

Atypical low-copy number plasmid segregation systems, all in one?

Patricia Siguier, Manuel Campos, François Cornet, Jean-Yves Bouet,

Catherine Guynet

► To cite this version:

Patricia Siguier, Manuel Campos, François Cornet, Jean-Yves Bouet, Catherine Guynet. Atypical low-copy number plasmid segregation systems, all in one?. Plasmid, 2023, 127, pp.102694. 10.1016/j.plasmid.2023.102694 . hal-04134336

HAL Id: hal-04134336 https://hal.science/hal-04134336

Submitted on 20 Jun 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Atypical low-copy number plasmid segregation systems, all in one?

3	Patricia Siguier ¹ , Manuel Campos ¹ , François Cornet ¹ , Jean-Yves Bouet ¹ and Catherine Guynet ^{1*}
4	¹ Laboratoire de Microbiologie et de Génétique Moléculaires, Centre de Biologie Intégrative (CBI),
5	Centre National de la Recherche Scientifique, Université de Toulouse, UPS, F-31000, Toulouse, France.
6	* Correspondence: Catherine.Guynet@univ-tlse3.fr, +33 5 61 33 59 15
7	KEY WORDS: segregation; partition; StbA; Par; plasmid R388; plasmid pSK1; hitchhiking; HTH
7 8	KEY WORDS: segregation; partition; StbA; Par; plasmid R388; plasmid pSK1; hitchhiking; HTH
7 8 9	KEY WORDS: segregation; partition; StbA; Par; plasmid R388; plasmid pSK1; hitchhiking; HTH
7 8 9 10	KEY WORDS: segregation; partition; StbA; Par; plasmid R388; plasmid pSK1; hitchhiking; HTH

12 ABSTRACT

Plasmid families harbor different maintenances functions, depending on their size and 13 copy number. Low copy number plasmids rely on active partition systems, organizing a 14 partition complex at specific centromere sites that is actively positioned using NTPase 15 proteins. Some low copy number plasmids lack an active partition system, but carry atypical 16 intracellular positioning systems using a single protein that binds to the centromere site but 17 18 without an associated NTPase. These systems have been studied in the case of the Escherichia coli R388 and of the Staphylococcus aureus pSK1 plasmids. Here we review these two systems, 19 which appear to be unrelated but share common features, such as their distribution on 20 21 plasmids of medium size and copy number, certain activities of their centromere-binding proteins, StbA and Par, respectively, as well as their mode of action, which may involve 22 dynamic interactions with the nucleoid-packed chromosome of their hosts. 23

24

Contents

27				
28	1.	Intro	oduction	. 4
29	2.	Gen	etic organizations and regulation of <i>par</i> and <i>stbA loci</i>	. 7
30	3.	Dist	ribution of single-protein partitioning systems	. 8
31	4.	Wha	at do proteins tell us about their functions?	10
32	4.	.1.	Binding to the centromere-like site	10
33	4.	.2.	Oligomerization	13
34	5.	Seg	regation mechanism models for single-protein systems	15
35	5.	.1.	Fate of the plasmids when cohabiting with chromosomal DNA	16
36	5.	.2.	"Chromosome hitchhiking" or "pilot fish" model	18
37	5.	.3.	StbA and the interplay between vertical segregation and horizontal transfer	20
38	6.	Con	clusion	21
39	7.	Mat	erials and methods	22
40	7.	.1.	StbA reference dataset	.22
41	7.	.2.	Enterobacterial StbA dataset	23
42	7.	.3.	Phylogenetic tree	23
43	8.	Ack	nowlegments	24
44	9.	Refe	erences	24
45				

47 **1. Introduction**

48 As non-essential extra-chromosomal DNA molecules, plasmids require special strategies for efficient replication and stable propagation in growing bacterial populations. These include 49 multimer resolution, addiction and partition systems, all of which have been identified in both 50 51 Gram-negative and Gram-positive bacteria. Some high copy number plasmids rely on a 52 stochastic distribution of their copies and do not require partition systems to ensure that the 53 fraction of cells without plasmids is low enough for their maintenance. In contrast, large low 54 copy number plasmids often require all three types of dedicated systems to be stably inherited (Sengupta and Austin, 2011). 55

Most well-studied low copy-number plasmids, which are maintained at less than 5-6 56 copies per chromosome, encode one or more partition system. Briefly, these systems 57 assemble a dedicated nucleoprotein complex around a centromere-like site, then separate 58 and position them at specific subcellular positions, allowing each daughter cell to receive at 59 60 least one copy after cell division. Partition systems described so far share common features: they include a *cis*-acting centromere-like site and contain two genes organized in tandem in 61 an autoregulated par operon that encode two trans-acting proteins. In all cases, one gene 62 encodes a centromere binding protein (CBP) forming a nucleoprotein complex at the 63 centromere (i.e. the partition/segregation complex) (for reviews: (Baxter and Funnell, 2014; 64 Bouet and Funnell, 2019; Gerdes et al., 2000). The other gene codes for an NTPase interacting 65 with the partition complex and essential for segregation. 66

The family of NTPase defines to which type belongs a given partition system. To date, three main types have been identified in bacteria. Type I systems (or ParABS), of which the well-studied models are the P1 and F plasmids are the paradigm, encode Walker-type ATPase

70 proteins (ParA). They are by far the most prevalent in low-copy-number plasmids and are the 71 only type present on chromosomes. They are divided into two sub-types, Ia and Ib, which 72 differ by the nature of the DNA-binding domain of their CBP (ParB), the size of the Par proteins and the mode of transcriptional autoregulation of the par operon. In type Ib, the CBPs include 73 74 a ribbon-helix-helix (RHH) domain and regulate transcription of the par operon (Golovanov et 75 al., 2003; Hayes, 2000). In type Ia, the CBPs contain a helix-turn-helix (HTH) domain and par 76 transcription is rather regulated by the associated NTPase (Friedman and Austin, 1988; Hirano 77 et al., 1998). Recently, type I ParB proteins have been shown to belong to a new family of cytosine triphosphate (CTP) dependent molecular switches, which is required for the partition 78 complex assembly (Jalal et al., 2021; Osorio-Valeriano et al., 2021; Soh et al., 2019). Type II 79 systems (or ParRMC), identified in the Escherichia coli plasmid R1, encode actin-like ATPases 80 81 (ParM) and CBPs containing a RHH DNA-binding domain that contributes to the operon transcriptional autoregulation (Gerdes et al., 2000; Jensen et al., 1994). Type III systems (or 82 TubZRC), exemplified by plasmid pXO1 of Bacillus anthracis, encode tubulin-like GTPases and 83 HTH-carrying CBPs (Larsen et al., 2007; Ni et al., 2010). 84

