Integrative conjugative elements of the ICEPan family play a potential role in Pantoea ananatis ecological diversification and antibiosis

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Pantoea ananatis is a highly versatile enterobacterium isolated from diverse environmental sources. The ecological diversity of this species may be attributed, in part, to the acquisition of mobile genetic elements. One such element is an Integrative and Conjugative Element (ICE). By means of in silico analyses the ICE elements belonging to a novel family, ICEPan, were identified in the genome sequences of five P. ananatis strains and characterized. PCR screening showed that ICEPan is prevalent among P. ananatis strains isolated from different environmental sources and geographic locations. Members of the ICEPan family share a common origin with ICEs of other enterobacteria, as well as conjugative plasmids of Erwinia spp. Aside from core modules for ICEPan integration, maintenance and dissemination, the ICEPan contain extensive non-conserved islands coding for proteins that may contribute toward various phenotypes such as stress response and antibiosis, and the highly diverse ICEPan thus plays a major role in the diversification of P. ananatis. An island is furthermore integrated within an ICEPan DNA repair-encoding locus umuDC and we postulate its role in stress-induced dissemination and/or expression of the genes on this island.

Keywords: Pantoea ananatis, integrative and conjugative element, ICEPan, stress response, antibiosis, umuDC

Introduction

Pantoea ananatis is a ubiquitous and versatile enterobacterial species, with strains isolated globally from a wide range of environmental sources. Most commonly isolated from plants, P. ananatis has been identified as the causative agent of diseases on a wide range of host plants, including agronomically important crops such as rice, corn, onion, and Eucalyptus (Coutinho and Venter, 2009). Other isolates represent non-pathogenic endo- or epiphytes, while a plant growth promoting P. ananatis strain has recently been characterized and patented (Coutinho and Venter, 2009; Kim et al., 2012). The potential of P. ananatis as an effective biological control agent of a number of phytopathogenic bacteria and fungi is also being investigated (Gasser et al., 2012). Furthermore,
**P. ananatis** has been associated with human disease (De Baere et al., 2004). The wide ecological distribution and versatile lifestyles of *P. ananatis* suggests that this bacterial species has undergone extensive genetic adaptation in order to effectively occupy and exploit its various ecological niches.

A key driver of rapid bacterial adaptation is the acquisition of genetic material through the horizontal exchange of mobile genetic elements. These elements, which include plasmids, phages, transposons and Integrative and Conjugative Elements (ICEs), influence bacterial fitness and allow microorganisms to occupy novel niches (Wozniak and Waldor, 2010; Aminov, 2011). ICE elements are a class of self-transmissible integrative elements found in numerous Gram-positive and Gram-negative bacterial taxa (Bi et al., 2012). At present, only a limited number of ICEs have been classified into ICE families, including the well-known SXT/R391 family found in *Vibrio* and *Providencia* spp. (Wozniak and Waldor, 2010). With the exponential increase of available genome sequences, it can be envisaged that many novel integrative and conjugative elements will be identified and novel ICE families described.

Typically, ICEs comprise of three core modules required for functioning of the element. The first module includes an integrase gene (xerC) which ensures the site-specific chromosomal integration of the ICE as well as effective excision of the element, where it may be aided by an excisionase or recombination directionality factor (Burrus et al., 2006; Wozniak and Waldor, 2010). Following excision, the ICE forms a circular extrachromosomal element through recombination between identical sequences at both ends (Burrus et al., 2006). The second core module is involved in the conjugal transfer of the circularized ICE. This module generally comprises a Type IV secretion system (T4SS), which ensures intimate contact between the donor and recipient for dissemination of the ICE (Wozniak and Waldor, 2010). The third core module is involved in ICE maintenance and includes regulatory proteins as well as toxin–antitoxin and partition systems which ensure that the ICE is vertically transmitted within a bacterial lineage (Wozniak and Waldor, 2009). Aside from core modules, ICEs contain an extensive array of cargo genes, which may contribute to diverse phenotypes (Wozniak and Waldor, 2010). These include genes coding for factors involved in pathogenesis, metabolic adaptation, the production of secondary metabolites and resistance to antibiotics and heavy metals (Burrus et al., 2002). Homologous recombination between ICEs may also occur in ICE recipients, leading to the formation of hybrid ICEs, which contribute to the diversity of ICE elements and potential accessory factors they encode (Wozniak and Waldor, 2010).

Previous comparative genomic analyses (De Maayer et al., 2014) revealed the presence of a large genomic island in five of eight compared *P. ananatis* genomes. Here we have characterized these genomic islands and show that they represent ICE elements, which are prevalent among strains of the species. The ICEPan elements comprise core modules interspersed with divergent cargo regions. Proteins encoded in these cargo regions may contribute to stress-response and production of antibiotic secondary metabolites. Finally, we identified a non-conserved island situated within the *umuC* gene involved in DNA damage repair and postulate its potential role in the dissemination or expression of the cargo genes.

### Methods

**In Silico Characterization of the ICEPan Elements**

ICE elements were identified on the genomes of five *P. ananatis* strains (Table 1) by localized tBlastN analysis with protein coding sequences from known ICE element core modules using BioEdit v 7.1.11 (Hall, 1999). The complete genome sequences of *P. ananatis* AJ13355 (NC_017531.1), LMG5342 (NC_016816.1) and PA13 (CP0003085.1), as well as the draft genomes of *P. ananatis* B1-9 (CAEI00000000) and BD442 (JMJL00000000) are publically available on the NCBI database under the given accession numbers. The full extent of the ICEPan elements was elucidated by BlastN with the full tRNA-Phe nucleotide sequence of the ICE-negative *P. ananatis* LMG20103 (PANA_0061) (De Maayer et al., 2010) to determine the ICEPan integration site. The sizes and G+C contents of the ICEPan Island (IR) and Core (CR) regions and other sequence manipulations, such as sequence alignments and localized Blast comparisons were performed using BioEdit (Hall, 1999). The CDS sets encoded on the ICEPan elements were standardized using the FgenesB ORF prediction server (http://www.softberry.com) and reciprocal best hit (RBH) BlastP analysis (Altschul et al., 1990; Moreno-Hagelsieb and Latimer, 2008). Core module CDSs in ICEPan elements were predicted by localized BlastP of the protein orthologs from the ICEberg server (Bi et al., 2012; http://db-mml.sjtu.

