that presented differences in two nucleotide positions (see alignment in Fig. S1, Supporting Information). The 'clinical' *intI1* allele (AF313471) was identified in BF25 and BF27 isolates which belonged to *Pseudomonas*. This allele had been previously found in other Gram-negative pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae* and *Aeromonas salmonicida* (accession numbers KU043115, CP018819, KX646543, KX364409, respectively). In turn, the novel 'environmental' *intI1* allele was identified in the present study for the first time in *Ochrobactrum* and *Microbacterium* genus.

Novel variable regions of class 1 integrons were found

To identify the variable region of class 1 integrons found in the six isolates, total DNA from each strain was PCR-amplified by using standard primers (see Table 1) and sequenced. As deduced from the sequences, four different gene cassette arrangements were found in the variable region of the six isolates: 5'CS-*aadA11c*Δ-3'CS, 5'CS-*aadA1b*-3'CS, 5'CS-*aadA2*-3'CS and 5'CS-*dfrB3-aadA1di-catB2-aadA6k*-3'CS (Fig. 1). Interestingly, the bioinformatic analysis of the gene cassette sequences revealed the presence of unique ARG cassette arrangements within the variable region, which were named In1368 and In1369 in INTEGRALL http://integrall.bio.ua.pt/ (Fig. 1). The remaining gene cassette arrangements were the already described 5'CS-*aadA1b*-3'CS (In93) and 5'CS-*aadA2*-3'CS (In127), with the same gene cassette alleles identified in the reference sequences AN: CP000645 and EU089667, respectively (http://integrall.bio.ua.pt/; Fig. 1). Class 1 integrons identified in *Ochrobactrum* sp. BF02, *Variovorax sp.* BF19, *Pseudomonas putida* BF25 and *Pseudomonas* spp. BF27 isolates contained each a single gene cassette related to the *aadA1* gene cassette family, with 92% (In1369, AN: KY047413), 99% (In93, AN: KY047414) and 89% identity (In127, AN: KY047415 and KY047416) with the reference *aadA1a* from In2 (AN: AF071413), while class 1 integrons from *Alcaligenes* sp. BF42 and *Microbacterium oxydans* BF44 contained 5'CS-*dfrB3-aadA1di-catB2-aadA6k*-3'CS (In1369, AN: KY047417 and KY047418, respectively), the same novel arrangement of ARG cassette (Fig. 1). This latter arrangement harbors two different *aadA* variants, the *catB2* gene cassette that encodes for chloramphenicol acetyltransferase previously described in Tn*2424* (Parent and Roy 1992),

