Physiological impact of transposable elements encoding DDE transposases in the environmental adaptation of *Streptococcus agalactiae*

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We have referenced and described *Streptococcus agalactiae* transposable elements encoding DDE transposases. These elements belonged to nine families of insertion sequences (ISs) and to a family of conjugative transposons (TnGBSs). An overview of the physiological impact of the insertion of all these elements is provided. DDE-transposable elements affect *S. agalactiae* in a number of aspects of its capability to adapt to various environments and modulate the expression of several virulence genes, the *scpB–lmB* genomic region and the genes involved in capsule expression and haemolysin transport being the targets of several different mobile elements. The referenced mobile elements modify *S. agalactiae* behaviour by transferring new gene(s) to its genome, by modifying the expression of neighbouring genes at the integration site or by promoting genomic rearrangements. Transposition of some of these elements occurs in vivo, suggesting that by dynamically regulating some adaptation and/or virulence genes, they improve the ability of *S. agalactiae* to reach different niches within its host and ensure the 'success' of the infectious process.

**Introduction**

Although *Streptococcus agalactiae* (group B *Streptococcus*) is a common inhabitant of the gastrointestinal or genital tracts of healthy women, it can cause invasive infections in susceptible hosts: newborns, pregnant women or immunocompromised nonpregnant adults. *S. agalactiae* is one of the leading causes of bacterial sepsis, pneumonia and meningitis in neonates in the United States and Europe. Contamination of newborns usually occurs just before or during delivery by inhalation or ingestion of contaminated vaginal secretions. Since the end of the twentieth century and the recommendation of intrapartum antibiotic prophylaxis for high-risk or colonized women, the incidence of early onset neonatal infection has decreased, however, and late onset neonatal infection has become more common. Contamination of late onset form probably occurs by the digestive route (Edwards et al., 2011; Farley, 2001; Jouard-Da Silva et al., 2008; Phares et al., 2008; Spellerberg, 2000a). First recognized as a pathogen in bovine mastitis, *S. agalactiae* was also reported to infect or colonize several other animal species, including fishes and aquatic mammals. It was first distinguished from other pathogenic streptococci by the cell-wall-associated group B carbohydrate. *S. agalactiae* is able to live and survive in a wide variety of environmental conditions. Besides its ability to develop in the gastrointestinal and genital tracts, *S. agalactiae* has also the capacity to colonize the throat or the oral and nasopharyngeal mucosa, and to infect the amniotic fluid, the blood, the cerebrospinal fluid or the mammary gland. It was also reported to be a food contaminant. Such ability to survive and develop in numerous different environments indicates a large capability of adaptation (Edwards et al., 2011; Evans et al., 2008; Farley, 2001; Frey et al., 2011; Richards et al., 2011; van der Mee-Marquet et al., 2008, 2009).

Horizontal gene transfer is one of the main mechanisms driving the evolution of bacterial species. Indeed, mobile genetic elements, which include DNA fragments of various types, such as insertion sequences (ISs), transposons (Tns), mobilizable transposons (MTns), integrons, integrative and conjugative elements (ICEs, also called conjugative transposons), plasmids, phagic DNA or group II introns, play a key role in bacterial adaptation to environmental conditions (Brüssow et al., 2004; Casacuberta & González, 2013; Labbate et al., 2009; Toro et al., 2007; Wiedenbeck & Cohan, 2011; Wozniak & Waldor, 2010). Genome analysis of multiple pathogenic isolates of *S. agalactiae* revealed the...
presence of 69 genomic islands that are absent in at least one of the genomes. Some of the genomic islands are characterized by an atypical nucleotide composition, suggesting possible acquisition by horizontal transfer. Genes associated with mobile and extrachromosomal elements are particularly abundant in these genomic islands, and are poorly represented within the core genome shared by all isolates (around 80 % of any single genome). The majority of the genetic traits linked to virulence, capsular serotype, adaptation and antibiotic resistance also belong to the dispensable genome. In addition, it was shown that phage-associated genes account for 10 % of all strain-specific genes (Tettelin et al., 2005).

In this review, we focused our interest on S. agalactiae mobile elements encoding DDE transposases. Transposases, the enzymes driving the transposition events of mobile genetic elements, are classified into different families according to the mechanism they catalyse. Among them, the DDE- and DDE-transposase family comprises phylogenetically diverse proteins that contain three conserved acidic residues that are required for the coordination of metal ions necessary for their activity. This family of transposases, that contains the enzymes driving the mobility of the majority of known transposable elements, is the best characterized. DDE transposases are modular proteins composed of multiple domains. Generally, one (or several) DNA-binding domain(s) located in the N-terminal part is separated by a linker from a C-terminal catalytic domain. DDE transposases are involved in different transposition mechanisms: replicative transposition (e.g. MuA, Tn3) or cut-and-paste transposition (e.g. Tn5, Tn7, Tn10) (Nesmelova & Hackett, 2010). In S. agalactiae, DDE transposase genes were found on ISs, transposons, a mobilizable transponson and on an atypical family of ICEs named TnGBSs (see below).

ISs are the simplest and smallest form of mobile genetic elements. They consist of short DNA sequences, generally from 700 to 2500 bp in length, able to transpose autonomously (Mahillon & Chandler, 1998). They only encode the proteins involved in the transposition process, in most cases a transposase, and are often flanked by two inverted repeats at their 5’- and 3’-termini (Mahillon & Chandler, 1998). Unit transposons are IS elements containing ‘passenger’ genes in addition to their transposase gene, while composite transposons contain one or more accessory genes flanked by two similar IS elements. Mobilizable transposons are genetic elements that unlike transposons or conjugative transposons (see below) are not self-transmissible. They require functions provided by a coresident conjugative transposon or plasmid for their excision and transfer into a new host (Rajeev et al., 2006). Conjugative transposons, or ICEs, were initially discovered due to their capacity to disseminate antibiotic-resistance genes. They combine recombination and conjugation modules. They encode their excision from a host replicon, their intercellular transfer by conjugation and their integration into a replicon of a recipient cell. Conjugative transposons have a major input in gene flow and genome dynamics in bacteria (Brochet et al., 2008a; Burrus et al., 2002a, b). The conjugative transposon Tn916 is one of the best characterized ICEs. About 80 % of human S. agalactiae isolates are resistant to tetracycline due to the presence of Tn916 or related ICEs (Achard & Leclercq, 2007; Le Bouguéne et al., 1990; Poyart et al., 2003). However, with the exception of the TnGBS conjugative transposons described below, transposition of Tn916 and of all S. agalactiae ICEs described so far is not mediated by a DDE transposase, but by a serine recombinase, such as Tn5397 from Clostridium difficile or a tyrosine recombinase such as Tn916 (Brochet et al., 2008a; Chuzeville et al., 2012; Da Cunha et al., 2013; Haenni et al., 2010; Puymège et al., 2013; Rajeev et al., 2009; Wang et al., 2006).

The impact of transposable elements in the environmental adaptation of prokaryote as well as eukaryote organisms has been extensively demonstrated (for a review see Casacuberta & González, 2013). Some of the accessory genes present on these transposable elements were found to confer to the genome of the organism some environmental adaptive advantage, such as a new metabolic property, a resistance to pollutants or antibiotics, or an increase of pathogenicity (Achard & Leclercq, 2007; Casacuberta & González, 2013). Transposable elements can also act by modifying the expression of neighbouring genes at the integration site, or by promoting huge genomic rearrangements when they are substrates for homologous recombination (Casacuberta & González, 2013; Mahillon & Chandler, 1998; Maruyama et al., 2009). Some well-described bacterial examples are the activation of the cryptic bgl and cel operons required for utilization of β-glucoside sugars in Escherichia coli by disruption of silencer elements around the promoter region by IS1, IS2 or IS5 transposition; IS1 insertion into the mgl locus improving the glucose transport and fitness of E. coli; ISS12 integration into the srpS gene increasing the resistance of Pseudomonas putida to toluene by derepressing the genes encoding an inducible solvent resistance pump; or IS6110 controlling transcription of Mycobacterium tuberculosis genes (including the phoP virulence gene) downstream of its integration site through an outward-directed promoter located at its 3’ end (Hall, 1998; Notley-McRobb & Ferenci, 1999; Parker & Hall, 1990; Safi et al., 2004; Soto et al., 2004; Wery et al., 2001).