### Table 1 | General characteristics of ICEPan-carrying *P. ananatis* strains and the ICEPan elements.

| Strain | Host       | Lifestyle        | Integration site | Size (kb) | G+C (%) | # CDSs | References       |
|--------|------------|------------------|------------------|-----------|---------|--------|-----------------|
| AJ13355| Soil       | Saprophyte       | 2                | 59.4      | 53.22   | 59     | Hara et al., 2011 |
| B1-9   | Onion      | Plant-growth promoter | 1              | 99.2      | 53.73   | 110    | Kim et al., 2012  |
| BD442  | Corn       | Pathogen         | 1                | 98.8      | 53.21   | 107    | Weller-Stuart et al., 2014 |
| PA13   | Rice       | Pathogen         | 1                | 107.2     | 53.87   | 108    | Choi et al., 2012 |
| LMG5342| Human      | Pathogen         | 1                | 110.3     | 53.26   | 113    | De Maayer et al., 2012 |

The *P. ananatis* strain names, their hosts and lifestyles are shown. The size of the complete ICEPan element for each strain, G+C contents and number of CDSs encoded on the elements are indicated. The integration site of the ICEPan elements in each strain, (Site 1, adjacent to yjO encoding a putative HTH-transcriptional regulator; Site 2, adjacent to serine phosphatase gene rbsU) are indicated.
The core module CDSs were further used to identify ICE-like elements in closely related organisms by BlastP comparison against the NCBI non-redundant protein sequences (nr) database. Average amino acid identities were calculated on the basis of localized RBH BlastP analyses (Moreno-Hagelsieb and Latimer, 2008), where the sum of the number of identities was divided by the sum of the total lengths of the aligned regions. Orthology of the CDSs encoded on the ICEPan IR regions to proteins encoded on other genomes was determined by BlastP comparison of the translated protein products against the NCBI nr database, while conserved domains were predicted using the Batch CD-search tool and the Conserved Domain Database (Marchler-Bauer and Bryant, 2004; Marchler-Bauer et al., 2009).

Phylogenetic Tree Construction

Phylogenetic trees were constructed, using the amino acid sequences of the chromosomal house-keeping markers AtpD, GyrB, InBf, and RpoB; ICEPan core CDSs and enterobacterial ICE core CDSs (ICEEpirCFBP5888, ICEEclATCC13047, YAPI, Ctnscr94, and ICEPanWPP163); ICEPan core CDSs and conjugative plasmid core CDSs (pEI70 and pEb102) and umuDC island CDSs. The amino acid sequences were concatenated and aligned using the MAFFT web server (Katoh and Standley, 2013). Tree construction was performed using MEGA v 5.2 (Tamura et al., 2011) with the Neighbor-joining approach, with complete gap deletion, Poisson correction and bootstrapping (n = 1000).

PCR Screening for ICEPan Elements in P. ananatis Strains

Forty-six P. ananatis strains of different geographic origins and sources of isolation (Table 2) were incubated overnight at 28°C and maintained on Luria Bertani (LB) agar. Genomic DNA was extracted using the ZR Fungal/Bacterial DNA MicroPrep™ kit (Zymo Research Corporation, California, USA), as per the manufacturer’s instructions. Strains were confirmed as belonging to the species P. ananatis by PCR amplification and Sanger sequencing of the 16S rRNA gene. The chromosomal DNAs were PCR amplified using the following primer sets: sit1F-sit1R and sit2F-sit2R, covering the hyp-tRNA-Phe-yydC region and hyp-tRNA-Phe-rbsU regions of ICEPan-negative P. ananatis strains for which genome sequences are available, respectively; xerC1F-xerC1R and xerC2F-xerC2R, within the XerC1 and XerC2 integrases of site 1 and site 2-integrated ICEPan elements, respectively; topBF-topBR, pilVF-pilVR, and tralF-tralR, designed on the nucleotide sequences of topB, pilV, and tral core module genes, respectively; mae1F-mae1R and ssFD-ssFDR, designed from the nucleotide sequences of ssFD in IR-4A and mae1 in IR-4B, respectively (Table S1).

PCR amplification was undertaken with a standardized PCR program (94°C for 5 min; 30 × (94°C for 1 min, 55°C for 1 min, 72°C for 1 min); 72°C for 5 min), and the resultant amplicons were visualized, after agarose gel electrophoresis with GelRed™ nucleic acids stain (Biotium, California, USA), using a UV transilluminator. The presence of an insert within tRNA-Phe site 1 or site 2 was confirmed by the absence of a band for Sit1 and/or Sit2, while the presence of an ICEPan element in these sites was determined on the basis of the presence of bands for the xerC1, xerC2, topB, pilV, and tral gene products. The presence of a putative antibiotic biosynthetic locus and the mae1-containing umuDC island was confirmed through the presence of a band for the ssFD and mae1 gene products, respectively.

Results and Discussion

General Properties of the P. ananatis Integrative Conjugative Elements

Analysis of eight P. ananatis genomes revealed the presence of integrative and conjugative elements integrated on the chromosomes of five strains (Table 1; Figure 1). These elements belong to a novel family, ICEPan, named in accordance with the nomenclatural system proposed by Burrus et al. (2002), to reflect the species, P. ananatis in which they were identified, and the strain numbers to distinguish between the different ICEPan elements. The ICEPan elements vary in size from 67.4 (ICEPanAJ13355) to 110 kb (ICEPanLMGS3542) and carry between 59 and 113 protein coding sequences (CDSs). The G+C contents of the ICEs (53.2–53.9%) are similar to those of the rest of the chromosome (53.5–53.7%) for the complete chromosomes of AJ13355, LMGS3542, and PA13. As is the case for the majority of genomic islands, it has been observed that most ICEs are integrated within tRNA genes, which serve as hotspots for recombination (Burrus et al., 2002; Boyd et al., 2009).

All five ICEPan elements are integrated into one of two copies of identical phenylalanine-specific tRNAs, pheU/V (Table 1) present on all the genomes. Similarly, the ICEs Ctnscr94 of Salmonella enterica subsp. enterica serovar Senftenberg 5494-57, YAPI of Yersinia pseudotuberculosis 32777 and ICEEc2 of Escherichia coli BEN374 (Hochhut et al., 1997; Collyn et al., 2002; Roche et al., 2010) are integrated into tRNA-Phe genes. It has been observed that ICE integrases may have evolved to drive ICE insertion within specific tRNA genes. This targeted integration may ensure that essential loci and highly expressed tRNAs, where ICE integration may affect cellular fitness, are avoided (Boyd et al., 2009). While four of the ICEPan elements were integrated in one tRNA-Phe site (Site 1; adjacent to yjdC encoding a putative HTH-transcriptional regulator), the ICEPanAJ13355 was integrated within the second identical tRNA-Phe gene copy (Site 2; adjacent to serine phosphatase gene rbsU). The ICE-associated integrase (XerC1) of the four ICEPan elements integrated in the first site shared 99.4–100% amino acid identity among them, while the ICEPanAJ13355 integrase (XerC2) shares lower sequence identity (58.5% average amino acid identity) with XerC1. Variability in the integrase sequence may thus dictate the ICEPan integration site.