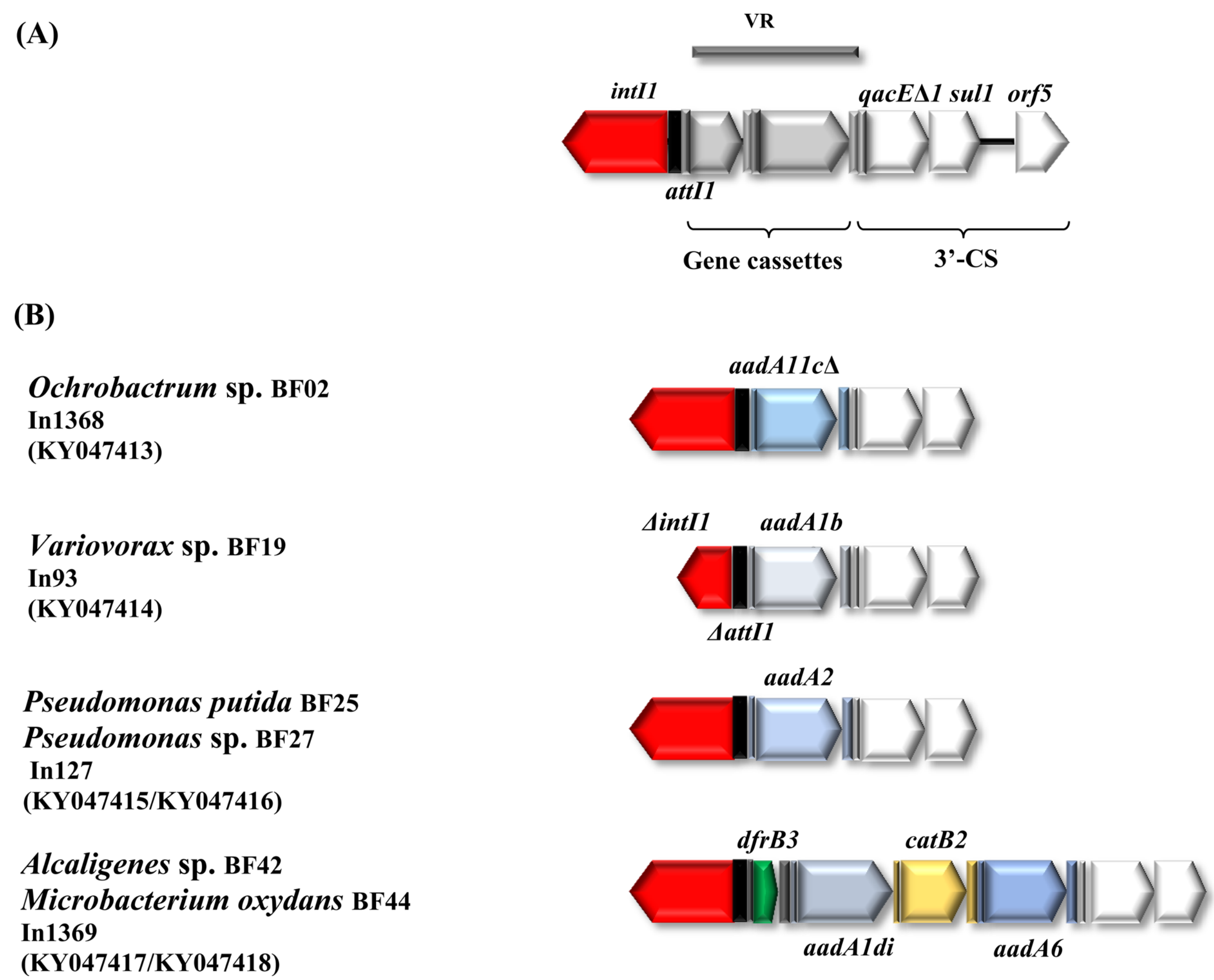

Figure 1. Schematic representation of regions surrounding class 1 integrons detected among the bacterial pool analyzed in the present study. (**A**) Representation of the typical structure of a class 1 integron. (**B**) Structure of the four integrons described in this study. VR: variable region.

and the *dfrB3* gene cassette that encodes for a dihydrofolate reductase previously found in class 1 integrons that mediate antibiotic resistance in the fish pathogen *Aeromonas salmonicida* worldwide, and also in multiresistant *Enterobacteriaceae* isolates from Bogota hospitals (accession numbers AF327729 and GQ150744, respectively). The first *aadA* variant in In1369 shows 99% identity with *aadA1a*, with three point mutations GAC→AAC, AAA→AGA and GTC→GTT at positions 205, 602 and 750, respectively. The first two codify for D69N and K149R, respectively, but the third one is a silent mutation. Following the nomenclature currently accepted (Novick *et al.* 1976; Partridge *et al.* 2009; INTEGRALL) and due to the high number of *aadA1* variants, a double-letter suffix is assigned to correctly identify the new ones (e.g. *aadA1a* … *aadA1z*, *aadA1aa* … *aadA1az*, *aadA1ba*, etc.); which in the case described above, the mutations lead to a new variant called *aadA1di*. The second *aadA* variant in this integron is *aadA6k*, with 99% identity to *aadA6* from In51 (AN: AF140629.1). It shows two point mutations, GAT→AAT and GAA→GAG at positions 175 and 243, respectively. The first mutation codifies for D59N and the second is silent. Sequence analysis of the other novel class 1 integron (In1368, AN: KY047413) showed maximal nucleotide identity (99%) with *aadA11c* (In720 AN: JN894689). The variant present in In1369 shows two point mutations, the insertion of a C and an A at positions 610 and 775, respectively. The first mutation is silent for the specific codon (CCG→CCC that codifies for a proline at position 204), but generates a frameshift leading to a change in the remaining 21 amino acids codified plus a premature stop codon that truncates the last 38 amino acids of the resulting protein. This last characteristic is highlighted by the Δ suffix in the *aadA11c*Δ variant present in this integron.