In this review, we have referenced and described S. agalactiae transposable elements encoding DDE transposases. These mobile elements were grouped into families following the classification used by the IS repository database, IS Finder (https://www-is.biotoul.fr//). IS elements are classified according to the type of transposase they encode, along with several other criteria including their size-range, the length of the direct repeats of flanking DNA they generate at the target site, the presence of terminal inverted repeats and the number of ORFs they encode. An old system of nomenclature consisting of a single number attributed to each IS element (for example IS861, see below) still coexists with a new, more informative system, that also indicates the first letters of the genus and of the species of the bacteria in
which the IS element was firstly identified [for example
ISSag1 (see below) for the first IS of S. agalactiae registered
under this nomenclature in the IS Finder database (https://
www-is.biotoul.fr/) by B. Spellerberg in 2000 (Mahillon &
Chandler, 1998; Siguer et al., 2006)].

Based on the literature and on in silico analysis, we have then provided an overview of the physiological impact
of the insertion of S. agalactiae mobile elements. We particularly focused our attention on the effect exerted by
these elements on the adaptation and survival capabilities of
S. agalactiae within the host during the infectious process.

Mobile genetic elements of the IS3 family

ISS861 was the first IS described in S. agalactiae. This
1442 bp IS was first identified in strain COH-1 (Rubens
et al., 1989; Table 1). In S. agalactiae strains, the number of
ISS861 copies varies between zero and nine (Dmitriev et al.,
2003; Rubens et al., 1989). ISS861 is flanked by 26 bp
imperfect inverted repeats and contains two overlapping
ORFs encoding 141 and 277 aa proteins. These ORFs were
found to be actively transcribed in vivo but in larger
transcripts, suggesting that adjacent genes are also tran-
scribed in a polycistronic message (Rubens et al., 1989).
In strain COH-1, one of the nine ISS861 copies is located
near genes involved in capsular synthesis. The capsule of
S. agalactiae is a major virulence factor involved in the
inhibition of the activation of the complement alternative
pathway, the impairment of the bactericidal function of
neutrophils, the selective internalization of the bacteria
into dendritic cells via caveolae-independent but lipid raft-
dependent pathways, and the intracellular survival of the
bacteria (Lemire et al., 2012). It has been proposed that
ISS861 could have a regulatory function on S. agalactiae
capsule expression, although no differences in copy number
or locations of ISS861 between differently encapsulated
strains could be found (Rubens et al., 1989; Sellin et al.,
1995). Nevertheless, in one non-typable strain isolated from
the blood of a patient, ISS861 was found to be inserted within
the capsular cpsD gene (Sellin et al., 2000). ISS861 was found
to be present in 59 to 76.9 % of strains of human origin and
in 28.7 to 37 % of bovine isolates (Dmitriev et al., 2003;
Héry-Arnaud et al., 2005; Shakleima et al., 2004). This IS is
highly associated (94 % of the tested strains) with human
isolates containing the bac gene which encodes a surface
protein that is able to bind the Fc portion of IgA and the
factor H. Like the capsule, the Bac protein (also called C
protein β antigen) is thought to help bacteria to escape from
the immune response, by inhibiting complement attack
(Areschoug et al., 2002; Kong et al., 2006).

ISSag1 was detected as part of the composite ISSag2-
flanked scpB–lmB transposon (see below; Bröker &
Spellerberg, 2004; Franken et al., 2001; Luan et al., 2003).
It is a 1251 bp long element flanked by 37 bp imperfect
inverted repeats. ISSag1 carries two ORFs. ORFA encodes
a protein of 96 aa containing a leucine zipper motif. ORFB is
disrupted by a stop codon in all the studied strains
(Franken et al., 2004). As this stop codon disrupts the
conserved core DDE catalytic triad of the transposase, it
is unlikely that ISSag1 represents a functional IS element
(Franken et al., 2004). When present in a S. agalactiae strain,
ISSag1 is found in only a single copy, which is always located
between phtD, the second ORF that is predicted to be
cotranscribed with the lmb gene, and the downstream
copy of ISSag2 (Fig. 1). In the sequenced strain A909, this
deregenerate element was named ISSag7 (SAK_1316, ORFA,
and SAK_1317, ORFB) (GenBank annotation; Tettellin et al.,
2005). ISSag1 was found to be mostly present in human
isolates and in nearly half of bovine isolates (Héry-Arnaud
et al., 2007). ISSag1 homologues were found in Streptococcus
dysgalactiae subsp. dysgalactiae (Table 1). In these latter
strains of animal origin, the transposase gene was not
interrupted by the stop codon identified in S. agalactiae and
multiple copies of the ISSag1 homologue could be detected
(Franken et al., 2004).

The scpB–lmB composite transposon (Fig. 1) is a 16 kb ge-
etic element containing genes encoding the pathogenicity
factors C5a peptidase (scpB) and the laminin-binding/zinc-
binding protein (lmB) (Bröker & Spellerberg, 2004; Franken
et al., 2001; Raganathan et al., 2013). ScpB mediates cleavage
of the chemotactic component C5a and binding to human
immobilized fibronectin, a component of the extracellular
matrix. ScpB also plays a role in the invasion of epithelial
cells (Beckmann et al., 2002; Cheng et al., 2002). The
surface-associated lipoprotein Lmb mediates binding of S.
agalactiae to human laminin, a major component of the
basement membrane, and promotes invasion of human
brain microvascular endothelial cells (Spellerberg et al.,
1999b; Tenenbaum et al., 2007).

The scpB–lmB transposon is flanked by two copies of the
insertion element ISSag2. The copy of ISSag2 downstream
of lmB is a 1220 bp long IS that is characterized by two
ORFs encoding the transposase by translational shifting
between the two ORFs (IS Finder database annotation;
Bröker & Spellerberg, 2004). It carries two imperfect
inverted repeats of 41 bp. In contrast, the ISSag2 copy
upstream of lmB does not harbour two regular inverted
repeats at its termini and the deduced protein of the second
ORF is truncated (Franken et al., 2001). The scpB–lmB
transposon could be mobilized from the chromosome of
strain O90R. In the mobilized strain, the downstream copy
of ISSag2 was deleted and the upstream copy persisted in
the chromosome. It was found that all the tested human
isolates of S. agalactiae had two copies of ISSag2, while in
strains of bovine origin, copy number ranged from zero to
two. While all of the strains of human origin that contained
two copies of ISSag2 also contained the scpB and lmB genes,
neither scpB nor lmB could be found in strains of bovine
origin harbouring only a single copy or no copy of ISSag2.
Moreover, S. agalactiae strains of bovine origin that
harbour a single copy of ISSag2 have the same genotype
as the O90R mobilized mutant. It was thus hypothesized
that horizontal gene transfer caused the exchange of scpB
and lmB, and that the ability of S. agalactiae strains to
colonize or infect human hosts is dependent on their presence (Franken et al., 2001).

In strain A909, the two elements (SAK_1314/SAK_1315 and SAK_1322/SAK_1323) flanking the scpB–lmB transposon were named ISSag4 (Fig. 1). These elements are 99.8% identical to ISSag2 (Tettelin et al., 2005). The scpB–lmB genomic region appears to be a hotspot of integration for mobile genetic elements, as a number of strains also contain ISSag1, or either the IS1548 element or the group II intron GBSi1 in this region (see below; Fig. 1). In the sequenced strain 2603V/R, a truncated copy of ISSag5 (sag1241–sag1242; see below) is also present next to the copy of ISSag2 located upstream of lmB (Fig. 1). A very close homologue of ISSag2 exists in S. dysgalactiae strains and has been designated ISSdy1 (Bröker & Spellerberg, 2004; Vasi et al., 2000). By BLASTN searches, we also identified homologues of ISSag2 in the genome of other Streptococcus species, such as Streptococcus pyogenes and Streptococcus iniae (Table 1).