The ICEPan Elements Contain Core Modules for Integration, Maintenance, and Conjugative Transfer

Comparison of the ICEPan elements of the five strains revealed that they comprise of four (ICEPanAJ13355) or five (ICEPanBJ1-9, ICEPanBD442, ICEPanLMGS3542, and ICEPanPA13) conserved syntic blocks, CR 1–5, with regions

edu.cn/ICEberg/).
### TABLE 2 | The presence or absence of ICEPan in *P. ananatis* strain isolated from different ecological sources.

| Strain Acc. | Origin | Host | sit1 | sit2 | xerC1 | xerC2 | topB | pilV | tral | ssfD | mae1 |
|-------------|--------|------|------|------|-------|-------|------|------|------|------|------|
| FBCC0024    | RSA    | Insect | +    | −    | −     | −     | −    | −    | −    | −    | −    |
| PA13        | Korea  | Rice | +    | −    | +     | −     | +    | +    | +    | −    | −    |
| B1–9        | Korea  | Tea  | +    | −    | +     | −     | +    | −    | −    | +    | −    |
| FBCC0053    | RSA    | Insect | +    | −    | +     | −     | +    | +    | +    | −    | +    |
| FBCC0083    | USA    | Onion | +    | −    | −     | −     | −    | +    | −    | +    | −    |
| BD442       | RSA    | Corn | +    | −    | −     | −     | −    | +    | −    | +    | −    |
| LMG5342     | USA    | Human | +    | −    | −     | −     | −    | +    | −    | +    | −    |
| LMG20105    | RSA    | Eucalyptus | + | − | + | − | − | + | − | − | − |
| FBCC0030    | RSA    | Insect | +    | −    | −     | −     | −    | +    | −    | −    | −    |
| LMG2678     | Zimbabwe | Wheat | +    | −    | −     | −     | −    | +    | −    | −    | −    |
| FBCC0094    | USA    | Onion | +    | −    | −     | −     | −    | +    | −    | −    | −    |
| 0197–28     | USA    | Sudangrass | + | − | − | − | − | + | − | + | − |
| 0696–21     | USA    | Sudangrass | +    | −    | −     | −     | −    | +    | −    | −    | −    |
| FBC0087     | USA    | Onion | +    | −    | −     | −     | −    | +    | −    | −    | −    |
| ICMP12183   | Brazil | Cassia | +    | −    | −     | −     | −    | +    | −    | −    | −    |
| BD301       | RSA    | Onion | +    | −    | −     | −     | −    | +    | −    | −    | −    |
| LMG2101     | India  | Rice | +    | −    | −     | −     | −    | +    | −    | −    | −    |
| BD377       | RSA    | Onion | +    | −    | −     | −     | −    | +    | −    | −    | −    |
| PA4         | RSA    | Onion | +    | −    | −     | −     | −    | +    | −    | −    | −    |
| DAR76141    | Aus    | Rice | −    | +    | +     | +     | +    | +    | +    | +    | +    |
| DARY76144   | Aus    | Rice | −    | +    | +     | +     | +    | +    | +    | +    | +    |
| RAMI7969    | Aus    | Rice | −    | +    | +     | +     | +    | +    | +    | +    | +    |
| FBC00583    | Uruguay | Eucalyptus | + | − | + | + | − | + | − | + | + |
| LMG2628     | Japan  | Banana | − | − | + | − | − | + | − | − | − |
| Yomagi–101  | Japan  | Artemisia sp. | − | + | − | − | + | − | + | − | + |
| AJ13355     | Japan  | Soil | −    | +    | −     | +     | +    | +    | +    | +    | +    |
| B731        | Brazil | Corn | −    | +    | −     | +     | +    | +    | +    | +    | +    |
| LMG2676     | USA    | Wheat | −    | +    | −     | +     | +    | +    | +    | +    | +    |
| ATCC35400   | USA    | Melons | − | − | + | − | − | + | − | + | + |
| LMG2675     | Europe | Wheat | − | + | − | − | + | − | − | − | − |
| BD588       | RSA    | Corn | −    | +    | −     | +     | +    | +    | +    | +    | +    |
| SUPP2582    | Japan  | Melon | −    | +    | −     | +     | +    | +    | +    | +    | +    |
| BD561       | RSA    | Corn | − | − | + | + | − | − | − | − | − |
| LMG2655T    | USA    | Pineapple | − | − | − | − | − | − | − | − | − |
| FBCC0116    | RSA    | Eucalyptus | − | − | − | − | − | − | − | − | − |
| LMG20103    | RSA    | Eucalyptus | − | − | − | − | − | − | − | − | − |
| LMG2666     | USA    | Pineapple | − | − | − | − | − | − | − | − | − |
| SUPP2219    | Japan  | Rice | − | − | − | − | − | − | − | − | − |
| LMG2807     | USA    | Cattleya sp. | − | − | − | − | − | − | − | − | − |
| BD622       | RSA    | Corn | − | − | − | − | − | − | − | − | − |
| FBC00367    | Thailand | Eucalyptus | − | − | − | − | − | − | − | − | − |
| SUPP1128    | Japan  | Melon | − | − | − | − | − | − | − | − | − |
| SUPP1179    | Japan  | Melon | − | − | − | − | − | − | − | − | − |
| SUPP2213    | Japan  | Rice | − | − | − | − | − | − | − | − | − |
| SUPP2219    | Japan  | Rice | − | − | − | − | − | − | − | − | − |

*P. ananatis* strains were obtained from various commercial and research culture collections: FBCC accessions – Forestry and Agricultural Biotechnology Institute Bacterial Culture Collection, T.A. Coutinho, University of Pretoria, South Africa; LMG accessions – BCCM/LMG culture collection, University of Gent, Belgium; BD and PA accessions – Plant Pathogen and Plant Protecting Bacteria (PPPBP) culture collection, ARC-PPLR, Pretoria, South Africa; ICMP accessions – International Collection of Microorganisms from Plants, Auckland, New Zealand; DAR and RAMI accessions – R. Colter, New South Wales Agricultural, Australia; 0198-28 and 0696-21 from D.A Cooksey, Department of Plant Pathology, University of California, USA; SUPP and Yomagi accessions – Y. Takikawa, Laboratory of Plant Pathology, Shizuoka University, Japan; Jan-97, and Jan-98 – R. Gitaitis, College of Agricultural and Environmental Sciences, University of Georgia, USA. Products were amplified by PCR with the primers indicated in Table S1. Gray blocks with a + indicate the presence of bands visualized with agarose electrophoresis for xerC1, xerC2, topB, pilV, tral, ssfD, and mae1 products and the absence of a product for sit1 or sit2. Strains indicated in bold are those for which genome sequences are available.
containing non-conserved genes, IR 1–4, interspersed between these blocks (Figure 1). The protein complements of the ICEPan CRs were compared to characterized ICEs to identify the three core functional modules within the CR blocks of the ICEPan elements.