85 In types I-III systems, plasmid segregation strictly relies on the activity of their NTPases, which provides energy to separate plasmid copies and to position them to specific subcellular 86 locations. Type II, which are the best characterized, and III systems use filamentation-based 87 mechanisms relying on the ATP-dependent polymerization of the NTPase to drive partition 88 complexes toward opposite cell poles, either via a pushing or a pulling mechanism, 89 respectively (Figure 1) (Aylett et al., 2011; Aylett and Löwe, 2012; Møller-Jensen et al., 2003; 90 91 Vecchiarelli et al., 2012). Although a mechanism involving similar dynamic NTPase filaments has been initially proposed for type I systems (Ebersbach et al., 2006; Ringgaard et al., 2009), 92 93 recent studies have shown that ParA ATPases rather promotes segregation by using a

Brownian-ratchet mechanism (Hu et al., 2017; Hwang et al., 2013; Le Gall et al., 2016; Lim et al., 2014; Vecchiarelli et al., 2014, 2013, 2012). This mechanism relies on ParA ATP-dependent nonspecific DNA binding activity to the bacterial nucleoid. Briefly, the partition complex binds to the nucleoid via ParA, which stimulates ParA release from the DNA. The ParA rebinding to the nucleoid is slow, such that a void of ParA is created on the nucleoid around the partition complexes. This asymmetric redistribution of unbound ParA induces the partition complexes to move away from each other, towards the ParA remaining bound to the nucleoid (Figure 1).

101 Partition systems are not mutually exclusive, since some naturally-occurring plasmids 102 carry two different systems, generally one of each type, such as plasmids R27 and pB171 (Ebersbach and Gerdes, 2001; Lawley and Taylor, 2003; Planchenault et al., 2020). Although 103 both systems contribute to plasmid stability for these plasmids, the type I system makes the 104 105 larger contribution. Besides, an number of sequenced low-copy-number plasmids do not 106 encode typical partition systems (types I-III), suggesting the existence of alternative systems (Planchenault et al., 2020). In that line, two additional and distinct segregation systems have 107 108 been highlighted. Although unrelated, they share the particularity of involving a single 109 plasmid-encoded DNA-binding protein: a CBP not associated with an NTPase (Guynet et al., 110 2011; Simpson et al., 2003). These proteins, which have no homology to each other or to other known partitioning proteins, are the Par and the StbA proteins of the staphylococcal plasmid 111 pSK1 and the Escherichia coli conjugative plasmid R388, respectively. Plasmids R388 and pSK1 112 are low-copy-number plasmids. R388 is maintained at about 5 copies per chromosome 113 (Guynet et al., 2011), and, to our knowledge, the copy number of pSK1 has not been 114 115 determined experimentally, but pSK1 minireplicons are also about 5 copies per chromosome (Grkovic et al., 2003). This range of copy number, which is theoretically not high enough for 116

faithful vertical transmission to rely on a stochastic distribution (Nordström and Austin, 1989),
requires an active segregation process.

Here, we present an update on the distribution and organization of the StbA system. We also look back at recent biochemical, structural and *in vivo* studies and discuss hypotheses on possible models of segregation of R388 and pSK1, as experimental plasmids for these unusual segregation pathways involving a single plasmid-encoded protein.

123

124 **2.** Genetic organizations and regulation of *par* and *stbA loci*

pSK1 Par is encoded by the par gene, which is divergently transcribed from the plasmid's 125 replication initiation gene rep (Figure 2A; (Firth et al., 2000)). The intergenic region between 126 127 rep and par genes contains several features involved in plasmid replication and stability. These include the promoter of rep, the promoter of an antisense RNA, complementary to the leader 128 region of rep (Prnal), mediating negative regulation of pSK1 copy number, and the 129 130 centromere-like site (Chan et al., 2022; Kwong et al., 2008). The origin of replication of pSK1 131 is located within the rep gene (Kwong et al., 2008; Liu et al., 2012). This organization of the 132 *rep-par* intergenic region is conserved among staphylococcal multiresistance plasmids (Kwong et al., 2008, 2004). This configuration, in which the partition locus and other maintenance 133 134 features are located near the rep locus, is widespread in plasmids. The centromere site, recognized by Par, is composed of seven repeats of a 12-bp DNA sequence with the consensus 135 sequence TTAGGYRSYWAR (Y=C/T, R=A/G, S=G/C, W=A/T) containing a palindrome 136 TTAG(X)₄CTAA ((Chan et al., 2022; Simpson et al., 2003); Figure 2A). The promoter of pSK1 par 137 138 has been identified among other putative promoters between the par and rep genes. The Ppar 139 - 35 sequence is located between repeats 3 and 4 of the centromere site, the -10 sequence

encompasses repeats 2 and 3, and the transcriptional start point is in repeat 2. As observed in many partition systems, Par autoregulates the expression of its own gene by binding the centromere repeats in the promoter region ((Chan et al., 2022), Figure 2A).

143 R388 StbA is encoded in the *stb* operon, which is divergently transcribed from the *trwABC* mobility operon (MOB) involved in conjugative DNA processing ((Fernández-López et al., 2006; 144 145 Guynet et al., 2011); Figure 2B). The intergenic region between the two operons contains the origin of conjugative transfer (oriT) and stbS, the centromere site. stbS is composed of two 146 147 sets of five repeats of a 9-bp DNA sequence (the *stbDR*) with the consensus (T/C)TGCATCAT 148 separated by 2 bp (Figure 2B). The PstbA – 35 and -10 sequences are predicted to be in the stbDRs of the centromere site. StbA was shown to repress its own promoter, as well as four 149 150 other promoters of plasmid R388. These latter contain two to five stbDR repeats, and makes 151 StbA an important transcriptional regulator of the plasmid (Fernandez-Lopez et al., 2014; 152 Quèbre et al., 2022). Except ardC, which encodes an antirestriction protein, necessary for 153 conjugation in certain conditions (González-Montes et al., 2020), the other genes regulated 154 by StbA have unknown functions. In addition to stbA, the stb operon contains two other genes, stbB and stbC, which are not involved in plasmid segregation in E. coli (Guynet et al., 2011). 155 156 StbB is involved in the control of conjugation (see below), while StbC functions remain 157 unknown.