The 1259 bp long element ISSag5 was identified in one copy in the chromosome of S. agalactiae strain A909. This element contains two ORFs (ORFA, SAK_0773; ORFB, SAK_0772). A putative ORFAB transposase could be reconstructed in silico by −1 frameshift (IS Finder database annotation; Tettelin et al., 2005). By BLASTN searches, we also found one copy of ISSag5 in the genome of some other S. agalactiae strains (e.g. 2603V/R, NEM316 or 09mas01883), and several copies of ISSag5 in the genome of other S. agalactiae (13 and 20 copies in the genome of the camel strains ILRI112 and ILRI005, respectively) or S. dysgalactiae subsp. equisimilis strains (Table 1). In all the above strains, incomplete ISSag5 elements (ORFA or ORFB only) that are signal scars of previous transposition events could also be identified in several positions of the chromosome. Finally, ISSag5 was also identified in one copy in the genome of several S. pyogenes strains (Table 1).

A degenerate element, ISSag6 (SAK_0695, ORFA; SAK_0696, ORFB) was also identified in one copy in the genome of strain A909. This insertion element should not be functional as its ORFB transposase contains premature stops and/or frameshifts, which are not the result of sequencing errors (GenBank annotations; Tettelin et al., 2005). By BLASTN searches, we also identified ISSag6 in the genome of other S. agalactiae strains (Table 1).

**Mobile genetic element of the IS5 family**

Tamura and collaborators brought to light that strain A909 contains multiple copies of a variant of the IS1381 element previously described in Streptococcus pneumoniae R6 (Sánchez-Beato et al., 1997; Tamura et al., 2000). This 900 bp long element, named IS1381A by the IS Finder database, belongs to the ISL2 group of the IS5 family (IS Finder database annotation). IS1381A carries two ORFs of
Table 1. Transposable elements encoding DDE transposases identified in *S. agalactiae*

<table>
<thead>
<tr>
<th>Transposable element (GenBank accession no.)</th>
<th>Strain*</th>
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<tbody>
<tr>
<td><strong>IS3 family</strong></td>
<td></td>
</tr>
<tr>
<td>IS861 (M22449)</td>
<td><em>S. agalactiae</em> COH-1, A909, 2603V/R, GD201008-001</td>
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<tr>
<td>ISSag1/ISsag7 (AF329276)</td>
<td><em>S. agalactiae</em> O90R, A909, 2603V/R, NEM316, 09mas18863, COH-1</td>
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<td>ISsag2/ISsag4 (AF329276)</td>
<td><em>S. agalactiae</em> subsp. <em>dygactiae</em> ATCC 27957</td>
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<tr>
<td>ISsag5 (CP000114)</td>
<td><em>S. agalactiae</em> A909, 2603V/R, NEM316, GD201008-001, 09mas18863, COH-1</td>
</tr>
<tr>
<td>ISsag6 (CP000114)</td>
<td><em>S. agalactiae</em> O90R, 2603V/R, NEM316, 09mas18863, COH-1</td>
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<tr>
<td>IS5 family</td>
<td></td>
</tr>
<tr>
<td>IS1381A (AF064785)</td>
<td><em>S. agalactiae</em> A909, 2603V/R, GD201008-001, 09mas18863, COH-1, S. <em>mitis</em> B6, S. <em>pneumoniae</em> G54, INV200, CGSP14</td>
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<tr>
<td><strong>IS30 family</strong></td>
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<tr>
<td>ISsag3 (CP000114)</td>
<td><em>S. agalactiae</em> A909, GD201008-001, 98-D60C, S. <em>lutetensis</em> 033</td>
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<td>ISsag9 (CP000114)</td>
<td><em>S. agalactiae</em> A909, 2603V/R, NEM316, GD201008-001, ILRI112, S. <em>mitis</em> Sf1</td>
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<td><strong>IS256 family</strong></td>
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<td>ISsag11 (AEXT01000002)</td>
<td><em>S. agalactiae</em> FSL S3-026, 2603V/R, GBS11, MG628</td>
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<td>Tn3706 (not deposited)</td>
<td><em>S. agalactiae</em> subsp. <em>equisimilis</em> RE378, GGS 124</td>
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<td><strong>IS982 family</strong></td>
<td><em>S. agalactiae</em> BS2001</td>
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<td>ISsag4 (AF165983)</td>
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<td><strong>IS1182 family</strong></td>
<td><em>S. agalactiae</em> serotype III, GB00965, MC627</td>
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<td>IS1563 (AY078457)</td>
<td><em>S. salivarius</em> 57.1, CCHSS3, JIM 8777</td>
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<td>IS1563-like (AY437915)</td>
<td><em>S. vestibularis</em> F0396, ATCC 49124</td>
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<td>ISsag8 (CP000114)</td>
<td><em>S. agalactiae</em> serotype III, <em>S. agalactiae</em> A909, GD201008-001</td>
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<td>Allobaculum stercoris* DSM13633</td>
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<td>Bifidobacterium <em>longum</em> subsp. <em>longum</em> BBM68</td>
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<td>Meganonas funiformis CAG:377</td>
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<td>Selenomonas bovis DSM 23594</td>
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<td>Veillonella magna DSM 19857</td>
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<td><em>S. agalactiae</em> Mc1, 2603V/R</td>
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<td><em>S. pyogenes</em> MGAS315, MGAS15252, MGAS1882, SF370</td>
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<td><strong>ISL3 family</strong></td>
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<td>merA–merR Tn (AE009948)</td>
<td><em>S. mitis</em> B6</td>
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<td><strong>DDE conjugative transposon family</strong></td>
<td><em>S. agalactiae</em> NEM316</td>
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Table 1. cont.

<table>
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<tr>
<th>Transposable element (GenBank accession no.)</th>
<th>Strain*</th>
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</thead>
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<tr>
<td>TnGBS2 (NC_004368)</td>
<td>S. agalactiae NEM316</td>
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</table>

*Non-exhaustive list of S. agalactiae strains and other bacterial species containing at least one copy of the transposable elements encoding a DDE transposase. Strains containing these elements were reported in the literature or were identified after BLASTN searches (bold text indicates strains in which the mobile element was first described). In the latter case, the strains indicated (non-bold text) contain a mobile element with more than 90% identical nucleotides and a coverage of more than 95% with respect to the element firstly described.

384 and 432 bp and a pair of 24 bp imperfect inverted repeats at its termini. It has been proposed that the transposase may be encoded by both ORFs due to a potential —1 frameshift (IS Finder database annotation). A DDE motif is found in the C-terminal moiety of the in silico reconstructed transposase. This DDE motif spans over almost the entire sequence of the peptide encoded by the 432 bp ORF.

Due to the multiple presence of IS1381 in S. agalactiae, Tamura et al. (2000) proposed to use this IS for typing S. agalactiae strains. IS1381 was found to be widespread among human clinical isolates (prevalence of about 70%), but is less frequently found among isolates of bovine origin (prevalence of about 15 to 26%) (Héry-Arnaud et al., 2005, 2007; Shakleina et al., 2004; Tamura et al., 2000). A comparative analysis of the phylogeny of a collection of human isolates by MultiLocus Sequence Typing (MLST) and by the prevalence of IS1381 showed that IS1381 may have been anciently acquired during the differentiation of the common ancestor of S. agalactiae strains (Héry-Arnaud et al., 2005). Besides being present in a lot of S. agalactiae strains, IS1381 elements are also found in numerous S. pneumoniae isolates and in one strain of Streptococcus mitis (Sánchez-Beato et al., 1997; our BLASTN searches). In S. pneumoniae and, to a lesser extent, in S. agalactiae, significant variations between the multiple copies of IS1381 present in a given strain are found, suggesting either occurrence of subsequent mutations after duplication of the IS element in the genome of the strain, or successive acquisitions of this mobile element from different bacterial donors (Sánchez-Beato et al., 1997; Table 1).