The tyrosine recombinase (XerC), which drives both the integration and excision of various ICEs including those of the SXT/R391 family (Boyd et al., 2009), is localized in CR-5 of the ICEPan. A large number of proteins with putative roles in the conjugative transfer of ICEPan are interspersed within the CRs. These include orthologs of the relaxase TraL (CR-5), Type IV coupling factor TraG (CR-4), conjugative transfer ATPase TraC (CR-3) and putative conjugative transfer proteins TraE and TraF (CR-5). Located within CR-2 are genes encoding orthologs of another conjugation coupling factor TraD, exported transglycosylase TraL and putative conjugative transfer protein TraW. CR-2 also includes a ~11 kb locus encoding 11 proteins, PilL-V, sharing 50 and 46.2% average amino acid identity with the Pil proteins of the ICEs CTnscr94 in S. enterica (CAX68107.1-CAX68117.1) and YAPI in Y. pseudotuberculosis (CAF28485.1-CAF28494.1), respectively (Figure 1). The encoded type IV pilus was initially thought to function as a virulence factor, with deletion of the Y. pseudotuberculosis pil locus resulting in decreased pathogenicity in mice which were infected orally (Collyn et al., 2002). However, more recent analysis of E. coli ICEEc2 has shown that this pilus plays a role in mating pair formation and conjugal transfer, and the decreased pathogenicity of the Y. pseudotuberculosis pil mutant may rather result from accessory elements on YAPI (Collyn et al., 2006; Roche et al., 2010). The chromosome partitioning protein ParA/Soj and plasmid maintenance protein ParB have been demonstrated to play a role in the maintenance and vertical transmission of the excised ICE (Woźniak and Waldor, 2009). Genes encoding orthologs of these proteins are localized within ICEPan CR-1. Our analyses thus revealed that the core modules for integration and excision, maintenance and conjugative dissemination are present in the ICEPan regions of four of the five sequenced ICEPan-containing strains, suggesting that they represent functional and transmissible ICEs. The absence of CR-1 and -2, which carry genes for ICE dissemination in the other strains, suggests that ICEPanAJ13355 has lost its functionality as a transmissible element. The absence of CR-1 is puzzling, as this region is involved in the vertical maintenance of the ICE element during cell division and replication. One possibility is that the distinct chromosomal integration site and/or the presence of integrase XerC2 within the ICEPanAJ13355 prevent its excision. In its un-excised form it may then be replicated as part of the chromosome.

The ICEPan Family Elements are Closely Related to other Enterobacterial ICEs and Share a Common Origin with Conjugative Plasmids of Erwinia amylovora and Erwinia billingiae

Nineteen CDSs conserved in all five ICEPan elements showed significant sequence identity to proteins encoded on various characterized enterobacterial ICEs, including Ctnscr94 of S. enterica subsp. enterica serovar Senftenberg 5494-57 and YAPI of Y. pseudotuberculosis 32777 (Table 3). The conserved ICEPan CDSs showed highest sequence similarity to those encoded on uncharacterized ICEs identified in the genomes of Enterobacter cloacae ATCC13047 (ICEEciATCC13047) and Erwinia piriformis CFBP5888⁻ (ICEEpiCFBP5888) (Table 3) (Ren et al., 2010; Smits et al., 2013). Alignment of the ICE loci (Figure 2) also indicated a high level of synteny between ICEPan and the enterobacterial ICEs. A phylogeny based on the concatenated protein products of the conserved ICE CDSs showed similar clustering to a phylogeny constructed on the basis of the concatenated amino acid sequences of four chromosomal
TABLE 3 | Average amino acid identities between ICE Pan and related ICEs.

|          | AJ13355 | B1-9 | BD442 | LMG5342 | PA13 | ECL | EPIR | CtnScr94 | YAPI | Pwa |
|----------|---------|------|-------|---------|------|-----|------|----------|------|-----|
| ICE Pan  | 90.0    | 90.3 | 89.4  | 89.1    | 75.4 | 74.6| 65.9  | 63.1     | 62.7 |     |
| ICE Pan  | 96.0    | 96.0 | 94.1  | 75.2    | 72.3 | 64.6| 62.8  | 62.2     | 62.2 |     |
| ICE Pan  | 95.2    | 93.4 | 75.3  | 72.7    | 64.6 | 62.4| 61.7  | 61.9     | 61.9 |     |
| ICE Pan  | 94.9    | 74.5 | 72.4  | 64.8    | 62.4 | 61.9| 62.7  | 62.2     | 62.2 |     |
| ICE Pan  | 74.4    | 71.6 | 64.7  | 62.1    | 64.0 | 62.7| 62.2  | 62.2     | 62.2 |     |
| ICE Pan  | 78.9    | 67.7 | 64.9  | 64.3    | 64.0 | 62.7| 62.7  | 62.2     | 62.2 |     |
| CTnScr94 | 60.6    | 61.2 | 60.6  | 61.2    | 62.7 | 62.2| 62.2  | 62.2     | 62.2 |     |
| YAPI     | 69.5    |      |       |         |      |     |       |          |      |     |
| ICE Pan  | WPP163  |      |       |         |      |     |       |          |      |     |

The average amino acid identities (%) between pair-wise compared translated ICE CDS sets was calculated on the basis of 16 conserved CDSs, where the sum of identities for each CDS was divided by the sum total of the alignment lengths.

FIGURE 2 | Schematic diagram of ICE Pan LMG5342 and closely related ICEs showing the extensive synteny that exists between the CRs. ICE Epi CFBP5888 and ICE Ecl ATCC13047 were identified from the genome sequences of E. piriformis CFBP 5888T and E. cloacae subsp. cloacae ATCC13047T, respectively. The Genbank files for CTnScr94 of S. enterica subsp. enterica serovar Senftenberg 5494-57 and YAPI of Y. pseudotuberculosis 32777 were obtained from the ICEberg database (Bi et al., 2012). Dark gray shaded regions and green arrows indicate the conserved CRs between the ICEs. Black arrows represent transposases.

house-keeping markers (Figure 3). This suggests that ICE Pan and the compared enterobacterial ICEs were derived from a common ancestor, and have undergone subsequent divergence in parallel with the bacterial chromosome.