Localization of integrons in bacterial genomes

Class 1 integrons correspond to functional platforms that are physically associated with mobile DNA elements as transposons which in turn can be carried by conjugative plasmids. Determination of the chromosomal or plasmid location of class 1 integrons was performed by PCR using *intI1* as a marker gene and DNA recovered from plasmids or chromosome, obtained as mentioned in Materials and Methods. Class 1 integrons were found in plasmids of five out of six isolates (*Ochrobactrum* sp. BF02, *P. putida* BF25, *Pseudomonas* sp. BF27, *Alcaligenes* sp. BF42 and *M. oxydans* BF44), while in *Variovorax sp.* BF19, the integron was located on the bacterial chromosome (data not shown). This is the first report of a class 1 integron in the *Variovorax* genus, although a chromosome-located class 1 integron has been previously reported in *Acidovorax* (Stokes *et al.* 2006) and in *Comamonas testosteroni* (AN: NC_013446.1), two genera belonging to the same family as *Variovorax* (*Comamonadaceae*). In the case of the *Variovorax* BF19 isolate, the IntI1 was truncated in the N-terminal domain, thus suggesting a lack of recombinational activity. On the other hand, the *intI1* found in plasmids of the other five isolates was probably complete (944 bp in length).

Independent spread of *qacE/qacE*Δ1 and *sul1* genes in the BPS

The incidence of ISCR1, *sul1* and *qacE/qacE*Δ1 genes, usually associated with class 1 integrons, was investigated in our plasmid-containing isolate collection. Identification of ISCR1, which is a marker for complex class 1 integrons (Quiroga *et al.* 2013), was performed with conventional primers (see Table 2). The *qacE/qacE*Δ1 gene confers resistance towards multiple chemical classes of biocides, including quaternary ammonium compounds, biguanides, acridines, diamidines, xanthenes and phenanthridines, while *sul*1 confers resistance to sulphonamides. In addition to the six class 1 integron-containing isolates, another three and six isolates resulted positive for *sul1* and *qacE/qacE*Δ1, respectively, in the collection (Table 2). Consistent with the findings from previous studies (Wang *et al.* 2007; Nardelli *et al.* 2012; Hoa *et al.* 2008; Wu *et al.* 2010), *sul1* and *qacE/qacE*Δ1 spread independently of class 1 integrons among bacterial genomes in the BPS bacterial community as well as in a variety of bacterial habitats.

DISCUSSION

In this work, novel environmental class 1 integrons were found spread in a collection of plasmid-carrying bacteria belonging to different genera from an on-farm BPS, a useful device of environmental and agricultural interest. As reported by Dealtry *et al.* (2016), the BPS in Kortrijk, Belgium, is a system that was set up in 2008 and treats about 15,000 liters of pesticide-contaminated water annually. The biomix of the BPS contains soil and manure (straw and animal feces). The influent water is contaminated with a broad variety of pesticides. No data are available on the actual amount and composition of the different pesticides in the influent water but approximately 70 different pesticides, mainly herbicides were added to the BPS with contaminated water. An overview of the pesticides detectable in BPS material was provided previously by Dealtry *et al.* (2014a) and Holmsgaard *et al.* (2017).

The analysis of both allelic variants of *intI1* and ARG cassettes within their corresponding variable regions has been recognized as a useful tool for understanding the capture and transit of antimicrobial resistance determinants from the environment by human anthropogenic habitats. It is accepted that antimicrobial resistance determinants found in clinical isolates come from extra clinical environments (Davies and Davies 2010; Cantas *et al.* 2013; Berglund 2015; von Wintersdorff *et al.* 2016).