In human clinical isolates of S. agalactiae, IS1381 insertion is strongly suspected to be involved in the regulation of bacterial adaptive capacity or virulence. The insertion of an IS1381-like element has been identified within the translational attenuator region of ermA of highly clindamycin-resistant isolates (Culebras et al., 2005). ermA encodes a methylase that modifies the 23S rRNA target of MLS (macrolides, lincosamide and streptogramin B) antibiotics, and consequently inhibits their binding (Leclercq, 2002). The insertion of the IS1381 element into the attenuator region presumably results in increased or constitutive expression of ermA in S. agalactiae, leading to antibiotic resistance (Culebras et al., 2005). IS1381 is also thought to be involved in the regulation of the expression of the haemolysin transporter of S. agalactiae (Spellerberg et al., 1999a). The genes necessary for the synthesis and export of this virulence factor are located in a 7 kb genomic region called the cyl locus (Spellerberg et al., 1999a). Naturally occurring non-haemolytic mutants of S. agalactiae have been described. Some of them harbour an IS1381 element in the cylA gene, which encodes a subunit of the haemolysin ABC transporter (Spellerberg et al., 1999a, 2000b). In three analysed epidemiologically unrelated strains, the IS1381-like element is inserted at the same position within cylA (473 bp downstream of the ATG codon), which suggests that this phenomenon is not scarce (Spellerberg et al., 1999a). Interestingly, the emergence of a subpopulation of non-haemolytic bacteria was suspected to occur during transmission of a haemolytic strain from a mother to her neonate (Sigge et al., 2008). This phenomenon was associated with an IS1381 insertion within cylA, indicating that this transposition event can occur during the infectious process (Sigge et al., 2008). Dynamic transposition of IS1381 in a S. agalactiae strain during mother-to-child transmission has previously been assumed by Tamura et al. (2000). In nontypable strains of S. agalactiae unable to produce the capsule, different polymorphisms have been described, consisting of the insertion of IS elements in the capsular cps locus (Ramaswamy et al., 2006; Sellin et al., 2000). Among other ISs, IS1381 should also be involved in the generation of uncapsulated variants of S. agalactiae as this IS was identified at the 3’ end of the cpsE gene of the capsular operon of a nontypable isolate (Ramaswamy et al., 2006). Finally, IS1381 also targets the genomic region encoding the Bac protein. The bac gene is present in numerous S. agalactiae isolates and is often comprised in a genomic region flanked by some IS elements (notably IS861, see above), and in particular by a copy of IS1381 four genes upstream (Nagano et al., 2006; our BLASTN analysis). This suggests the involvement of horizontal gene transfer in the acquisition of this virulence gene. In a human vaginal isolate of S. agalactiae showing a low Bac expression level, an IS1381 element was also found inserted in the opposite orientation within the promoter region upstream of the bac gene (Nagano et al., 2006). The authors suggested that IS1381 may provide a promoter with a lesser transcriptional activity than that of the bac gene, since IS1381 contains in its 5’ end sequences resembling the E. coli −35 and −10 consensus, with a spacer of 23 bp (Nagano et al., 2006). Interestingly, in another S. agalactiae isolate, the integration of IS1381 had previously been identified within the coding sequence of bac, suggesting that this genetic region is specifically targeted by this IS element (Kong et al., 2002).
Whole genome sequencing of several \textit{S. agalactiae} isolates allowed the identification of additional IS1381 targets. Some of them might have an impact on the pathogenicity and/or on the adaptive ability of the strains. Thus, in strain \textit{A909}, \textit{IS1381} interrupts the ORF encoding the cell-wall-anchored surface adhesin \textit{BibA} (Tettelin \textit{et al.}, 2005). \textit{BibA} is a multifunctional protein involved in the virulence of \textit{S. agalactiae} by conferring to the bacteria both an increased resistance to opsonophagocytic killing by human neutrophils, and so a better survival in human blood, and a greater adhesion to host cervical and lung epithelial cells (Santi \textit{et al.}, 2007). In strain 2603V/R, \textit{IS1381} interrupts a gene (\textit{sag2004}/\textit{sag2001}) predicted to encode a conjugal transfer protein belonging to the \textit{ConE/YddE} family (Tettelin \textit{et al.}, 2002). In \textit{Bacillus subtilis}, \textit{ConE} is an ATPase of the \textit{FtsK/HerA} superfamily that is required for the conjugal transfer of \textit{ICEBs1} (Berkmen \textit{et al.}, 2010). This suggests that \textit{IS1381} might be involved in the regulation of horizontal gene transfer. Furthermore, \textit{IS1381} also targets two genes involved in the resistance to environmental stress. Indeed, in strains \textit{A909} and \textit{GD201008-001}, \textit{IS1381} interrupts \textit{opuD} that encodes a BCCT transporter of the osmoregulotant glycine betaine, which takes part in osmoregulation in \textit{B. subtilis} (Kappes \textit{et al.}, 1996; Liu \textit{et al.}, 2012; Tettelin \textit{et al.}, 2005). In strains 2603V/R and GD201008-001, \textit{IS1381} is also found in the intergenic region between two genes predicted to encode a \textit{Na}\textsuperscript{+/H}\textsuperscript{+} antiporter and a GMP synthase (\textit{guaA}) (Liu \textit{et al.}, 2012; Tettelin \textit{et al.}, 2002). In these last cases, \textit{IS1381} is located such that the putative promoter present at its 5' end could have an impact on the transcriptional activity of the gene encoding the \textit{Na}\textsuperscript{+/H}\textsuperscript{+} antiporter. As \textit{Na}\textsuperscript{+/H}\textsuperscript{+} exchangers are involved in resistance to alkaline and salt stresses in some bacterial species, it might be thought that \textit{IS1381} insertion at this locus could have an impact on the resistance of the strains to these environmental stresses (Padan \textit{et al.}, 2005; Waditee \textit{et al.}, 2002; Zuleta \textit{et al.}, 2003).

### Mobile genetic elements of the IS256 family

The \textit{ISSag3} element, encoding a 340 aa long transposase, is related to but different from \textit{ISSag2}. It is present in one copy in the complete sequenced genome of the human isolate \textit{A909} and of the fish isolate \textit{GD201008-001} (Liu \textit{et al.}, 2012; Tettelin \textit{et al.}, 2005). \textit{ISSag3} is flanked by 27 bp imperfect repeats, but no direct repeats were identified (\textit{IS Finder database annotations}). \textit{ISSag3} was also identified on the 8992 bp DNA fragment of strain 98-D60C reported by Dmitriev and collaborators to contain the \textit{bac} gene and being highly similar to pathogenicity islands (Dmitriev \textit{et al.}, 2006). By \textit{BLASTN} searches, we also identified \textit{ISSag3} in the genome of \textit{Streptococcus lutetiansis} 033 (Table 1).

Another insertion element, \textit{ISSag9}, encoding a 388 aa long transposase of the \textit{IS30} family, was also found in three copies (\textit{SAG0434}, \textit{SAG0447}, \textit{SAG0452}) in the genome of strain \textit{A909} (Tettelin \textit{et al.}, 2005). \textit{ISSag9} is flanked by 23 bp imperfect inverted repeats (\textit{IS Finder database annotation}). \textit{BLASTN} searches also distinguished \textit{ISSag9} in the completely sequenced genome of other \textit{S. agalactiae} strains, and in the genome of \textit{S. iniae} SF1 (Table 1). It was observed that \textit{ISSag9} is much more prevalent among human than among bovine isolates of \textit{S. agalactiae} (Héry-Arnaud \textit{et al.}, 2007).

### Mobile genetic elements of the IS256 family

The sequence of the genome of the \textit{S. agalactiae} strain \textit{S3-026} isolated from a cow with mastitis revealed the presence of several copies of an \textit{IS} of the \textit{IS256} family (Richards \textit{et al.}, 2011). The \textit{IS Finder} database annotated this \textit{IS} in the genome of several \textit{S. agalactiae} strains, but also in the genome of \textit{S. dysgalactiae} subsp. \textit{equisimilis} (Table 1). The \textit{ISSag11} transposase possesses 93% similar amino acids with the \textit{IS905} transposase of \textit{Lactococcus lactis} subsp. \textit{cremonis} FL5935 (\textit{IS Finder database annotation}). The right inverted repeat of \textit{IS905} harbours a −35 box capable of participating in promoter activity (Dodd \textit{et al.}, 1994). To our knowledge, an increase in the transcription of downstream genes after \textit{ISSag11} insertion has not yet been described in \textit{S. agalactiae}. \textit{ISSag11} is able to insert between genes (for example between the \textit{SAG0447} and \textit{SAG0449} genes of strain 2603V/R; Tettelin \textit{et al.}, 2002) but also in ORFs. Notably, a capsular polysaccharide polymerase, a lantibiotic synthesis \textit{SpaB} family protein and the IIC component of a phosphoenolpyruvate: carbohydrate phosphotransferase (PTS) system have been found to be split into two by insertion of \textit{ISSag11} (Rato \textit{et al.}, 2013; Richards \textit{et al.}, 2011). Inactivation of proteins after \textit{ISSag11} insertion can thus result in the modification of virulence, competitiveness and fitness properties of \textit{S. agalactiae} strains. Some strains of \textit{S. agalactiae} also possess a mutated \textit{ISSag11} sequence, giving rise to a truncated transposase (for example \textit{SAG0434} of strain 2603V/R; Tettelin \textit{et al.}, 2002).