BlastP analyses of the translated ICE Pan CDSs against the NCBI non-redundant (nr) protein database revealed a number of orthologous CDSs occurring in the conjugative plasmids of E. amylovora ACW56400 (pEI70 – NC_018999.1; ~65 kb) and E. billingiae Eb661 (pEB102 – NC_014304.1; ~102 kb) (Figure 4) (Kube et al., 2010; Llop et al., 2011). Between 36 and 54 of the 114 proteins encoded on pEB102 (60.3% average amino acid identity) and 35–53 of the 70 proteins encoded on pEI70 (60.1% average amino acid identity) shared orthology with proteins encoded on the ICE Pan elements. The lower number of orthologous CDSs shared between pEI70/pEB102 and ICE Pan AJ13355 is due to the absence of CR-1 and -2 from the latter element. Among the conserved plasmid/ICE CDSs are those in the maintenance (ParAB) and conjugative transfer (TraDEFGILW) modules. With the exception of a shared Pil ortholog, no other orthologs of proteins encoded in the ICE Pan pil locus are present in pEB102 or pEI70, suggesting that Pil pilus-associated conjugative transfer is specific to ICE elements. Also absent from the conjugative plasmids is an ortholog of the integration and excision module protein XerC indicative of pEI70 and pEB102 being maintained as plasmids rather than being chromosomally integrated. The presence of a number of orthologous CDSs within the ICE Pan loci and the plasmid pEI70 and pEB102 and the presence of these orthologs in synteny blocks suggest an archaic origin for the ICE Pan family elements and the Erwinia plasmids (Figure 4). A phylogeny constructed on the basis of the amino acid sequences of 21 CDSs conserved in pEI70, pEB102, all five ICE Pan elements, ICE Ecl ATCC13047 and ICE Epi CFBP5888 showed that ICE Pan is more closely related to the enterobacterial ICE elements than pEB102 and pEI70 (Supplementary Figure S1). This is incongruent with the phylogeny on the basis of the house-keeping markers, which thus suggests that the common ancestor of the ICE/plasmids pre-dates species divergence.

ICE Pan Elements are Prevalent among P. ananatis Strains Isolated from Different Sources

The prevalence of ICEs in P. ananatis was determined through PCR amplification with primers designed on the basis of
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FIGURE 3 | Phylogeny of ICE\textsubscript{Pan} and related ICEs and comparison to chromosomal house-keeping marker phylogeny. Alignments were done with MAFFT (Katoh and Standley, 2013) using the concatenated amino acid sequences of the ICE elements (19 conserved CDSs) and house-keeping markers (AtpD, GyrB, InfB, RpoB). Phylogenies were constructed using MEGA v 5.2 (Tamura et al., 2011), using the Neighbor-joining algorithm with complete gap deletion, Poisson correction and bootstrap analysis (n = 1000).

FIGURE 4 | Schematic diagram of ICE\textsubscript{PanLMG5342} and the phylogenetically related conjugative plasmids pEI70 of \textit{E. amylovora} ACW65400 and pEb102 of \textit{E. billingiae} Eb661. ICE\textsubscript{PanLMG5342} is aligned with the plasmids pEI70 of \textit{E. amylovora} ATCC56400 (NC_018999.1) and pEb102 of \textit{E. billingiae} Eb661 (NC_014304.1). Dark gray shaded regions and green arrows indicate the conserved CRs between the ICEs. The conserved umuC island is marked by red arrows, with the umuC fragments and umuD genes colored in yellow, and the plasmid replication gene repA in pEI70 and pEb102 is colored purple.

Conserved ICE protein-based phylogeny
- 19 CDS

P. ananatis B1-9
P. ananatis BD442
P. ananatis LMG5342
P. ananatis PA13
P. ananatis AJ13355
\textit{E. piriformis} CFBP5888
\textit{S. enterica} Senftenberg CTnscr94
P. \textit{wasabiae} WPP163
\textit{Y. pseudotuberculosis} 32777 YAPI

Conserved ICE genes and integration sites (Table S1). Initially, two sets of primers sit1F/R and sit2F/R were designed to cover the two genomic tRNA-Phe regions on the basis of conserved flanking sequences in strains which did not have an insertion at these sites. No amplicons were observed in 31 out of 46 strains (67.4% of strains tested), indicating that a large insert is integrated at one or both of the tRNA-Phe sites. Of these, 11 insertions occur in the tRNA-Phe site occupied by ICE\textsubscript{PanB1-9}, ICE\textsubscript{PanBD442}, ICE\textsubscript{PanLMG5342} and ICE\textsubscript{PanPA13} (sit1), nine solely in the tRNA-Phe site of ICE\textsubscript{PanAJ13355} (sit2) while 11 strains had large insertions integrated at both sites (Table 2). This would suggest that in the latter strains, a third functional copy of the tRNA-Phe gene exists, or that the tRNA-Phe gene(s) is located at a different site. As these primers only give an indication of the presence of an insertion at a given tRNA-Phe site, further primers were designed on the basis of conserved genes in the ICE\textsubscript{Pan} elements.

PCR amplification with primers designed on the conserved sequences of the ICE\textsubscript{PanB1-9}, ICE\textsubscript{PanBD442}, ICE\textsubscript{PanLMG5342}, and ICE\textsubscript{PanPA13} integrase (xerC1F/R) produced amplicons in six additional strains, while those based on the ICE\textsubscript{PanAJ13355} integrase (xerC2F/R) amplified products in six more strains. Further primers designed on the basis of the CR genes coding for topoisomerase B (topB), type IV pilus shufflon (pilV) and relaxase/helicase (tral) amplified products in 22, 23, and 20 strains, respectively (Table 2). There was some variability in the presence of the conserved ICE genes among the strains. For example, nine strains were positive for one or more of the tral, pilV, topB gene fragments, while they were negative for both the XerC1 and XerC2 integrase primer sets. Similarly, while ICE\textsubscript{PanAJ13355} was missing the core regions containing the topB (CR-1) and pilV (CR-2) genes, products were obtained for either or both of the gene fragments in six strains which were positive for the ICE\textsubscript{PanAJ13355} integrase XerC2. In addition to the variability observed among ICE\textsubscript{Pan} elements of the sequenced strains, there thus appears to be further variability among the ICE elements of other \textit{P. ananatis} strains. Variability in the conserved sequences, as was observed for the distinct integrases, may also result in no amplification occurring for conserved ICE genes among \textit{P. ananatis} strains. Nevertheless, 24 out of 46 (52% of...
tested strains) *P. ananatis* strains were positive for two or more of the conserved ICE genes tested (Table 2), which suggests that integrative conjugative elements are relatively common among strains of the species.