In this regard, class 1 integrons both play a major role in adaptation in clinically relevant bacteria and are not limited to clinical settings, thus representing an adequate model system to evaluate the flow of antimicrobial resistance determinants (Álvarez *et al.* 2016; Gomez-Alvarez *et al.* 2016; Chamosa *et al.* 2017). While it is known that these elements are more prevalent in bacteria that live in niches under antibiotic pressure, the distribution of class 1 integrons has been studied in different environments (Rosser and Young 1999; Nandi *et al.* 2004; Nemergut, Martin and Schmidt 2004; Gaze *et al.* 2005; Gillings 2017). There is increasing evidence proving that these elements are highly distributed outside the clinic, even in environments with a low human impact (Hardwick *et al.* 2008; Wright *et al.* 2008; Gaze *et al.* 2011; Nardelli *et al.* 2012. Jones-Dias *et al.* 2016; Chamosa *et al* 2017; Gillings 2017). Class 1 integrons have been shown to be involved in the process of swapping genetic material and in the spread of antibiotic resistance genes in environmental bacteria (Stalder *et al.* 2012; Alvarez *et al.* 2016; Chamosa *et al.* 2017). In this regard, class 1 integrons played an important role in the diversification of the broad-host range plasmids belonging to the IncP-1ε group as previously shown by Heuer *et al.* (2012). In addition, IncP1ε plasmids are important vectors for horizontal transfer of antibiotic resistance in manure slurries and in agricultural soils. Among the analyzed isolates *Alcaligenes* sp. BF42 carries an IncP-1 plasmid and *Pseudomonas putida* BF25 carries an IncP-7 plasmid (Martini *et al.* 2015), these types of plasmids have previously been found to be abundantly present in pesticide-polluted environments (Dunon *et al.* 2013; Jechalke *et al.* 2013). Moreover, previous reports have shown the BPS as a 'hot spot' for these type of plasmids (Dealtry *et al.* 2014).

Little is known concerning how novel ARG cassettes within integrons and/or novel alleles of *intI1* are selected in clinical samples. The study of integrons presence and structures in diverse environments is an important challenge. Since scarce attention has been paid to pesticide-amended agricultural environments, and the few reported studies have been limited to investigate the sole presence of class 1 integrons but not their structure and composition (Jechalke *et al.* 2014; Dealtry *et al.* 2014b), this work represents the first study showing a detailed bioinformatics-based analysis of class 1 integrons in this type of environment.

The analysis based on the detection of *intI1* and subsequent flanking sequences revealed the presence of four class 1 integrons in six out of the 35 isolates analyzed. Each one of these four class 1 integrons harbors at least one *aadA*-like gene cassette. These results are in agreement with two previous reports (Jechalke *et al.* 2014; Dealtry *et al.* 2014b) that identified the presence of *intI1* as well as *aadA*-like gene cassettes by using total DNA from a BPS biomix sample exposed to different pesticides. None of these studies, however, aimed at investigating either the alleles of such genes or the type of *intI1* allele. The finding of four class 1 integrons (In93, In127, In1368 and In1369) and six ARG cassettes in our plasmid-containing bacterial isolates indicates that not only *aadA*-like gene cassettes but indeed a variety of AGR cassettes in different arrangements can be present in the BPS bacterial communities. The finding of four *aadA* alleles in our bacteria collection also highlights the possible role of the BPS as reservoirs related to *aadA*-like genes. It would be interesting to investigate whether the function conferred by the *aadA* family of genes which encodes an aminoglycoside-3´-adenylyltransferase that confers resistance to streptomycin and spectinomycin by adenylation is involved in some processes inherent to the rates of sorption or biodegradation in the on-farm BPS. One point that deserves to be underlined is the finding of class 1 integron In1369 in the Gram-positive *Microbacterium oxydans* BF44 isolate. Only a few class 1 integrons have been reported in Gram-positive bacterial genera, including *Corynebacterium*, *Streptococcus*, *Enterococcus*, *Staphylococcus*, *Aerococcus* and *Brevibacterium* (Deng *et al.* 2015). Interestingly, the same class 1 integron In1369 was also found in *Alcaligenes* spp. BF42 in the present study, an event that is likely the result of lateral antimicrobial resistance genetic transfer among Gram-negative and Gram-positive strains, indicating how active can the transfer of DNA be even among non-related taxa. Since only the truncated *intI1* in *Variovorax* BF19 was found located on the chromosome, probably plasmids from the environment are playing a significant role in the dissemination of complete *intI1*, as evidenced in this study.