\textit{IS256} can also be found associated with the \textit{aadA–aphD} gene encoding a bifunctional enzyme conferring resistance to gentamicin, tobramycin and kanamycin to form a composite transposon. The name of this transposon is \textit{Tn5281} in \textit{Enterococcus faecalis}, \textit{Tn4001} in \textit{Staphylococcus aureus} and \textit{Tn4031} in \textit{Staphylococcus epidermidis} (\textit{IS Finder database annotation}). The \textit{S. agalactiae} strain \textit{B128} possesses a chromosomal transposon of this family designated \textit{Tn3706}, and also numerous copies of an \textit{IS256}-like element (Horada \textit{et al.}, 1996; Table 1). This transposon encodes resistance to gentamicin/kanamycin and was found to transpose into the broad-host-range conjugative plasmid \textit{pIP501}. In \textit{pIP501}, \textit{Tn3706} is composed of a central fragment containing the \textit{aaac6–aph2z} resistance gene flanked by two tandemly repeated copies of \textit{IS256} at its 5’ extremity and a single inverted copy of \textit{IS256} at its 3’ extremity. The hybrid replicon \textit{pIP501} is unstable following conjugative transfer between \textit{S. agalactiae} strains. It was proposed that this
Mobile genetic element of the IS982 family

ISSa4 was identified by Spellerberg et al. (2000b). This 963 bp IS encodes a 287 aa transposase homologous to the IS982 transposase of L. lactis. Similar elements were also identified in the genomes of S. dysgalactiae subsp. equisimilis (our BLASTN searches; Table 1). The ISSa4 transposase end is in the right inverted repeat. ISSa4 is flanked by 25 bp perfect inverted repeats and leads to the duplication of a 9 bp target at the insertion site. ISSa4 could be detected only in strains isolated after 1996, suggesting a recent acquisition of this IS by S. agalactiae (Spellerberg et al., 2000b). Based on the prevalence of ISSa4 in MLST groups, other authors also proposed that ISSa4 was recently acquired by S. agalactiae (Héry-Arnaud et al., 2005). ISSa4 was found in 18.6 % of human isolates and in 47.5 % of bovine isolates (Dmitriev et al., 2004; Shakleina et al., 2004). The copy number of ISSa4 varies significantly between strains. Some target sites for insertion of ISSa4 were identified and include the cylB gene encoding the membrane spanning domain of the haemolysin ABC transporter, a putative pathogenicity island, housekeeping genes and intergenic regions. No significant similarity was observed in the sequences targeted by ISSa4 (Dmitriev et al., 2004; Spellerberg et al., 2000b). ISSa4 has been associated with the pathogenic function of S. agalactiae as a non-haemolytic isolate with ISSa4 insertion 87 bp downstream of the start codon of the cylB gene was identified. As for several IS elements of the IS982 family, ISSa4 has also been predicted to provide a −35 hexamer in both inverted repeat regions, which could lead to the generation of hybrid promoters with resident −10 sequences located at the target site (López de Felipe et al., 1996; Spellerberg et al., 2000b). This feature suggests that insertion of ISSa4 upstream of ORFs could modulate their transcription, and thus could influence changes in the virulence or adaptive properties of S. agalactiae.

Mobile genetic elements of the IS1182 family

IS1563 was identified by Takahashi and collaborators during the analysis of restriction digestion patterns (RDPs) of phylogenetic lineages of clinical isolates of S. agalactiae. This 1592 bp long IS encodes a 467 aa transposase and carries a pair of 15 bp inverted repeats at its termini. Its transposase shares homology with the IS1562 transposase of S. pyogenes, hence its designation as IS1563 (Takahashi et al., 2002). Similar IS elements were also identified in the sequenced genomes of Streptococcus salivarius and Streptococcus vestibularis (our BLASTN searches; Table 1). IS1563 was found inserted between the ftsY gene and the group II intron GBSi1 in the AW-10 locus of the RDP type II-2 strains (Bohnsack et al., 2008; Takahashi et al., 2002). The GBSi1 target sequence in RDP type II-2 strains is the 3’ inverted repeat and the 3’ direct repeat of IS1563. The 5’ direct repeat of IS1563 is contained within the 3’ end of ftsY and is separated from the 5’ inverted repeat of IS1563 by a 7 bp non-coding spacer, whereas the 3’ inverted repeat and the 3’ direct repeat are adjacent. It was proposed that IS1563 was originally present within the ancestral AW-10 locus, and that a large part of the IS was deleted from most of the S. agalactiae lineages, leaving the vestigial 5’ direct repeat, the 7 bp spacer, an inverted repeat and the 3’ direct repeat (Bohnsack et al., 2002; Luan et al., 2003; Takahashi et al., 2002).

An IS1563-like IS with identical direct and inverted repeat sequences to IS1563, but with a predicted amino acid sequence only 75 % identical to that of IS1563, has also been identified in a bovine isolate of S. agalactiae (Table 1). This IS1563-like element is located upstream of GBSi1 exactly as IS1563 is found upstream of GBSi1 in the AW-10 locus in RDP type II-2 strains (Bohnsack et al., 2004).

The sequencing of the genome of strain A909 revealed the presence of an isoform of the IS1563-like element. This isoform was named ISSag8 by the IS Finder database. There are five and seven copies of ISSag8 in the chromosome of the completely sequenced genomes of the human isolate A909 and of the fish isolate GD201008-001, respectively (Liu et al., 2012; Tettelin et al., 2005; Table 1). The translational stop site for the ISSag8 transposase is located outside of the IS element (National Center for Biotechnology Information annotations for genes SAK_0320, SAK_0564, SAK_0671, SAK_0941 and SAK_1877).

Mobile genetic element of the IS1595 family

The 1724 bp long mobilizable transposon MTnSag1 (also annotated MTnLnu) was found to be inserted within the cpsE gene belonging to the capsular synthesis operon of the S. agalactiae clinical strain UCN36 (Achard et al., 2005). MTnSag1 was reported to encode a transposase related to the InsAB′ protein of IS1. A DDE motif was found in the C-terminal moiety of the transposase, whereas helix–turn–helix and zinc finger motifs characteristic of IS1 transposases were identified in the N-terminal part of the protein (Achard & Leclercq, 2007). However, further analysis revealed that the transposase of MTnSag1 possesses an active site with a DDN7K and not a DDE chemistry (Sigui er et al., 2009).

MTnSag1 was thus classified in the IS1595 family (ISPna2 group) and renamed ISSag10 or tISSag10 to indicate the presence of a transposable passenger gene (Sigui er et al., 2009; IS Finder database). tISSag10 carries the passenger gene (InuC), which encodes a lincosamide O-nucleotidyldtransferase conferring resistance to lincomycin (Achard & Leclercq, 2007). MTnSag1 (tISSag10) was also recently identified in the chromosome of the Streptococcus anginosus clinical isolate UCN93 (Gravey et al., 2013). Our BLASTN searches do not allow the identification of MTnSag1 (tISSag10) in the genome of other S. agalactiae strains; however, it was found in the genome of several other bacterial species (Table 1).
MTnSag1 (tISSag10) possesses a pair of 25 bp imperfect inverted repeats at its termini. It can be mobilized by the Tn916 conjugative transposon to \( S. agalactiae \) strains. A circular form of MTnSag1 (tISSag10) was detected, but only in the surrogate host \( E. coli \), and an origin of transfer (oriT) was located at the 3' end of the \( lnuC \) gene. It was proposed that MTnSag1 (tISSag10) can be transferred by conjugation and that its acquisition by \( S. agalactiae \) might be efficient for the spread of lincomycin resistance since the majority of \( S. agalactiae \) strains possess Tn916-like genetic elements (Achard & Leclercq, 2007). However, the insertion of MTnSag1 (tISSag10) in the capsular operon of strain UCN36 has interrupted the \( cpsE \) gene; as a consequence the strain was non-typable (Achard et al., 2005). It is thus probable that gain of lincomycin resistance by strain UCN36 is concomitant with a reduction of its virulence.