**ICEPan Elements Carry Extensive Non-conserved Cargo Genes with a Potential Role in Fitness, Stress Response, and Antibiosis**

The ICEPan elements of all five strains contain four large non-conserved cargo regions, IR 1–4 (Figure 1). IR4 has further been subdivided into IR-4A and IR-4C, separated by a region flanked by the *umuD* and partial *umuC* genes, IR-4B, which is discussed in further detail below. The IRs contribute between 45.7% (ICEPanBD442) and 65.7% (ICEPanAJ13355) of the total ICEPan size (Table 4). The G+C contents of the ICE cargo and core regions were determined. This showed that while the G+C contents of ICEPan (average G+C content: 53.3%) is similar to that of the chromosomes (53.7%), the G+C content of the core regions (56.9%) is somewhat higher and that of the IRs is lower (50.0%) than that of the chromosome (Table 4). This suggests that the current ICEPan structures may have arisen through distinct horizontal acquisition events of the core and island regions. Between 44 (ICEPanAJ13355) and 67 (ICEPanLMG5342) CDSs are encoded in IR1-4. The previous pan-genome comparison of eight *P. ananatis* strains revealed a sizeable accessory genome (1690 CDS—30.4% of the pan-genome) for the species (De Maayer et al., 2014). A total of 124 distinct CDSs are encoded in the IRs of the ICEPan elements, suggesting this genomic element contributes substantially to the accessory fraction of the pan-genome and that ICEPan plays a major role in the diversification of *P. ananatis*.

Several genes within the ICEPan IRs encode orthologs of characterized stress response mechanisms (Table S2). One means of stress response utilized by Gram-negative bacteria is through the production and activation of alternative RNA polymerase σ factors. These regulate the transcription of a large number of different genes that allow the cell to tolerate or counteract various stresses including starvation, osmotic stress, and oxidative and DNA damage (Bougdour and Gottesman, 2007). It is imperative that σ factor concentrations, activity and stability are tightly regulated. Several σ factor stabilizing proteins have been identified including the ATP-dependent protein ClpXP and the anti-adaptor protein IraP which stabilizes σ factor during phosphate starvation (Bougdour and Gottesman, 2007). Genes within the IR-4A of ICEPanPA13 and ICEPanBD442 encode orthologs of ClpXP, while an IraP ortholog is encoded in IR-1 of ICEPanBD442. Another means of stress response is through the expression of Universal Stress Proteins (Usp). UspA of *E. coli* is produced in response to starvation, osmotic and heat shock, as well as exposure to heavy metals and antimicrobial agents (Kvint et al., 2003). Orthologs of UspA are encoded within the IR-4B of all ICEPan elements with the exception of ICEPanBD442. Bacteria associated with plants are frequently exposed to reactive oxygen species such as superoxide anions and hydroperoxides which form an integral part of plant defense responses. A gene localized within the IR-1 of ICEPanBD442 encodes an ortholog of OhrR, a sensor and regulator of organic hydroperoxide resistance (Panmanee et al., 2006). Within IR-4B of this same strain a gene encoding an ortholog of the σ5-regulated manganese-catalase KatN, which has been demonstrated to degrade hydroperoxides into water and oxygen in *S. enterica*, is present (Robbe-Saule et al., 2001). As is the case for the katN gene in *S. enterica*, the ICEPanBD442 gene is localized adjacent to genes showing extensive sequence identity to *yciEFG*, which are likewise thought to play a role in oxidative stress response (Hindupur et al., 2006). Upstream of the ICEPanBD442 katN gene, a gene encoding an ortholog of the oxidoreductase YdeP involved in acid resistance in *Shigella flexneri* (Oglesby et al., 2005), is present.

Aside from roles in stress response, several ICEPan CDSs may contribute to the fitness of the ICE carrying strains. A locus within IR-4C of ICEPanPA13 (Pagr_3794-Pagr_3798) encodes five proteins sharing 61.8% average amino acid identity with a predicted iron transport system in *Agrobacterium tumefaciens* 5A (AT5A_20056.1-AT5A_20076.1). This locus may allow *P. ananatis* PA13 to compete for this limiting nutrient within the plant host. Also interspersed among the IR-1, -4, and -5 of all ICEPan elements is a gene encoding an ortholog of *S. enterica* *umuD* and partial *umuC* genes, IR-4B, which is discussed in further detail below.

**Role in Fitness, Stress Response, and Antibiosis**

**TABLE 4 | Characteristics of the ICEPan and its core (CR) and island (IR) regions.**

| ICE          | CR Size (kb) | CR Size (nt) | IR Size (nt) | CR % Total | IR % Total | G+C | G+C | Genomic G+C (%) | # CDSs | # CDSs | # CDSs |
|--------------|--------------|--------------|--------------|------------|------------|-----|-----|----------------|--------|--------|--------|
| ICEPanAJ13355 | 59.4         | 20.4         | 39.0         | 34.32      | 65.68      | 52.67| 56.76| 50.53          | 53.76  | 67     | 23     | 44     |
| ICEPanB1-9   | 99.2         | 52.7         | 46.5         | 53.11      | 46.89      | 53.27| 56.87| 50.12          | 53.62  | 111    | 47     | 64     |
| ICEPanBD442  | 98.8         | 53.7         | 45.2         | 54.3       | 45.7       | 53.22| 56.88| 48.87          | 53.78  | 109    | 47     | 62     |
| ICEPanLMG5342| 110.3        | 51.9         | 58.4         | 47.05      | 52.95      | 53.22| 56.96| 49.89          | 53.45  | 114    | 47     | 67     |
| ICEPanPA13   | 107.2        | 53.5         | 53.7         | 49.91      | 50.09      | 53.84| 57.02| 50.67          | 53.66  | 111    | 47     | 64     |