158

3. Distribution of single-protein partitioning systems

160 The *par* locus was essentially found in Staphylococcal plasmids. These usually range in size 161 from small (usually less than 10 kb) plasmids replicating by a rolling circle mechanism, to larger 162 low-copy-number theta-replicating plasmids (generally 15 to 60 kb). These latter usually carry

antibiotic resistance determinants and are referred to as staphylococcal multiresistance 163 plasmids, which are divided into three groups based on resistance phenotypes and 164 165 conjugative properties: the pSK41-like conjugative plasmids, the nonconjugative antimicrobial and heavy-metal resistance plasmids, and the pSK1 family (Firth et al., 2000; Shearer et al., 166 167 2011). The *par* locus distribution has been investigated only in staphylococcal genomes so far. 168 In 2011, a survey of plasmids of a large number of natural isolates of staphylococci reported 169 93 fully sequenced plasmids ranging from 1290 bp to 64909 bp, of which about 60 were > 10 170 kb (Shearer et al., 2011). All the 29 small plasmids of less than 10 kb appeared to lack any known partition system. In contrast, the vast majority of large plasmids harbored segregation 171 functions. Of these, genes homologous to the pSK1 par locus were the most frequent, present 172 in about 80% of plasmids of more than 10 kb (Figure 3). Interestingly, par was mostly present 173 174 in medium-sized plasmids (20 to 37 kb). Largest plasmids (above 38 kb) carried either a type 175 II (all pSK41-like conjugative plasmids), or a type Ib partitioning system.

176 Homologs of the other single-protein mediated segregation system, StbA, were found in 177 about 14 % of the plasmids from a database containing 971 secondary replicons from Enterobacteria selected for their representativity based on the diversity of their replication 178 179 and transfer machineries and the genus they belong to (Planchenault et al., 2020). We further searched for StbA homologs in all Enterobacterial plasmids present in RefSeq (Materials and 180 methods). In agreement with our previous results, StbA was found in 17% of plasmids (957 of 181 5820), the large majority (92 %) ranging from 20-kb to 150-kb (Figure 3). Thus, as Par homologs 182 in Staphylococci, StbA is preferentially found in medium-sized plasmids in enterobacteria. 183

184 We next built a phylogenetic tree based on amino acid sequences of homologs of StbA, 185 from previously identified alleles ((Guynet et al., 2011), Figure 4, Materials and methods). StbA family members fall into four major groups. Groups 1, 2 and 4 seem to be restricted to Y- and
 β-proteobacteria and group 3 also includes cyanobacteria (Table S1).

Overall, these data indicate that the Par and StbA proteins are widespread, at least in Staphylococcal and Proteobacterial species, respectively. They also suggest that their function is well adapted to medium size plasmids (i.e., ranging from 20-kb to 40-kb and to 150-kb, respectively).

192

4. What do proteins tell us about their functions?

194 **4.1. Binding to the centromere-like site**

Recent data, mostly from structural and *in vitro* approaches, have provided information on how the partition complex assembles, which is the first step in the segregation process. The crystal structures of the pSK1 Par and the R388 StbA N-terminal DNA-binding domains (residues 1-53, (PDB ID: 8CSH) and residues 1-75 (PDB ID: 7PC1), referred to as StbA₁₋₇₅ throughout the text, respectively) have been solved. They display a winged-helix-turn-helix (wHTH) for pSK1 and a typical HTH for StbA, but without structural homology to other characterized CBPs or to each other (Chan et al., 2022; Quèbre et al., 2022).

Par is a 245-residues protein predicted to contain three domains: a N-terminal HTH domain, a C-terminal disordered domain, which frame a short disordered region and a central coiled coil (CC) region that might be involved in oligomerization of the protein (Chan et al., 2022), Figure 5A).The structure of Par 53 N-terminal residues bound to a 18-mer DNA site consists in two Par subunits. These show no contacts with each other, and the α -helix 2 inserts into the major groove of the duplex DNA, which consist of a half-site of a centromere repeat

(Figure 5B, (Chan et al., 2022)). Further studies indicate that Par is able to bind to at least one centromere consensus half sequence for high affinity binding *in vitro*. In addition, the organization of the centromere in seven contiguous repeats but separated by 1 to 19-bp or even overlapped suggests that one Par DNA binding entity in bound to each repeat. Notably, most contacts between Par and the DNA are hydrophobic and water mediated, without highly specific hydrogen bond-base interactions, which might indicate that Par is able to make contacts with DNA sites that deviate from the consensus (Chan et al., 2022).

215 R388 StbA is a small protein of 110 residues that was shown to harbor two domains, a 216 pretty conserved N-terminal half composed of the HTH, and a non-conserved C-terminal half with a predicted disordered region (residues 69 to 108)((Quèbre et al., 2022) Figure 5A). The 217 218 HTH domain is a typical HTH with three α helices, of which α 3 is supposed to be the recognition 219 helix responsible for the specific binding to the major groove of DNA. StbA N-terminal HTH 220 domain (StbA1-75) contains the DNA binding activity required for specific binding to the stbDR 221 sequences and for plasmid segregation (Quèbre et al., 2022). In vitro experiments (EMSA) 222 further strongly suggest that FL StbA, as well as StbA1-75, binds to the stbDRs with high 223 cooperativity resulting in the binding of two StbA HTH domains to every two stbDRs. Since no 224 structure of StbA in the presence of DNA is yet available, we attempted to generated a model of StbA1-75 bound to DNA (Figure 5C). StbA N-terminal domain HTH domain is structurally 225 related (DALI search) to the wHTH domain of several members of the PadR family transcription 226 227 regulators, except that it lacks the C-terminal wing. The wHTH domain of the protein Rv3488 of Mycobacterium tuberculosis H37Rv (PDB ID: 5ZHC) showed the strongest structural 228 229 similarity to StbA (Quèbre et al., 2022). We thus used Rv3488 as a query structure for a Vast+ 230 search in order to find protein structures, in complex with a DNA substrate, which have the 231 closest similar 3D shape (Madej et al., 2020). We found a replication terminator protein of