**Mobile genetic element of the ISAs1 family**

The IS1548 element was identified by Granlund and collaborators in hyaluronidase-negative \( S. agalactiae \) strains of serotype III responsible for endocarditis and septicaemia cases in adults (Granlund et al., 1998). In these strains, the hyaluronidase gene (\( hylB \)) is interrupted by a 1317 bp long IS called IS1548 (Granlund et al., 1998). IS1548 belongs to the ISAs1 family, which includes several other IIS and the H-repeats that form part of some rearrangement hotspot elements in \( E. coli \) (Rh), and whose members are often found associated with cell surface component encoding genes (Mahillon & Chandler, 1998; Granlund et al., 1998; IS Finder database annotations). IS1548 has 19 bp imperfect inverted repeats at its termini and encodes two putative proteins of 377 and 62 aa. The amino acid sequence of the protein encoded by the first ORF of IS1548 has similarities with the H-repeats, and displays 52 % similarity with the transposase of IS1358, an IS element present in \( Vibrio cholerae \) (Granlund et al., 1998). DDE-transposase 1 associated superfamily motifs (pfam 13808) are found in the N-terminal moiety and in the middle of this 377 aa protein.

Studies of the prevalence of IS1548 showed that it is also present in some \( S. agalactiae \) strains of serotype II (Granlund et al., 1998). IS1548 was found to be inserted in the genome of 12 to 36 % of human isolates of \( S. agalactiae \), and in 29 % of the strains responsible for neonatal meningitis (Bidet et al., 2003; Dmitriev et al., 2003; Héry-Arnaud et al., 2005). IS1548 is significantly linked to the \( S. agalactiae \) phylogenetic subdivision A1x that corresponds to the clonal complex 19 (CC19), which is associated with strains responsible for neonatal meningitis and endocarditis (Héry-Arnaud et al., 2005). Based on this association, Luan and collaborators proposed to use IS1548 as a genetic marker for the identification of serotype III strains belonging to the CC19 (Luan et al., 2005). IS1548 is not frequently found among \( S. agalactiae \) isolates of bovine origin (prevalence of 9 to 16 %), and when present in these strains, this insertion element was never found to target the \( hylB \) gene (Dmitriev et al., 2003; Shakleina et al., 2004; Sukhnanand et al., 2005). The presence of IS1548 has also been identified in the genome of feline, canine and equine isolates of \( S. agalactiae \) (Yildirim et al., 2002a, 2002b). IS1548 is also widespread in \( S. pyogenes \) isolates, and present in \( S. dysgalactiae \) subsp. \( equisimilis \) (Granlund et al., 1998; Table 1).

Several data indicate that IS1548 is able to modulate the virulence properties of \( S. agalactiae \). As discussed above, one of the preferential insertion target of IS1548 is the hyaluronidase-encoding gene. Hyaluronidase or hyaluronate lyase is a virulence factor of several mucosal pathogens. It is able to degrade hyaluronic acid, a major component of the extracellular matrix composed of high-molecular-mass polysaccharide, into disaccharides. Hyaluronidase activity thus supplies some nutrients to the bacteria and decreases the surrounding viscosity (King et al., 2004). Several streptococci, including \( Streptococcus suis \), \( S. agalactiae \) and \( S. pneumoniae \), produce a cell-surface-associated hyaluronidase (King et al., 2004; Polissi et al., 1998; Pritchard & Lin, 1993). In \( S. suis \), an HylB activity is detectable in numerous isolates associated with pneumonia, whereas no HylB activity is found in most of the strains isolated from invasive disease (King et al., 2004). This variation in hyaluronidase activity is correlated with a huge sequence diversity of the \( hyl \) gene (King et al., 2004). Similarly, in \( S. pneumoniae \), the gene encoding the hyaluronidase is important for virulence in a mouse pneumonia model, although it is not the case in a mouse septicaemia model (Polissi et al., 1998). This negative correlation between hyaluronidase expression and the ability of \( S. suis \) and \( S. pneumoniae \) to cause an invasive infection is consistent with the fact that the majority of \( S. agalactiae \) strains isolated from the blood of adult patients suffering from endocarditis do not have any hyaluronidase activity. In virtually all the cases, this defect is correlated with the insertion of IS1548 within the \( hylB \) gene (Granlund et al., 1998). Furthermore, it was found that the majority of \( S. agalactiae \) invasive strains isolated from the cerebrospinal fluid of infected neonates harbour IS1548 within \( hylB \) (Rolland et al., 1999). Nevertheless, high HylB levels or the presence of an intact \( hylB \) gene were also reported in isolates of \( S. agalactiae \) causing invasive disease. These contradictory reports point out that the exact involvement of HylB in systemic infections needs further investigations (Bohnsack et al., 2001; Musser et al., 1989; Rojo et al., 2008). IS1548 possesses another preferential insertion target just upstream of the \( lmb \) gene encoding a bifunctional laminin-binding protein/zinc-binding protein (Granlund et al., 1998, 2001; Fig. 1). IS1548 insertion at this site is responsible for an increased transcriptional activity of the \( lmb \) gene, probably due to the presence of a predicted promoter at the 3' end of IS1548, and for an increased binding of the strain to laminin (Al Safadi et al., 2010). IS1548 is also able to insert into the \( cpsD \) gene of the \( cps \) capsular locus. This insertion engenders a \( S. agalactiae \) variant defective for capsule production (Sellin et al., 2000).

In addition to inserting into the above genes, IS1548 also targets the \( covS \) gene in \( S. pyogenes \) (Engleberg et al., 2001).
In streptococci, the CovRS system (Cov for control of virulence, also called CsrRS) is a two-component system composed of a membrane histidine kinase and of a cytoplasmic response regulator, which influences the transcription of about 15% of the genome (Graham et al., 2002). It is particularly known to negatively control the expression of several virulence genes, including the capsular synthetic genes, sgaA encoding the streptolysin S and speB encoding the pyrogenic exotoxin B (Heath et al., 1999). Mutants in the covRS locus, regardless of whether they are constructed in vitro or whether they occur spontaneously in animal models during experimental infections, render bacteria more virulent and highly invasive (Engleberg et al., 2001; Garcia et al., 2010; Heath et al., 1999). In S. agalactiae, the inactivation of covRS increases the expression of surface adhesins, and thus adhesion of the bacteria to human vaginal, cervical and respiratory epithelial cells, as well as to constituents of the extracellular matrix (Park et al., 2012).

Bacterial adhesion to mucosal epithelia is the first step necessary for the establishment of an invasive infection. Moreover, capsule synthesis allows bacteria to evade the immune system in the blood during invasion. This could explain how covRS inactivation by spontaneous mutations may enable bacteria to trigger a systemic infection. These mutations, that occur rarely in vivo, are likely to favour the emergence of a subpopulation of bacteria with the capacity to invasively colonize their host. Whereas most spontaneous mutations of covR or covS consist of substitution, insertion or deletion of a few bp, IS1548 insertions were also identified within covS in two human clinical isolates of S. pyogenes during experimental murine skin infections (Engleberg et al., 2001). This dynamic insertion of IS1548 into covS might allow the bacteria to switch between commensalism and pathogenicity. Indeed, Treviño et al. (2009) showed that covS mutants of S. pyogenes, albeit more virulent and invasive, are less competitive than wild-type strains during growth in human saliva, which models the ecological niche of the bacteria during their commensal state in the upper respiratory tract or during pharyngeal infections. We previously identified two conserved motifs close to the IS1548 region in S. agalactiae, suggesting that this gene might also be targeted by IS1548 in this species (Fléchard et al., 2013a).