Both types of clinical and environmental alleles of the *intI1* were detected in our plasmid-carrying bacteria collection. The class 1 integron In29 bearing a same 'clinical' allele (AF313471) was identified in the strains BF025 and BF27 which probably belonged to two different species of the genus *Pseudomonas*, evidencing another lateral antimicrobial resistance genetic transfer event in the BPS system. Both *Pseudomonas* isolates have not only the same 'clinical' allele, but also they carry the *aadA*2 gene cassette with the typical 3´-CS which is usually found in clinical samples suggesting that probably come nosocomial samples or from human or animal microbiota. The finding of the novel 'environmental' *intI1* allele for the first time in *Ochrobactrum*, *Alcaligenes* and *Microbacterium* genus harboring different variable regions (Fig. 1) evidence how the resistome works in several steps: novel 'environmental' *intI1* alleles spread among non-clinical strains (as in *Ochrobactrum*, *Alcaligenes* or *Microbacterium* from this work), until they are selected and maintained in a particular habitat (such as the BPS), where again they can disseminate among different species (certainly among *Ochrobactrum*, *Alcaligenes* and *Microbacterium* in the BPS from this work). From the time when two arrays of ARG cassettes were found associated with the novel *intI1* allele, it is likely that the rearrangement of ARG cassettes occurred in the BPS. In addition, considering that these novel 'environmental' *intI1* alleles are associated with ARG cassettes in the variable region, it can be supposed that these variants of class 1 integrons could be later disseminated to clinical habitats. On the other hand, since In1368 and In1369 possess the typical 3´-CS, it can be also presumed that part of the integron come from clinical samples released into the environment, where in turn it has picked up an uncommon *aadA* gene cassette, followed by some form of rearrangement that lead to the finding of a novel *intI1* allele. Whatever the events that led to the formation of these new integrons, the fact is that the environment is polluted with clinical integrons.

These findings show BPSs as potential reservoirs of novel-class 1 ARG cassette-containing integrons. Interestingly, integrons that are contaminating the inputs for the BPS appear to be undergoing further change in the BPS: acquisition and swapping of ARG cassettes, movement of integrons between different genera and movement of ARG cassettes into chromosomal class 1 integrons. In addition, the incidence of *intI1* in bacterial isolates from the BPS was relatively high (17%) in comparison with previous works analyzing non-clinical settings (Rosewarne *et al.* 2010; Nardelli *et al.* 2012). Future studies on deeper bacterial DNA sequencing or a complete metagenomic sequencing analysis will probably help to better understand and predict the roles of different bacterial and plasmids groups in the potential of integrons dissemination and on the pesticides biodegradation processes in the BPS.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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REFERENCES

Álvarez VE, Quiroga MP, Castro GA *et al.* Molecular ecology of Class 1 Integrons in Patagonia as model system for understanding the rise of antibiotic resistance isolates around the world. In: Olivera NL, Libkind D, Donati E (eds). *Biology and Biotechnology of Patagonian Microorganisms*. Springer, 2016, 111–25.

Allen HK, Donato J, Wang HH *et al.* Call of the wild: antibiotic resistance genes in natural environments. *Nat Rev Microbiol* 2010;**8**:251–9.

Amos GCA, Gozzard E, Carter CE *et al.* Validated predictive modelling of the environmental resistome. *ISME J* 2015;**9**:1467–76.

Angebault C, Andremont A. Antimicrobial agent exposure and the emergence and spread of resistant microorganisms: issues associated with study design. *Eur J Clin Microbiol* 2013;**32**:581–95.

Arduino SM, Roy PH, Jacoby GA *et al.* blaCTX-M-2 is located in an unusual class 1 integron (In35) which includes Orf513. *Antimicrob Agents Ch* 2002;**46**:2303–6.

Arduino SM, Catalano M, Orman BE *et al.* Molecular epidemiology of *orf*513-bearing class 1 integrons in multiresistant clinical isolates from Argentinean hospitals. *Antimicrob Agents Chemother* 2003;**47**:3945–9.