232 Bacillus subtilis, arranged in a dimer, in complex with part of one of its DNA termination sites (PDB ID: 1F4K; Figure S1 (Vivian et al., 2007)), and used it to build a model of DNA-bound StbA 233 234 (Figure 5C). With all the caution in the different interpretations that working with models requires, some interesting observations can be made. In the model, StbA assembles as a dimer 235 and, as expected, the recognition α 3-helix of each monomer inserts into the major groove of 236 237 the DNA. Noteworthy, both monomers contact the DNA on the same side. This is consistent 238 with the organization of stbS in two arrays of five 9-bp stbDRs spaced by 2-bp, thus 239 corresponding to a complete helix turn. This is reminiscent of the ParR proteins (type II CBPs) from plasmids pB171 and pSK41. These cooperatively assemble into a continuous structure 240 on their cognate centromeres, which are organized very similarly to stbS (Møller-Jensen et al., 241 242 2007; Schumacher et al., 2007). The putative oligomerization of the N-terminal domain of StbA 243 as suggested by the model will be discussed below.

The potential roles of the other domains of both proteins on binding to the DNA have been investigated. For both proteins, Par and StbA, the C-terminal disordered region does not appear to be involved in centromere binding (Chan et al., 2022; Quèbre et al., 2022). However, in the case of Par, deletion of the central CC domain or mutation at a specific position predicted to interfere with dimer formation (see below) led to reduced binding to the centromere *in vitro*. This suggests that, although the CC domain does not directly contact DNA, oligomerization of Par has an impact on DNA binding affinity (Chan et al., 2022).

252

4.2. Oligomerization

Protein oligomerization is proved to be an important property of proteins involved in segregation functions, whether for the assembly of large nucleoprotein partition complexes or the process generating the driving force that promotes adequate plasmid positioning.

Par protein structural data indicate that there are no interactions between the two Par 256 wHTH (Figure 5B), which is confirmed by size exclusion chromatography (SEC) (Chan et al., 257 2022). In silico predictions combined with SEC, circular dichroism and microscopy assays also 258 show strong evidence converging on the presence of a CC domain in the central domain of 259 260 Par, mediating the formation of Par dimer-of-dimers (Chan et al., 2022). Besides, wild type Par expressing GFP at its C-terminus forms distinct fluorescent foci in S. aureus cells. Foci of GFP-261 tagged Par carrying a mutation in the wHTH domain that abolishes DNA binding are more 262 263 diffuse but visible, suggesting that Par multimerizes in the absence of DNA binding, although 264 potential aggregation artifacts cannot be ruled out. A model of the full-length (FL) Par protein bound to the DNA was proposed by combining the crystal structure of the wHTH, AlphaFold 2 265 266 modeling of the central CC and the disordered domains, and docking the ends of the domain from each dimer (Chan et al., 2022). It shows that the CC domain mediates the formation of 267 268 Par A dimers, which assemble in dimer-of-dimers through interactions between the C-terminal 269 ends of the CC domains. Altogether, these data strongly suggest that the oligomerization 270 mediated by the CC domain is important in Par segregation function.

271 Regarding StbA, a body of evidence points to an oligomerization of the protein, but the 272 involvement of each domain, the N-terminal HTH and the C-terminal disordered domain, as 273 well as the oligomerization level, are not clear. StbA, as most bacterial transcription factors, 274 including the PadR proteins to which it is structurally most related, is likely to dimerize. In that

275 line, SEC analysis and bacterial two-hybrid assays indicate that FL StbA forms dimers (Quèbre 276 et al., 2022). Crystallographic data of StbA N-terminal HTH domain (StbA₁₋₇₅) showed that the asymmetric unit contained one monomer, which correlates with SEC assays showing that it is 277 278 a monomer in solution. This suggests that the N-terminal domain of StbA is not involved in 279 oligomerization of the protein, yet bacterial two-hybrid assays show that StbA₁₋₇₅ interacts 280 with itself. Besides, the fact that it exhibits specific and cooperative binding to the stbDRs 281 properties similar to those of FL StbA suggests that interactions between StbA₁₋₇₅ monomers might be promoted or stabilized by binding to the DNA (Quèbre et al., 2022). In agreement 282 with this, EMSA experiments indicate that neither StbA nor StbA1-75 are able to form stable 283 284 complexes with a single *stbDR* site, leading to the hypothesis that two StbA HTH domains bind to every two stbDRs. StbA and StbA1-75 form specific high molecular weight complexes in the 285 286 presence of DNA carrying stbDRs in vitro, suggesting that the proteins are capable of higher 287 order oligomerization.

288 Structural and modeling data suggest roughly three different models that could explain 289 how StbA₁₋₇₅ may form dimers. The first one is predicted from the nature of the crystals. Indeed, although the asymmetric unit (a.s.u.) contained a single StbA1-75 monomer, the 290 crystals showed a threefold symmetry between three identical subunits (Quèbre et al., 2022). 291 292 The N-terminus of helix $\alpha 1$ of one monomer packed into a hydrophobic pocket formed with residues from helices the $\alpha 1$ and $\alpha 2$ helices of the second monomer (Figure 6A). The second 293 model follows AlphaFold 2 predictions using StbA1-75 as a query (Figure 6B). The putative 294 dimerization interface is similar, but in contrast to the first model, the dimer assembles in a 295 296 head-to-tail fashion. A loop located within helix $\alpha 1$ allows the N-terminus of helix $\alpha 1$ of the second monomer to contact helices $\alpha 1$ and $\alpha 2$ of the first monomer. The third model arises 297 298 from our model based on structural homologies with a replication terminator protein (see

above, Figure 5C), in which monomers interact through their α 1 helices. All three models suggest that the long α 1 helix has an important role for StbA oligomerization.