The sizes (number of nucleotides) and proportions (%) of the CRs and IRs in relation to the total ICEPan element, G+C contents (%), number and proportions (%) of CDSs on the ICE, CRs, and IRs are shown. The average G+C content of the genomes are given, with a indicating those strains for which the genomic G+C contents were determined on the basis of available contigs for draft genomes.
such as siderophores and antibiotics on these elements (Ghinet et al., 2011). The ICEPan sequences were compared against the antiSMASH (antibiotics and secondary metabolite analysis shell) server (Blin et al., 2013). A ∼7.8 kb locus encoding seven proteins involved in the biosynthesis of a predicted secondary metabolite is present in the IR-4A regions of ICEPanB1-9, ICEPanPA13 and ICEPanAJ13355 (Figure 1; Table S2). The encoded proteins share 99.3–99.9% average amino acid identity among the three strains. Two proteins encode orthologs of the fosmymycin biosynthetic proteins Fom1 and Fom2 of Streptomyces fradiae (ACG70831.1-ACG70832.1—37.6% average amino acid identity). Fom1 encodes a phosphoenolpyruvate (PEP) mutase which catalyzes the first step of fosmymycin biosynthesis, converting PEP into phosphopyruvate (PnP), which is consequently converted by PnP decarboxylase (Fom2) into phosphonoacetaldelyde (Wodyer et al., 2006). Two further proteins share 48.3% average amino acid identity to amidotransferase SsfD (ADE35421.1) and acyl carrier protein SsfC (ADE35420.1) of Streptomyces sp. SF2575 which are required for the production of the malonamate starter unit of tetracyclines (Pickens et al., 2010). Another protein shows weak sequence identity (34.5% average amino acid identity) to StrU of Streptomyces griseus (CAH94303.1), a dehydrogenase involved in the final processing of streptomycin. Also encoded in ICEPan IR-4A of these three strains is an ortholog of the AfsA protein of Streptomyces virginiae MAF10-06014 (BAA23611.1). AfsA is a diffusible γ-butyrolactone autoregulator which regulates the production of secondary metabolites in Streptomyces sp. (Healy et al., 2009). The presence of this locus in the ICEPan IR-4A region suggests that P. ananatis AJ13355, PA13 and B1-9 are capable of producing a secondary metabolite with a potential role in antibiosis. Amplification with primers designed on the basis of the ssjD nucleotide sequences showed that two additional rice-pathogenic strains from Australia and one Eucalyptus-pathogenic strain from South Africa, may potentially encode this putative secondary metabolite biosynthetic locus (Table 2). Within IR-1 of ICEPanB1-9 are two genes encoding proteins with weak sequence identity (31.3% average amino acid identity) to the alveicin bacteriocin A Aat (CDD cl04134: proteins with weak sequence identity (31.3% average amino acid identity)). By contrast the umuC genes were aligned (Figure 5B). This showed that the integron is inserted at amino acid position 88 in the UmuC protein of the Erwinia plasmids pEB102 and pEI70 and all ICEPan with the exception of ICEPanBD442. In the latter, the insertion is integrated in UmuC after position 37.

The ICEPanBD442 insertion encodes 11 proteins not encoded in the other ICEPan, while 11 CDSs are conserved among the other four ICEPan (99.2% average amino acid identity) (Table S2). The ICEPanBD442 umuDC island CDSs include the catalase KatN, YciEFG and YdeP orthologs, which may play a role in oxidative and acid stress response as discussed above, as well as a methyl-accepting chemotaxis protein (Figure 5A). Proteins encoded within ICEPanAJ13355, ICEPanB1-9, ICEPanLMG5342, and ICEPanPA13 umuDC region include an ortholog of the Mae1 protein in the yeast Schizosaccharomyces pombe which plays a role in the uptake of the dicarboxylic acid substrates L-malate, succinate, and malonic acid that are central to the tricarboxylic acid (TCA) pathway (Grobler et al., 1995). Also encoded within this island are orthologs of the 2,3-bisphosphoglycerate-independent phosphoglyceratemutase GpmA and enolase Eno which catalyze the reversible conversion of 3-phospho-D-glycerate to PEP, involved in the glycolysis and gluconeogenesis (Nurmohamed et al., 2010). The umuDC island-encoded enzymes thus play a likely role in energy production and conversion. Also included in the region is a gene (ppgC) encoding a manganese-dependent inorganic pyrophosphatase, which removes pyrophosphate generated as a by-product of many biosynthetic and metabolic reactions, as well as a gene encoding the universal stress protein UspA, which is discussed above. Finally, orthologs of the recombinases XerC and XerD are encoded adjacent to the small 5′ fragment of umuC.

The 11 translated CDSs in the ICEPan elements of AJ13355, B1-9, LMG5342, and PA13 share 99.3% average amino acid identity, similar to the average amino acid identity for the core genome CDS sets (99.3%) (De Maayer et al., 2014). By contrast the IR-4B CDSs share much higher average amino acid identity (~92.0%) with orthologs in P. vagans C9-1, E. billingiae Eb661, and E. amylovora ACW56400 than the genomic average (84.8, 76.0, and 76.3%, respectively) (Table S3). Most striking is the comparison of the 11 umuC-inserted CDSs on the chromosome of P. vagans C9-1 and those encoded on pEB102 and pEI70, which share an average amino acid identity error-prone replication across the damaged DNA lesion, also known as SOS mutagenesis (Hare et al., 2006). The presence of UmuDC orthologs further supports a role for ICEPan in stress response. BlastN analysis of the ICEPan IR-4B CDS sets revealed that the umuC gene is disrupted by a non-conserved insertion (Figure 5A). A similar disruption of the umuC gene could be observed on the chromosome of Pantoea vagans C9-1 and the Erwinia plasmids pEB102 and pEI70. This insertion is ∼11 kb in size in ICEPanB1-9, ICEPanAJ13355, ICEPanLMG5342, and ICEPanPA13 with an average G+C content of 60.3%, substantially higher than the average G+C% for the ICEPan element (53.3%) and the chromosome (Figure 5A). By contrast the umuC insertion in ICEPanBD442 is ∼13.7 kb in size with a G+C content of 49.0%. The amino acid sequences of the disrupted umuC genes were aligned (Figure 5B). This showed that the integron is inserted at amino acid position 88 in the UmuC protein of the Erwinia plasmids pEB102, pEI70 and all ICEPan with the exception of ICEPanBD442. In the latter, the insertion is integrated in UmuC after position 37.

A Non-conserved Island is Integrated into the umuDC Locus

Localized within IR-4 of the ICEPan of all five strains are genes encoding proteins with extensive sequence identity to the SOS damage response proteins UmuC and UmuD. In response to DNA damage, as a result of genotoxic stresses such as UV irradiation or exposure to mitomycin C, these two proteins effect

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identity of 99.7 and 100%, respectively (Table S3), which is substantially higher than the genomic average amino acid identities (76.4, 77.2, and 81.0% average amino acid identity for *P. vagans* C9-1 vs. *E. billingiae* Eb661 and *E. amylovora* ACW56400, and Eb661 vs. ACW56400, respectively). Alignment of the nucleotide sequences for these regions shows only three nucleotide polymorphisms between the *umuDC* islands of *P. vagans* C9-1 and *E. amylovora* pEI70, while those of C9-1 and *E. billingiae* Eb661 share 100% nucleotide identity. This suggests very recent inter-genera horizontal exchange of the *umuDC* island regions between the plasmids of *E. amylovora* ACW56400 and *E. billingiae* Eb661, ICEPanAJ13355, ICEPanB1-9, ICEPanLMG5342, and ICEPanPA13 and the chromosome of *P. vagans* C9-1 (Supplementary Figure S2).