We propose that IS1548 may also modulate metal homeostasis in S. agalactiae. Indeed, we recently identified two IS1548 integration sites in the adcRCB operon encoding the zinc-dependent AdcR repressor, and the ATPase and permease subunits of a putative zinc/manganese ABC transporter (Fléchard et al., 2013a). We also identified an IS1548 insertion in a region very close to and upstream of adcA, encoding the high affinity zinc-binding protein of the Adc transporter (Fléchard et al., 2013b). Moreover, the laminin-binding protein Lmb, encoded by a gene that is one of the preferential targets of IS1548 in S. agalactiae, exhibits significant homologies to AdcA, and was shown to bind a zinc divalent cation in S. agalactiae (Granlund et al., 1998, 2001; Ragunathan et al., 2009). Furthermore, IS1548 insertions identified within the adcRCB operon spontaneously appeared during growth in vitro, suggesting, as is the case with covS, that a bacterial subpopulation carrying the mutation could emerge during the infectious process (Fléchard et al., 2013a). This subpopulation might expand in the presence of selective pressure, which could be the local zinc concentration. Indeed, during S. agalactiae invasive infections, the bacteria cross successively anatomical sites and fluids with greatly varying zinc concentrations. The possibility of modulating the expression of zinc transporters by the dynamic insertion of IS1548 may confer an adaptive advantage to S. agalactiae allowing the 'success' of its infection (Fléchard et al., 2013a). Furthermore, the IS1548 covS target described above is also the principal sensor for extracellular Mg\(^{2+}\) in S. pyogenes, another diveral metallic cation (Gryllos et al., 2007). Finally, we also identified IS1548 in the cpdB gene in two meningitic strains of S. agalactiae (Fléchard et al., 2013b). This gene encodes a putative \(2',3'-\)cyclic-nucleotide \(2'-\)phosphodiesterase, whose homologue in S. suis (called SntA) is anchored to peptidoglycan by sortase A and possesses an RGD motif that may directly interact with integrin receptors of mammalian cells (Osaki et al., 2002). In S. suis, cpdB has been shown to be induced under iron starvation (Li et al., 2009). Interestingly, a homologue of cpdB is also upregulated in the presence of a chelator of divalent cations or during incubation in porcine cerebrospinal fluid in Haemophilus parasuis, an opportunistic bacteria responsible for a systemic disease in swine (Metcalf & Maclnnes, 2007). These data indicate that IS1548 insertion within cpdB might modulate S. agalactiae response to metals and its survival in cerebrospinal fluid during meningitis.

We also suggest that IS1548 could be involved in the regulation of the genetic competence of streptococci. Several studies based on the search for transformation-deficient mutants revealed the involvement of genes encoding high-affinity zinc transporters in the genetic competence of streptococci and other Gram-positive bacteria (Dintilhac et al., 1997; Loo et al., 2003; Ogura, 2011). The adcCBA operon (called adc for adhesin competence repressor) was identified as being indispensable for the competence of S. pneumoniae (Dintilhac et al., 1997). The addition of zinc to the medium almost totally restores the transformability of the mutants and is required soon after contact of cells with the CSP competence-stimulating peptide (Dintilhac et al., 1997). In Streptococcus gordonii, a transpositional adcR polar mutant is also defective in competence and impaired in biofilm formation (Loo et al., 2003). Finally, in B. subtilis, transpositional mutants of the genes encoding the ZnuABC high-affinity zinc transporter and of zosA encoding a P-type ATPase for zinc incorporation exhibit low transformability (Ogura, 2011). These defects are due to an impaired expression of late competence genes and are rescued by the addition of zinc (Ogura, 2011). Thus, zinc transport appears to be necessary for the late stages of competence in
some Gram-positive bacteria including streptococci. As described above, IS1548 often targets zinc-related genes and in particular those encoding the adc high-affinity transporter (Fléchard et al., 2013a). In S. pyogenes strains SF370 and MGAS315, IS1548 is also found in the close vicinity of the comX.1 gene encoding a putative sigma factor involved in the transcription of late competence genes. Nevertheless, in these two strains, IS1548 seems unlikely to interfere directly with comX.1 expression due to its location downstream of this gene (Beres et al., 2002; Ferretti et al., 2001). While pyogenic streptococci have not yet been shown to be spontaneously transformable under laboratory conditions, the genomes of all of the sequenced streptococcal strains contain at least one homologue of the gene encoding the master regulator of competence ComX, as well as the competence effector genes. This suggests that S. pyogenes and S. agalactiae could also become transformable under some specific conditions (Mashburn-Warren et al., 2012). It is thus possible that IS1548 takes part in this process leading to the adaptation of part of the bacterial population to particular environmental conditions, such as those encountered during host infection.

In addition to the numerous targets of IS1548 described above, we also identified an IS1548 insertion site in the intergenic region upstream of murB, a S. agalactiae gene encoding an UDP-N-acetylpyruvoylglucosamine reductase (Fléchard et al., 2013a). This enzyme, encoded by an essential gene in bacteria, is involved in an early step of peptidoglycan synthesis (El Zoibiley et al., 2003). IS1548 insertion upstream of murB was found to be widespread among CC19 clinical strains, suggesting a possible role in bacterial pathogenicity (Fléchard et al., 2013a). Furthermore, IS1548 is oriented toward the murB gene, which might lead to an increased transcription of murB due to the presence of a putative promoter located at the 3’ end of the IS element, as previously described for the IS1548 insertion site upstream of lmb (Al Safadi et al., 2010). In Bacillus anthracis, the downregulation of a murB homologue using targeted antisense RNA is responsible for hypersensitivity of the strain to β-lactam antibiotics (Kedar et al., 2007). This suggests that the potential upregulation of murB by IS1548 could cause a decrease in S. agalactiae susceptibility to β-lactams, although this class of antibiotics is currently still efficient in first-line chemoprophylaxis.

Altogether, the above data indicate that IS1548, which is associated with some types of clinical isolates, is likely to play an important role in the pathogenesis of streptococci. This involvement in bacterial virulence may occur both by modulating the expression of some virulence factors, or by allowing the bacteria to better adapt to their environment during the infectious process.

**Mobile genetic element of the ISL3 family**

A transposable element that also encodes two genes (merA and merR) for a mercuric resistance operon was identified in two copies (sag2022 to sag2024 and sag1253 to sag1255) in strain 2603V/R (Stapleton et al., 2004; Tettelin et al., 2002). This element encodes a 417 aa long transposase of the ISL3 family and carries a pair of 24 bp inverted repeats at its termini. In one of the two copies of this element, an 8 bp direct repeat sequence consistent with movement of the transposon was also identified next to the inverted repeats (Stapleton et al., 2004). Stapleton and collaborators also identified a similar transposable element in a S. gordoni strain and in S. mitis NCTC 12261. The same authors reported as well that Streptococcus oralis 1601A and S. mitis 26410 possess the same transposon, but with deletion of most of the transposase gene (Stapleton et al., 2004). Mutations in this transposase gene should not be unusual as our BLASTN searches showed that S. mitis strain B6 carries this transposon with an authentic stop codon in the transposase gene (National Center for Biotechnology Information annotation for gene smi_0887; Table 1).

The predominant mechanism of mercury resistance in bacteria is the enzymic reduction of mercury by the mercuric reductase enzyme encoded by the merA gene. This gene is usually part of an operon encoding in addition proteins involved in mercury transport, regulation of mer gene expression (principally merR, but also merD) and in some cases, mercuric lyase enzymes (encoded by the merB genes) conferring, in combination with MerA, organomercuric-labile resistance (Mathema et al., 2011; Stapleton et al., 2004). The above strains containing the merA–merR transposon lack the genes encoding proteins involved in mercury transport. This absence may explain why the merA-positive strains identified by Stapleton and collaborators exhibit only a twofold to fourfold decrease in mercury susceptibility compared to merA-negative mercury-sensitive isolates (Stapleton et al., 2004). Mercury in dental amalgam should have made a selective pressure for the occurrence of the merA–merR transposon in oral streptococci. It is possible that this transposon was then transferred horizontally to S. agalactiae strain 2603V/R. Although S. agalactiae is normally a resident of the gastrointestinal tract, it is also able to colonize the throat of human adults. Faecal–oral transmission of S. agalactiae may occur as hand-to-mouth and aerosol contaminations are common for streptococci (Manning et al., 2004; van der Mee-Marquet et al., 2008). It is worth noting that one copy of this ISL3-related transposon (sag1253 to sag1255) is inserted in a putative ICE of strain 2603V/R (ICESa2603), which also contains genes involved in cation and cadmium transport (Davies et al., 2009; Tettelin et al., 2002). To our knowledge, it is nevertheless not known if strain 2603V/R exhibits a decrease in its susceptibility to heavy metals.