The contribution of StbA C-terminal domain in the oligomerization of the protein is 301 302 unknown. One might expect that, as with the characterized PadR family proteins to which StbA is structurally related, it would mediate dimerization through interactions with the HTH 303 304 domain of the other monomer, but there is no evidence for this yet. Its disordered nature 305 probably correlates with the failure to crystallize FL StbA despite numerous attempts, as well 306 as the very low confidence in all predictions with AlphaFold 2, which does not allow to propose 307 a 3D structure model of the FL protein. Although the C-terminal domain is not required for the formation of specific StbA-stbDRs complexes in vitro, it is clearly necessary for StbA activities 308 in vivo. Indeed, StbA1-75 exhibits only partial activities in segregation and subcellular 309 310 positioning, as well as reduced activity in repressing the stbDR-carrying promoters of plasmid 311 R388. The C-terminus of StbA might thus stabilize interactions between StbA and the stbDR sites, and/or promote interactions with other partners, as discussed below (Quèbre et al., 312 2022). 313

314

5. Segregation mechanism models for single-protein systems

Although the pSK1 Par and R388 StbA segregation systems are unrelated, both involve a single plasmid-encoded CBP, and thus share the absence of the NTPase encoded together with the CBP in all partition systems described so far. This raises the question, how a single DNA binding protein can ensure both the assembly of the segregation complex and the addressing of the plasmid to both daughter cells.

322

5.1. Fate of the plasmids when cohabiting with chromosomal DNA

pSK1 minireplicons and R388 plasmids have been localized in live cells, using a TetR-323 GFP/tetO fluorescent repressor-operator system in S. aureus (Chan et al., 2022; Lau et al., 324 2003), and a ParB_{P1}-GFP/parS system in *E. coli* (Guynet et al., 2011; Li and Austin, 2002; Quèbre 325 et al., 2022), respectively. The presence of Par correlates with a weak mobility of pSK1 326 327 minireplicons foci, mostly two in number per cell, and which are confined in restricted areas 328 and separate into two or more foci in dividing cells (Figure 7A). For plasmid R388, most cells 329 carrying R388 exhibit between 4 and 6 foci of fluorescent-tagged plasmid (Guynet et al., 2011). This roughly corresponds to the copy number of R388, suggesting that most foci contain a 330 single copy of the plasmid. Foci appear to be evenly assorted within nucleoid area (Figure 7B). 331

In both cases, deletion of the CBP leads to a decrease in the number, as well as aberrant 332 333 localization or variations in the dynamics of fluorescent-tagged plasmids, which is associated 334 with plasmid instability. In the absence of Par, most cells do not contain any foci. In focicontaining cells, foci are highly mobile and do not separate, hence the absence of faithful 335 336 inheritance in the daughter cells upon division (Figure 7C). The absence of StbA, which does not affect plasmid copy-number, correlates with a significant decrease in the number of foci 337 338 (between 1 and 3), which are clustered in nucleoid-free spaces, mostly at one cell pole (Figure 7D, (Guynet et al., 2011). 339

These observations are reminiscent of the non-uniform plasmid distribution in the cell driven by entropic forces that tend to physically separate plasmids from the chromosome, resulting in plasmid exclusion from nucleoid space. Entropy had also been proposed previously as the driving force behind the spontaneous unmixing of daughter chromatids leading to chromosome segregation in dividing bacteria (Jun and Wright, 2010). High copy number 345 plasmids (>15 copies), for which no segregation systems have been identified, have a hybrid 346 distribution, composed of both large clusters and single random molecules. They are located mainly in the nucleoid-free area, at the poles and around the nucleoid periphery with 347 348 occasional movements between poles, which is conducive for efficient random assortment to 349 the daughter cells at cell division (Hsu and Chang, 2019; Reyes-Lamothe et al., 2014; Wang, 350 2017). Besides, experimental studies in E. coli as well as in silico simulations demonstrated 351 that DNA circles devoid of partition system, resulting either from excised chromosomal circles 352 or natural plasmids, are unmixed from the chromosome but maintained at the nucleoidcytoplasm transition (Planchenault et al., 2020). This unmixing strongly depends of the 353 354 replicon size. While plasmids below 25 kb diffuse rapidly across the chromosome, which would ensure efficient random segregation, largest plasmids suffer the highest missegregation and 355 356 require the presence of a partitioning system for stability (Planchenault et al., 2020). In the same vein, when mixed in cavities provided by artificial nanofluidic model systems, plasmid 357 molecules are found excluded from large DNA molecules (Liu et al., 2022). Polymer physics 358 359 further predicts that shape anisotropy of the cell influences the organization of DNA molecules in bacterial compartments, such that unmixing would be significantly increased in rod-shaped 360 361 bacteria compared to round-shaped bacteria (Jun and Mulder, 2006; Jun and Wright, 2010; 362 Liu et al., 2022).

These data can be accounted for when considered in light of the distributions of pSK1(Δpar) and R388($\Delta stbA$) minireplicons. Both make foci that appear to be released from the nucleoid. Eviction appears stronger in rod-shaped *E. coli* cells for R388($\Delta stbA$) plasmids, which are found primarily at the cell poles and, as far as can be deducted from 2-dimension images, at the nucleoid edges, than in round-shaped *S. aureus* cells for pSK1(Δpar) minireplicons, which appear to form clusters and foci that are more mobile than in the

presence of Par (Figures 7C and 7D). The Par and StbA proteins can therefore be considered as sub-cellular positioning systems that act to localize plasmids molecules to the nucleoid, which could allow plasmids to counter entropy. A potential strategy for efficient segregation and consistent with these observations would be the tethering of plasmids to the bacterial nucleoid by physical interactions, which would be ensured by the activities of Par and StbA.

374

375

5 5.2. "Chromosome hitchhiking" or "pilot fish" model

376 This idea of a physical association of plasmids with the host chromosome for their stable propagation, is reminiscent of the partitioning systems of eukaryotic extra-chromosomal 377 elements, exemplified by the 2-micron yeast plasmid and episomes of certain mammalian 378 379 viruses (reviewed in (Sau et al., 2019)). This is the so-called 'hitchhiking' model, positing that plasmids utilize the chromosomes as vehicles for segregation. Although the physical 380 381 association between the partition proteins of these elements and chromosomes has been established, the molecular basis of the interactions, as well as direct evidence of the 382 383 hitchhiking mechanism, are still missing.