Barbolla R, Catalano M, Orman BE *et al.* Class 1 integrons increase trimethoprim-sulfamethoxazole MICs against epidemiologically unrelated *Stenotrophomonas maltophilia* isolates. *Antimicrob Agents Chemother* 2004;**48**:666–9

Berendonk TU, Manaia CM, Merlin C *et al.* Tackling antibiotic resistance: the environmental framework. *Nat Rev Microbiol* 2015;**13**:310–17.

Berglund B. Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. *Infect Ecol Epidemiol* 2015;**5**:28564.

Cantas L, Shah SQA, Cavaco LM *et al.* A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota. *Front Microbiol* 2013;**4**, DOI: 10.3389/fmicb.2013.00096.

Chamosa LS, Álvarez VE, Nardelli M *et al.* Lateral Antimicrobial Resistance Genetic Transfer is active in the open environment. *Sci Rep* 2017;**7**:513.

Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev* 2010;**74**:417–33.

Dealtry S, Ding G-C, Weichelt V *et al.* Cultivation-independent screening revealed hot spots of IncP-1, IncP-7 and IncP-9 plasmid occurrence in different environmental habitats. *PLoS One* 2014a;**9**:e89922.

Dealtry S, Holmsgaard PN, Dunon V *et al.* Shifts in abundance and diversity of mobile genetic elements after the introduction of diverse pesticides into an on-farm biopurification system over the course of a year. *Appl Environ Microb* 2014b;**80**:4012–20.

Dealtry S, Nour EH, Holmsgaard PN *et al.* Exploring the complex response to linuron of bacterial communities from biopurification systems by means of cultivation-independent methods. *FEMS Microbiol Ecol* 2016;**92**, DOI: 10.1093/femsec/fiv157.

de la Cruz F, Davies J. Horizontal gene transfer and the origin of species: lessons from bacteria. *Trends Microbiol* 2000;**8**: 128–33.

Deng Y, Bao X, Ji L *et al.* Resistance integrons: class 1, 2 and 3 integrons. *Ann Clin Microb Anti* 2015;**14**:45.

Dunon V, Sniegowski K, Bers K *et al.* High prevalence of IncP-1 plasmids and IS1071 insertion sequences in on-farm biopurification systems and other pesticide-polluted environments. *FEMS Microbiol Ecol* 2013;**86**:415–31.

Fletcher S. Understanding the contribution of environmental factors in the spread of antimicrobial resistance. *Environ Health Prev* 2015;**20**:243–52.

Garcillán-Barcia MP, Francia MV, de La Cruz F. The diversity of conjugative relaxases and its application in plasmid classification. *FEMS Microbiol Rev* 2009;**33**:657–87.

Gaze WH, Abdouslam N, Hawkey PM *et al.* Incidence of class 1 integrons in a quaternary ammonium compound-polluted environment. *Antimicrob Agents Ch* 2005;**49**:1802–7.

Gaze WH, Zhang L, Abdouslam NA *et al.* Impacts of anthropogenic activity on the ecology of class 1 integrons and integron-associated genes in the environment. *ISME J* 2011;**5**:1253–61.

Gillings M, Boucher Y, Labbate M *et al.* The evolution of class 1 integrons and the rise of antibiotic resistance. *J Bacteriol* 2008;**190**:5095–100.

Gillings MR. Integrons: past, present, and future. *Microbiol Mol Biol Rev* 2014;**78**:257–77.

Gillings MR. Class 1 integrons as invasive species. *Curr Opin Microbiol* 2017;**38**:10–5.

Gillings MR, Gaze WH, Pruden A *et al.* Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME J* 2015;**9**:1269–79.

Gillings MR, Holley MP, Stokes HW. Evidence for dynamic exchange of qac gene cassettes between class 1 integrons and other integrons in freshwater biofilms. *FEMS Microbiol Lett* 2009;**296**:282–8.

Gomez-Alvarez V, Pfaller S, Pressman JG *et al.* Resilience of microbial communities in a simulated drinking water distribution system subjected to disturbances: role of conditionally rare taxa and potential implications for antibiotic-resistant bacteria. *Environ Sci-Wat Res* 2016;**2**:645–57.

Gravel A, Fournier B, Roy PH. DNA complexes obtained with the integron integrase *IntI1* at the *attI1* site. *Nucleic Acids Res* 1998;**26**:4347–55.