**DDE conjugative transposons family**

Genomic island X (TnGBS2) and pNEM316-1 (TnGBS1) of strain NEM316 were reported to be members of an atypical family of conjugative transposons named TnGBSs, which associates DDE transposition and conjugation (Table 1). We briefly described these elements below as
this new family of conjugative transposons has been recently reviewed extensively (Da Cunha et al., 2013).

TnGBS1 (47 kbp; 49 genes) present in three copies in strain NEM316 is flanked by 26 bp imperfect inverted repeats and 9 or 10 bp direct repeats. It encodes a transposase (gbs0410) containing a domain weakly similar to the rve domain of retrovirus integrases that are DDE transposases (Brochet et al., 2009; ICEberg database annotations http://db-mml.sjtu.edu.cn/ICEberg/). The origin of transfer of TnGBS1 was used to construct a mobilizable vector to introduce plasmid DNA from S. agalactiae into Streptococcus gallolyticus and construct targeted deletion mutants in the latter species (Danne et al., 2013).

TnGBS2 (33.5 kbp; 36 genes) present in one copy of strain NEM316 is delineated by inverted repeats similar to those of TnGBS1 and is flanked by a 9 bp repeated sequence. TnGBS2 contains a paralogue (gbs1118) of the TnGBS1 transposase (Brochet et al., 2009; ICEberg database annotations http://db-mml.sjtu.edu.cn/ICEberg/). Transposition of TnGBS1 and TnGBS2 occurs in intergenic regions, 15 or 16 bp upstream of the −35 sequence of ςA-dependent promoters, suggesting an association between transcription and transposition (Brochet et al., 2009; Guérillot et al., 2013). Transposition of TnGBS1 and TnGBS2 catalyses the formation of a circular intermediate that is substrate for a subsequent conjugative intercellular transfer. For their propagation, both ICEs combine replication and transposition functions, but are stabilized by integration into the genome (Brochet et al., 2009; Guérillot et al., 2013). TnGBS2 was also reported to promote the conjugative transfer of chromosomal DNA (Brochet et al., 2008b).

TnGBS1 and TnGBS2 encode only five homologous proteins: the transposases, three surface exposed LPxTG proteins similar to surface exclusion protein and proteins homologous to aggregation factors encoded by several conjugative plasmids from Gram-positive bacteria. TnGBS1 and more particularly TnGBS2 were found to be widely distributed among S. agalactiae isolates, but are more frequently detected in strains of bovine origin than in human isolates. It was proposed that this divergence in prevalence might be explained by the fact that TnGBSs may provide a selective advantage in the rumen of polygastric mammals (Brochet et al., 2009). Some functions encoded by TnGBS1 and TnGBS2 might affect the phenotype of S.

Table 2. Mobile elements encoding DDE transposases involved in environmental adaptation and virulence

<table>
<thead>
<tr>
<th>DDE transposase mobile element</th>
<th>Role in environmental adaptation and/or virulence</th>
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<tbody>
<tr>
<td><strong>IS3 family</strong></td>
<td></td>
</tr>
<tr>
<td>IS861 scpB–lmB Tn</td>
<td>Capsule expression</td>
</tr>
<tr>
<td>ISSag2</td>
<td>C5a cleavage, human laminin and fibronectin binding, epithelial cell invasion, zinc binding</td>
</tr>
<tr>
<td><strong>IS5 family</strong></td>
<td></td>
</tr>
<tr>
<td>IS1381A</td>
<td>Clindamycin resistance, haemolysin transport, capsule expression, bac expression</td>
</tr>
<tr>
<td><strong>IS256 family</strong></td>
<td></td>
</tr>
<tr>
<td>ISSag1 Tn3706</td>
<td>aaot6-aph2’” gentamicin/kanamycin-resistance gene mobilization</td>
</tr>
<tr>
<td><strong>IS982 family</strong></td>
<td></td>
</tr>
<tr>
<td>ISSa4</td>
<td>Haemolysin transport</td>
</tr>
<tr>
<td><strong>IS1595 family</strong></td>
<td></td>
</tr>
<tr>
<td>MTnSag1 (ISSag10)</td>
<td>Capsule expression, lincomycin resistance</td>
</tr>
<tr>
<td><strong>ISAs1 family</strong></td>
<td></td>
</tr>
<tr>
<td>IS1548</td>
<td>Hyaluronidase expression, capsule expression, laminin/Zn-binding protein (Lmb) expression</td>
</tr>
<tr>
<td><strong>ISL3 family</strong></td>
<td></td>
</tr>
<tr>
<td>merA–merR Tn</td>
<td>Metal resistance, peptidoglycan synthesis, β-lactam-antibiotic susceptibility</td>
</tr>
<tr>
<td><strong>DDE conjugative transposons</strong></td>
<td></td>
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<tr>
<td>TnGBS1</td>
<td>Conjugative transfer of chromosomal DNA</td>
</tr>
<tr>
<td>TnGBS2</td>
<td>Conjugative transfer of chromosomal DNA</td>
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agalactiae under certain circumstances. In particular, the aggregation proteins expressed by these DDE conjugative transposons are related to the streptococcal antigen I/II-family protein (Guérillot et al., 2013). In streptococci, members of this family of adhesins have been shown to mediate attachment of the bacteria to host structures, to be involved in biofilm formation and host colonization, and to exert an immunomodulatory function (Brady et al., 2010; Maddocks et al., 2011).

A large number of ICEs similar to TnGBS1 and TnGBS2 subfamilies were identified by systematic genome analysis (Da Cunha et al., 2013). Both subfamilies encode different conjugation modules: a relaxase and its cognate origin of transfer (oriT) site, which performs a single-strand nick on the circularized ICE; a VirD4 coupling protein recruiting the single-stranded ICE covalently bound to the relaxase to a pore forming type IV secretion system; a type IV secretion system actively pumping the ICE into the recipient cell (Guérillot et al., 2013; Llosa et al., 2002; Walden et al., 2010). TnGBSs are almost exclusively found in streptococci and have a restricted host range. However, as revealed by the analysis of TnGBS1 and TnGBS2 transfer to various bacterial species, this host restriction is not due to a transfer incompatibility linked to the conjugation machineries, but presumably to their ability to replicate within non-streptococci hosts for transient maintenance after their transfer (Guérillot et al., 2013).

Concluding remarks
The data described in this review highlight the fact that DDE-transposable elements impact S. agalactiae in various aspects of its capability of adaptation to various environments and modulate its virulence properties; some virulence genes even being the targets of several different mobile elements (Table 2). Transposition of some of these elements has been shown to occur in vivo, suggesting that they could regulate some adaptation and/or virulence genes in a dynamic manner, and thus increase the capability of S. agalactiae strains to cross the subsequent anatomical sites encountered within their host during the infectious process. The degree of specificity of insertion of transposable elements encoding DDE transposases varies among characterized elements. The transposition machinery can recognize low-stringency consensus sequences (e.g. Mu phage), targets containing the TA dinucleotide (e.g. IS630), GC- or AT-rich regions (e.g. IS186 and IS1, respectively), specific sequences (e.g. Tn7 and the attTn7 site of the E. coli glnS terminator), consensus sequences, some of which being palindromic (e.g. Tn5, Tn10 or IS10), regions with a high homology to the extremities of the transposable element (e.g. Tn3) or the extremities of other already integrated similar elements (e.g. IS21 or IS30) (Casadaban et al., 1981; Craig, 1997; Goryshin et al., 1998; Halling & Kleckner, 1982; Olasz et al., 1997; Reimmann & Haas, 1987; Sengstag et al., 1986; Tenzen & Ohtsubo, 1991; Zerib et al., 1985). In S. agalactiae, it remains to be seen whether the transposition events described in this review affect all categories of genes whatever their function or whether they specifically affect sets of genes belonging to the same functional class. In the first hypothesis, the transposition events would have been selected according to the selection pressure encountered by the bacteria at different stages of the infection and to their benefit for the strain. In the second hypothesis, specific environmental conditions would have induced the transposition of the mobile elements by a mechanism of adaptive mutation.

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References


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