384 The hitchhiking model has also been proposed for both pSK1 and R388 plasmids (Chan et al., 2022; Guynet et al., 2011). It was also called the 'pilot-fish' model, where plasmid copies 385 386 of R388 resemble pilot-fishes, who take advantage of the bow wave created by the swimming of big marine predators, which would be the chromosome in the case of plasmids (Guynet and 387 388 de la Cruz, 2011). In this model, Par and StbA CBPs would bind to their respective centromeres and the resulting segregation complex would associate with the nucleoid. This could be done 389 390 through direct interactions with chromosomal DNA sequences, or through indirect 391 interactions involving one or more unknown chromosome-associated host factors.

392 Figure 7E(i) represents direct binding of Par and StbA CBPs to a discreet DNA site on the 393 chromosome. According to the model of the pSK1 Par dimer-of-dimers bound to DNA, one Par dimer could bind to the centromere site, while the other dimer would interact with the host 394 chromosome. Searches of the S. aureus genomes showed that it carries many candidate 395 396 sequences for Par binding. Indeed, more than 20 sites contain 9 of the 12-bp of the 397 centromere consensus repeat of pSK1, and there are likely many more sites, given that 398 experimental data show that Par is able to bind to one centromere consensus half site, and probably to DNA sites that deviate from the consensus, as suggested by the absence of specific 399 hydrogen-bond base interactions (Chan et al., 2022). How CBPs binding to multiple sites 400 scattered around the chromosome could ensure faithful segregation of the plasmids to new 401 402 daughter cells remains however unclear. The case of StbA appears to be different. Even if 403 more than 350 matches to the 9-bp stbDR repeats sequences are found on the E. coli chromosome, the combination of two *stbDRs* in direct repetition separated by 2 bp, required 404 to detect binding in vitro (Quèbre et al., 2022), is not found. 405

406 Another way to mediate plasmid attachment to the chromosome would be through the 407 intervention of another protein. In this view, Par and StbA C-terminal domains might be 408 involved in interactions with a host-encoded protein that would make the link between the segregation complex and the nucleoid (Figure 7E(ii)). No potential host partners have been 409 identified so far by genome-wide assays: in S. aureus using the yeast two-hybrid, and in E. coli 410 using the bacterial two-hybrid screen. Attempted to specifically reveal interactions between 411 412 StbA and E. coli nucleoid-associated proteins (NAPs) by bacterial two-hybrid assays were also 413 unsuccessful. A clue could have been given by the structural similarities between the Par DNAbinding domain with the wHTH of the Bacillus subtilis protein RacA. RacA plays a critical role 414 415 in DNA segregation during sporulation by tethering the chromosome at the poles, presumably

by binding to centromere-like DNA sites near the origin of replication and then interacting
with the pole-located DivIVA protein via its C-terminal domain. (Ben-Yehuda et al., 2005, 2003;
Chan et al., 2022; Schumacher et al., 2016). However, yeast-two hybrid assays did not reveal
any interaction between Par and the *S. aureus* DivIVA (Chan et al., 2022).

The above hitchhiking models rely on interactions between partition complexes and the host nucleoid, requiring a certain degree of specificity hardly compatible with the broad host range of some plasmids as R388 (Fernández-López et al., 2006). Alternatively, plasmid copies may position relative to each other to optimize their distribution. Such a process could rely on repulsion forces between partition complexes by an unknown mechanism. Plasmid copies would thus occupy the largest possible volume, while remaining bound to the nucleoid by non-specific interactions, ensuring adequate positioning upon cell division (Figure 7E(iii)).

427

428 5.3. StbA and the interplay between vertical segregation and horizontal

429 transfer

430 If the segregation process strives to keep plasmid copies in the centre of the cell, this calls 431 into question the ability of conjugative plasmids such as R388 to undertake conjugation, which takes place at the membrane. Conjugation is the process by which plasmids are transferred 432 433 from a donor bacterium to a recipient bacterium through a conjugative pore (T4SS, type IV secretion system), which is established at the bacterial membrane between the two cells in 434 physical contact (Cabezón et al., 2015; Llosa et al., 2002; Virolle et al., 2020). In this context, 435 436 the stb operon the first evidence for a mechanistic interplay between segregation (vertical 437 transfer) and conjugation (horizontal transfer). Indeed, StbA is not only the sole plasmidencoded protein involved in R388 segregation, but is also an inhibitor of conjugation. StbA 438

439 inactivation makes R388 a super-spreader plasmid with up to 50-fold higher transfer 440 frequency than the wild type, whereas StbA presence makes the second protein encoded in the opreron stb, StbB, strictly required for conjugative transfers (Guynet et al., 2011). Note 441 442 that as mentioned above, StbB, which contains a variant Walker A nucleotide triphosphate-443 binding motif related to that found in the Soj/MinD superfamily of ATPases, including type I 444 par motor proteins, is not involved in R388 segregation. Strikingly, the activities of StbA and 445 StbB in controlling vertical and horizontal transfer of R388 correlate with subcellular localization of plasmid copies: StbA-mediated segregation of R388 and inhibition of 446 447 conjugation correlates with the confinement of plasmids to nucleoid areas, whereas StbB 448 stimulation of conjugation correlates with the presence of plasmid copies in nucleoid-free zones at the membrane T4SS. Plasmid R388 thus appears to encode in the same operon two 449 450 proteins that act to position plasmids. These counteract each other, and go against entropic forces, which tend to position plasmids at the edge of the nucleoid, i.e. neither at the nucleoid 451 nor near the membrane, where StbA and StbB position plasmids, respectively. The StbAB 452 453 system can illustrate evolutionary parsimony applied to plasmid physiology, using only two actors to ensure faithful plasmid propagation. 454

455

456 **6. Conclusion**

Despite recent progress in characterizing the proteins involved in the two unrelated oneprotein plasmid segregation systems described so far, the mechanisms underlying the assembly of their segregation complex, as well as how the plasmid copies separate and strategically position themselves to ensure their stable maintenance in progeny are not yet elucidated. No host protein that might play a role analogous to that of the NTPases of typical partition systems, by an as yet unknown mechanism, has been identified. Polymer physics