While the functions encoded in the *umuDC* islands of the ICEPan, *P. vagans* C9-1 chromosome and *Erwinia* plasmids, remain to be elucidated, we hypothesize two potential reasons for the integration of this island into the *umuC* gene. Firstly, as the *umuDC* is transcriptionally upregulated in response to stress, the insertion into the *umuC* reading frame may result in the genes on the island being expressed. This hypothesis is supported by observations in *Acinetobacter baylyi* ADP1, where a truncated *umuC* is also present and *umuDC* regulates the expression of DNA-damage inducible gene *ddrR* ([Hare et al., 2006](#)). The truncated *umuC* may thus serve as a DNA-damage inducible regulator for genes on the island which may potentially play a role in stress response. Alternatively, the recombinases encoded by the *xerC* and *xerD* genes located directly upstream of the truncated 5′ region of *umuC* ([Figure 5A](#)) may function in a similar fashion to the integration and excision core module tyrosine recombinase XerC in ICEPan CR-5. The stress-induced upregulation of *umuC* may thus drive the excision and dissemination of the island and its encoded genes. A BlastP analysis with the UmuC and UmuD protein sequences against the ICEberg dataset showed that orthologs were present in 21 out of 270 complete ICE
elements. Of these, an island insertion was observed in the umuC gene of 10 ICE elements, integrated mostly in ICEs of Vibrio spp., but also one Pseudomonas aeruginosa and one Streptococcus pyogenes ICE (Table S4). The islands inserted into the umuC gene in the ICE elements of these microorganisms carried genes implicated in mercury resistance and resistance to several antibiotics including tetracycline, chloramphenicol/florfenicol and streptomycin (Beaber et al., 2002; Battle et al., 2008). In the context of the above hypotheses, the umuC island may thus play a major role in the regulation and/or dissemination of important phenotypes from both a clinical and ecological perspective.

Conclusions

ICEs of the novel ICEPan family are a common feature among P. ananatis strains isolated from various environmental sources and hosts, and with different lifestyles. In silico characterization of the ICEPan elements showed similar ICEs are found in other enterobacteria and that these ICEs, and conjugative plasmids in E. billingiae and E. amylovora, are likely derived from a common ancestor. ICEPan contain the core modules required for their chromosomal integration, maintenance and dissemination. The presence of these core elements, however, does not provide definitive proof that the ICEPan elements can excise, circularize and integrate within the chromosome. Currently, assays are being performed to determine whether ICEPan represent functional integrative and conjugative elements, particularly ICEPanAJ13355, which lacks two of the core modules. Analyses of the ICE cargo genes suggest a likely role in response to various stresses they may encounter in the environment, including oxidative, pH, and genotoxic stresses. ICEPan elements could therefore play a role in facilitating P. ananatis survival under these stresses and provide a potential mechanism for this species to exploit novel ecological niches. With recent interest in the use of P. ananatis as biological control agent against phytopathogenic bacteria and fungi, the ICEPan elements, and the putative antibiotic biosynthetic loci encoded on these elements, provide potential targets for the exploration of P. ananatis antibiosis phenotypes. Perhaps the most pertinent finding in the ICEPan elements is the integration of an island in the umuDC locus, which shows evidence of recent horizontal acquisition between different Pantoea species and related genera. Here, we postulate on a potential role of umuDC in the dissemination and/or expression of phenotypes encoded on this island. Islands integrated into this locus are furthermore found in other bacterial taxa and encode phenotypes such as antibiotic and heavy metal resistance. It can be reasonably expected that similar features will be identified in the ICEs of many other bacterial taxa whose genomes are sequenced. Considering the clinical and ecological relevance of the phenotypes encoded in umuDC, this phenomenon warrants further attention.

Author Contributions

PM, SV, BD, TS, and TC conceived the study. PM, WC, DM, JB, and TS performed experiments and analyses, PM, SV, BD, DC, TS, and TC wrote the original manuscript. All authors contributed to and approved of the final version.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2015.00576/abstract

References

Altschul S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. J. Mol. Biol. 215, 403–410.
Aminov, R. I. (2011). Horizontal gene exchange in environmental microbiota. Front. Microbiol. 2:158. doi: 10.3389/fmicb.2011.00158
Baker, M. D., Wolanin, P. M., and Stock, J. B. (2006). Signal transduction mechanisms in bacterial chemotaxis. Bioessays 28, 9–22. doi: 10.1002/bies.20343
Battle, S. E., Meyer, F., Rello, J., Kung, V. L., and Hauser, A. R. (2008). Hybrid pathogenicity island PAGI-5 contributes to the highly virulent phenotype of a Pseudomonas aeruginosa isolate in mammals. J. Bacteriol. 190, 7130–7140. doi: 10.1128/JB.00785-08
Beaber, J. W., Hochhut, B., and Waldor, M. K. (2002). Genomic and functional analyses of SXT, an integrating antibiotic resistance gene transfer element derived from Vibrio cholerae. J. Bacteriol. 184, 4259–4269. doi: 10.1128/JB.184.5.4259–4269.2002
Bi, D., Xu, Z., Harrison, E. M., Tai, C., Wei, Y., He, X., et al. (2012). ICEberg: a web-based resource for integrative and conjugative elements found in Bacteria. Nucleic Acids Res. 40, 621–626. doi: 10.1093/nar/gkr846
Blin, K., Medema, M. H., Kazempour, D., Fischbach, M. A., Breitlin, R., Takano, E., et al. (2013). antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res. 41, 204–212. doi: 10.1093/nar/gkt449
Bougouard, A., and Gottesman, S. (2007). ppGpp regulation via anti-adapter protein IraP. Proc. Natl. Acad. Sci. U.S.A. 104, 12896–12901. doi: 10.1073/pnas.0705361104
Boyd, E. F., Almagro-Moreno, S., and Parent, M. A. (2009). Genomic islands are dynamic, ancient integrative elements in bacterial evolution. Trends Microbiol. 17, 47–53. doi: 10.1016/j.tim.2008.11.003

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Woodyer, R. D., Shao, Z., Thomas, P. M., Kelleher, N. L., Blodgett, J. A. V., Metcalf, W. W., et al. (2006). Heterologous production of fosfomycin and identification of the minimal biosynthetic gene cluster. Chem. Biol. 13, 1171–1182. doi: 10.1016/j.chembiol.2006.09.007

Wozniak, R. A. F., and Waldor, M. K. (2009). A toxin-antitoxin system promotes the maintenance of an integrative conjugative element. PLoS Genet. 5:e1000439. doi: 10.1371/journal.pgen.1000439

Wozniak, R. A. F., and Waldor, M. K. (2010). Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. Nat. Rev. Microbiol. 8, 552–563. doi: 10.1038/nrmicro2382

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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