Hardwick SA, Stokes HW, Findlay S *et al.* Quantification of class 1 integron abundance in natural environments using real-time quantitative PCR. *FEMS Microbiol Lett* 2008;**278**: 207–12.

Harrison E, Brockhurst MA. Plasmid-mediated horizontal gene transfer is a coevolutionary process. *Trends Microbiol* 2012;**20**:262–7.

Heuer H, Binh CT, Jechalke S *et al.* IncP-1ε plasmids are important vectors of antibiotic resistance genes in agricultural systems: diversification driven by Class 1 Integron gene cassettes. *Front Microbiol* 2012;**3**:2.

Hoa PT, Nonaka L, Viet PH, Suzuki S. Detection of the *sul1*, *sul2*, and *sul3* genes in sulfonamide-resistant bacteria from wastewater and shrimp ponds of north Vietnam. *Sci Total Environ* 2008;**405**:377–84.

Holmsgaard PN, Dealtry S, Dunon V *et al.* Response of the bacterial community in an on-farm biopurification system, to which diverse pesticides are introduced over an agricultural season. *Environ Pollut* 2017;**229**:854–62.

Jechalke S, Schreiter S, Wolters B *et al.* Widespread dissemination of class 1 integron components in soils and related ecosystems as revealed by cultivation-independent analysis. *Front Microbiol* 2013;**4**:420.

Jechalke S, Schreiter S, Wolters B *et al.* Widespread dissemination of class 1 integron components in soils and related ecosystems as revealed by cultivation-independent analysis. *Front Microbiol* 2014;**4**:420.

Jones-Dias D, Manageiro V, Ferreira E *et al.* Architecture of class 1, 2, and 3 integrons from gram negative bacteria recovered among fruits and vegetables. *Frontiers in microbiology* 2016;**7**, Doi: 10.3389/fmicb.2016.01400.

Klümper U, Riber L, Dechesne A *et al.* Broad host range plasmids can invade an unexpectedly diverse fraction of a soil bacterial community. *ISME J* 2015;**9**:934–45.

Koczura R, Mokracka J, Taraszewska A *et al.* Abundance of Class 1 integron-integrase and sulfonamide resistance genes in river water and sediment is affected by anthropogenic pressure and environmental factors. *Microb Ecol* 2016;**72**: 909–16.

Martínez JL. Natural antibiotic resistance and contamination by antibiotic resistance determinants: the two ages in the evolution of resistance to antimicrobials. *Front Microbiol* 2012; **3**:1.

Martini MC, Albicoro FJ, Nour E *et al.* Characterization of a collection of plasmid-containing bacteria isolated from an on-farm biopurification system used for pesticide removal. *Plasmid* 2015;**80**:16–23.

Mazel D, Dychinco B, Webb VA, Davies J. Antibiotic resistance in the ECOR collection: integrons and identification of a novel *aad* gene. *Antimicrob Agents Chemother* 2000;**44**:1568–74.

Mazel D. Integrons: agents of bacterial evolution. *Nat Rev Microbiol* 2006;**4**:608–20.

Messie N, Roy PH. Integron integrases possess a unique additional domain necessary for activity. *J Bacteriol* 2001;**183**: 6699–706.

Monkiedje A, Zuehlke S, Maniepi SJN *et al.* Elimination of racemic and enantioenriched metalaxyl based fungicides under tropical conditions in the field. *Chemosphere* 2007;**69**:655–63.

Nandi S, Maurer JJ, Hofacre C *et al.* Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. *P Natl Acad Sci USA* 2004;**101**:7118–22.

Nardelli M, Scalzo PM, Ramírez MS *et al.* Class 1 integrons in environments with different degrees of urbanization. *PLoS One* 2012;**7**:e39223.

Nemergut DR, Martin AP, Schmidt SK. Integron diversity in heavy-metal-contaminated mine tailings and inferences about integron evolution. *Appl Environ Microb* 2004;**70**: 1160–8.

Nield BS, Holmes AJ, Gillings MR *et al*. Recovery of new integron classes from environmental DNA. *FEMS Microbiol Lett*, 2001;**195**:59–65

Novick RP, Clowes RC, Cohen SN *et al*. Uniform nomenclature for bacterial plasmids: a proposal. *Bacteriol Rev* 1976;**40**:168–89.