463 studies could also potentially help in the understanding of the behavior of selfish elements 464 and chromosomal DNA in the very crowded yet dynamic bacterial compartment. Although a hitchhiking mode of segregation is appealing for the pSK1 and R388 plasmid, no direct or 465 466 indirect evidence for an association with the chromosome or any other host factor exists. The 467 next step forward would be to identify potential plasmid tethering sites on the nucleoid and 468 /or host factors if any. This mode of segregation would involve DNA-protein and protein-469 protein interactions that also have to be characterized. Whatever the molecular basis 470 governing the one-protein segregation systems, it seems to be adapted to medium-sized plasmids. These plasmids may be small enough not to interfere with chromosome dynamics, 471 either physically due to their size, or metabolically due to the number of genes they carry. 472 They may thus keep a medium copy number (about 5 copies per chromosome in both cases), 473 474 allowing stable inheritance to rely on an even subcellular assortment using the nucleoid as a 475 scaffold, with no need of active transportation of plasmid copies before cell division.

476

- 477 **7. Materials and methods**
- 478 **7.1. StbA reference dataset**

The sequence of StbA alleles described in (Guynet et al., 2011) was used as a query in an iterative BLASTP search at NCBI (Genbank non-redundant database, May 2019). These alleles were those found in plasmids R388, R46, NAH7, pTF-FC2, and RP4. Since the full sequence of the latter was not available, the StbA allele of plasmid pBS228, which was identical, was used, and the StbA allele of plasmid pET49 was added following the iterative search. For each potential non-redundant StbA sequence, the corresponding plasmid sequence was downloaded and annotated using SnapGene software (www.snapgene.com), and oriTfinder (https://tool-mml.sjtu.edu.cn/oriTfinder/oriTfinder.html) was used to determine possible oriT
site, relaxases, and type IV secretion system genes. Hypothetical StbA sequences were
retained if at least, either *stbB* or the MOB gene encoding the relaxase was present nearby.

489

490

7.2. Enterobacterial StbA dataset

An HMM profile was constructed using a MAFFT alignment of the conserved N-terminal domains of the StbA reference set. This HMM was then used as a query to search for StbA homologs in the full set of Enterobacteria plasmids (5820 plasmids) available in RefSeq (May 2019). We obtained 977 results for StbA in 957 plasmids (16 plasmids carried 2 StbA copies and 2 plasmids had 3 copies).

496

497 **7.3. Phylogenetic tree**

StbA sequences were analyzed via NGPhylogeny (https://ngphylogeny.fr/). They were aligned 498 499 using MAFFT (Katoh and Standley, 2013) and fitted with TrimAl (Capella-Gutiérrez et al., 2009). The tree was built from the StbA reference dataset, to which we added 18 sequences 500 (highlited in blue in Table S1) selected from previous results (Planchenault et al., 2020) for a 501 502 better representation of all StbA sequences. A phylogenetic tree was constructed with PhyML 503 (maximum-likelihood) (Guindon et al., 2010) (evolutionary model LG; Tree topology search: SPR (Subtree Pruning and Regraphing)) and visualized with iTOL ((Letunic and Bork, 2021), 504 https://itol.embl.de). 505

507 8. Acknowlegments

508 We thank all members of the Gedy team and to numerous colleagues for fruitful discussions

and contributions to our understanding of the Stb system. This work was supported by French

510 National Research Agency, grant number ANR-18- CE35-0008.

511

512 9. References

513	Aylett, C.H.S., Löwe, J., 2012. Superstructure of the centromeric complex of TubZRC plasmid
514	partitioning systems. Proc Natl Acad Sci U S A 109, 16522–16527.
515	https://doi.org/10.1073/pnas.1210899109
516	Aylett, C.H.S., Löwe, J., Amos, L.A., 2011. New insights into the mechanisms of cytomotive actin and
517	tubulin filaments. Int Rev Cell Mol Biol 292, 1–71. https://doi.org/10.1016/B978-0-12-
518	386033-0.00001-3
519	Baxter, J.C., Funnell, B.E., 2014. Plasmid Partition Mechanisms. Microbiol Spectr 2.
520	https://doi.org/10.1128/microbiolspec.PLAS-0023-2014
521	Ben-Yehuda, S., Fujita, M., Liu, X.S., Gorbatyuk, B., Skoko, D., Yan, J., Marko, J.F., Liu, J.S.,
522	Eichenberger, P., Rudner, D.Z., Losick, R., 2005. Defining a centromere-like element in
523	Bacillus subtilis by Identifying the binding sites for the chromosome -anchoring protein RacA.
524	Mol Cell 17, 773–782. https://doi.org/10.1016/j.molcel.2005.02.023
525	Ben-Yehuda, S., Rudner, D.Z., Losick, R., 2003. RacA, a bacterial protein that anchors chromosomes to
526	the cell poles. Science 299, 532–536. https://doi.org/10.1126/science.1079914
527	Bouet, JY., Funnell, B.E., 2019. Plasmid Localization and Partition in Enterobacteriaceae. EcoSal Plus
528	8. https://doi.org/10.1128/ecosalplus.ESP-0003-2019
529	Cabezón, E., Ripoll-Rozada, J., Peña, A., de la Cruz, F., Arechaga, I., 2015. Towards an integrated
530	model of bacterial conjugation. FEMS Microbiol Rev 39, 81–95.
531	https://doi.org/10.1111/1574-6976.12085
532	Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T., 2009. trimAl: a tool for automated alignment
533	trimming in large-scale phylogenetic analyses. Bioinformatics 25, 1972–1973.
534	https://doi.org/10.1093/bioinformatics/btp348
535	Chan, H.Y., Jensen, S.O., LeBard, R.J., Figgett, W.A., Lai, E., Simpson, A.E., Brzoska, A.J., Davies, D.S.,
536	Connolly, A.M., Cordwell, S.J., Travis, B.A., Salinas, R., Skurray, R.A., Firth, N., Schumacher,
537	M.A., 2022. Molecular Analysis of pSK1 par: A Novel Plasmid Partitioning System Encoded by
538	Staphylococcal Multiresistance Plasmids. J Mol Biol 434, 167770.
539	https://doi.org/10.1016/j.jmb.2022.167770
540	Coppens, L., Lavigne, R., 2020. SAPPHIRE: a neural network based classifier for σ 70 promoter
541	prediction in Pseudomonas. BMC Bioinformatics 21, 415. https://doi.org/10.1186/s12859-
542	020-03730-z
543	Ebersbach, G., Gerdes, K., 2001. The double par locus of virulence factor pB171: DNA segregation is
544	correlated with oscillation of ParA. Proc Natl Acad Sci U S A 98, 15078–15083.
545	https://doi.org/10.1073/pnas.261569598
546	Ebersbach, G., Ringgaard, S., Møller-Jensen, J., Wang, Q., Sherratt, D.J., Gerdes, K., 2006. Regular
547	cellular distribution of plasmids by oscillating and filament -forming ParA ATPase of plasmid
548	pB171. Mol Microbiol 61, 1428–1442. https://doi.org/10.1111/j.1365-2958.2006.05322.x