Nour EH, Elsayed TR, Springael D *et al*. Comparable dynamics of linuron catabolic genes and IncP-1 plasmids in biopurification systems (BPSs) as a response to linuron spiking. *Appl Microbiol Biot* 2017;**101**:4815–25.

Orman BE, Pineiro SA, Arduino S *et al*. Evolution of multiresistance in nontyphoid *Salmonella* serovars from 1984 to 1998 in Argentina. *Antimicrob Agents Chemother* 2002,**46**: 3963–70.

Parent R, Roy PH. The chloramphenicol acetyltransferase gene of Tn2424: a new breed of cat. *J Bacteriol* 1992;**174**:2891–7.

Partridge SR, Tsafnat G, Coiera E *et al*. Gene cassettes and cassette arrays in mobile resistance integrons. *FEMS Microbiol Rev* 2009;**33**:757–84.

Perry J, Wright G. The antibiotic resistance “mobilome”: searching for the link between environment and clinic. *Front Microbiol* 2013;**4**:138.

Quiroga MP, Andres P, Petroni A *et al*. Complex Class 1 Integrons with Diverse Variable Regions, Including aac(6’)-Ib-cr, and a Novel Allele, qnrB10, Associated with ISCR1 in Clinical Enterobacterial Isolates from Argentina. *Antimicrob Agents Chemother* 2007;**51**:4466–70.

Quiroga MP, Arduino SM, Merkier AK *et al*. Distribution and functional identification of complex class 1 integrons. *Infect Genet Evol* 2013;**19**:88–96.

Rosewarne CP, Pettigrove V, Stokes HW *et al*. Class 1 integrons in benthic bacterial communities: abundance, association with Tn402-like transposition modules and evidence for coselection with heavy-metal resistance. *FEMS Microbiol Ecol* 2010;**72**:35–46.

Rosser SJ, Young H-K. Identification and characterization of class 1 integrons in bacteria from an aquatic environment. *J Antimicrob Chemoth* 1999;**44**:11–8.

Rowe-Magnus DA, Guerout AM, Ploncard P *et al*. The evolutionary history of chromosomal super-integrons provides an ancestry for multiresistant integrons. *P Natl Acad Sci USA* 2001;**98**:652–7.

Shintani M, Sanchez ZK, Kimbara K. Genomics of microbial plasmids: classification and identification based on replication and transfer systems and host taxonomy. *Front Microbiol* 2015;**6**:242.

Sniegowski K, Bers K, Van Goetem K *et al*. Improvement of pesticide mineralization in on-farm biopurification systems by bioaugmentation with pesticide-primed soil. *FEMS Microbiol Ecol* 2011;**76**:64–73.

Stalder T, Barraud O, Casellas M *et al*. Integron involvement in environmental spread of antibiotic resistance. *Front Microbiol* 2012;**3**:119.

Stokes HW, Nesbø CL, Holley M *et al*. Class 1 integrons potentially predating the association with Tn402-like transposition genes are present in a sediment microbial community. *J Bacteriol* 2006;**188**:5722–30.

Sukul P, Zühlke S, Lamshöft M *et al*. Dissipation and metabolism of 14 C-spiroxamine in soil under laboratory condition. *Environ Pollut* 2010;**158**:1542–50.

von Wintersdorff CJH, Penders J, van Niekerk JM *et al*. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front Microbiol* 2016;**7**, DOI: 10.3389/fmicb.2016.00173.

Wang C, Cai P, Guo Y *et al*. Distribution of the antiseptic-resistance genes *qacE*Δ*1* in 331 clinical isolates of Pseudomonas aeruginosa in China. *J Hosp Infect* 2007;**66**:93–5.

Wright MS, Baker-Austin C, Lindell AH *et al*. Influence of industrial contamination on mobile genetic elements: class 1 integron abundance and gene cassette structure in aquatic bacterial communities. *ISME J* 2008;**2**:417–28.

Wu S, Dalsgaard A, Hammerum AM *et al*. Prevalence and characterization of plasmids carrying sulfonamide resistance genes among Escherichia coli from pigs, pig carcasses and human. *Acta Vet Scand* 2010,**52**:47.