549 Fernandez-Lopez, R., Del Campo, I., Revilla, C., Cuevas, A., de la Cruz, F., 2014. Negative feedback and 550 transcriptional overshooting in a regulatory network for horizontal genetransfer. PLoS Genet 10, e1004171. https://doi.org/10.1371/journal.pgen.1004171 551 552 Fernández-López, R., Garcillán-Barcia, M.P., Revilla, C., Lázaro, M., Vielva, L., de la Cruz, F., 2006. 553 Dynamics of the IncW genetic backbone imply general trends in conjugative plasmid 554 evolution. FEMS Microbiol Rev 30, 942–966. https://doi.org/10.1111/j.1574-555 6976.2006.00042.x Firth, N., Apisiridej, S., Berg, T., O'Rourke, B.A., Curnock, S., Dyke, K.G.H., Skurray, R.A., 2000. 556 557 Replication of Staphylococcal Multiresistance Plasmids. J Bacteriol 182, 2170–2178. 558 Friedman, S.A., Austin, S.J., 1988. The P1 plasmid-partition system synthesizes two essential proteins 559 from an autoregulated operon. Plasmid 19, 103–112. https://doi.org/10.1016/0147-560 619x(88)90049-2 561 Gerdes, K., Møller-Jensen, J., Bugge Jensen, R., 2000. Plasmid and chromosome partitioning: surprises from phylogeny. Mol Microbiol 37, 455-466. https://doi.org/10.1046/j.1365-562 563 2958.2000.01975.x 564 Golovanov, A.P., Barillà, D., Golovanova, M., Hayes, F., Lian, L.-Y., 2003. ParG, a protein required for 565 active partition of bacterial plasmids, has a dimeric ribbon-helix-helix structure. Mol Microbiol 50, 1141–1153. https://doi.org/10.1046/j.1365-2958.2003.03750.x 566 567 Grkovic, S., Brown, M.H., Hardie, K.M., Firth, N., Skurray, R.A., 2003. Stable low-copy-number 568 Staphylococcus aureus shuttle vectors. Microbiology (Reading) 149, 785–794. 569 https://doi.org/10.1099/mic.0.25951-0 570 Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms 571 and methods to estimate maximum-likelihood phylogenies: assessing the performance of 572 PhyML 3.0. Syst Biol 59, 307–321. https://doi.org/10.1093/sysbio/syq010 Guynet, C., Cuevas, A., Moncalián, G., de la Cruz, F., 2011. The stb operon balances the requirements 573 574 for vegetative stability and conjugative transfer of plasmid R388. PLoS Genet 7, e1002073. 575 https://doi.org/10.1371/journal.pgen.1002073 Guynet, C., de la Cruz, F., 2011. Plasmid segregation without partition. Mob Genet Elements 1, 236-576 577 241. https://doi.org/10.4161/mge.1.3.18229 578 Hayes, F., 2000. The partition system of multidrug resistance plasmid TP228 includes a novel protein 579 that epitomizes an evolutionarily distinct subgroup of the ParA superfamily. Mol Microbiol 580 37, 528–541. https://doi.org/10.1046/j.1365-2958.2000.02030.x 581 Hirano, M., Mori, H., Onogi, T., Yamazoe, M., Niki, H., Ogura, T., Hiraga, S., 1998. Autoregulation of 582 the partition genes of the mini-F plasmid and the intracellular localization of their products in 583 Escherichia coli. Mol Gen Genet 257, 392-403. https://doi.org/10.1007/s004380050663 584 Hsu, T.-M., Chang, Y.-R., 2019. High-Copy-Number Plasmid Segregation-Single-Molecule Dynamics in 585 Single Cells. Biophys J 116, 772–780. https://doi.org/10.1016/j.bpj.2019.01.019 586 Hu, L., Vecchiarelli, A.G., Mizuuchi, K., Neuman, K.C., Liu, J., 2017. Brownian ratchet mechanisms of 587 ParA-mediated partitioning. Plasmid 92, 12-16. 588 https://doi.org/10.1016/j.plasmid.2017.05.002 589 Hwang, L.C., Vecchiarelli, A.G., Han, Y.-W., Mizuuchi, M., Harada, Y., Funnell, B.E., Mizuuchi, K., 2013. 590 ParA-mediated plasmid partition driven by protein pattern self-organization. EMBO J 32, 591 1238-1249. https://doi.org/10.1038/emboj.2013.34 592 Jalal, A.S., Tran, N.T., Stevenson, C.E., Chimthanawala, A., Badrinarayanan, A., Lawson, D.M., Le, T.B., 593 2021. A CTP-dependent gating mechanism enables ParB spreading on DNA. Elife 10, e69676. 594 https://doi.org/10.7554/eLife.69676 595 Jensen, R.B., Dam, M., Gerdes, K., 1994. Partitioning of plasmid R1. The parA operon is autoregulated 596 by ParR and its transcription is highly stimulated by a downstream activating element. J Mol 597 Biol 236, 1299-1309. https://doi.org/10.1016/0022-2836(94)90059-0 598 Jun, S., Mulder, B., 2006. Entropy-driven spatial organization of highly confined polymers: lessons for 599 the bacterial chromosome. Proc Natl Acad Sci U S A 103, 12388–12393. 600 https://doi.org/10.1073/pnas.0605305103

601 Jun, S., Wright, A., 2010. Entropy as the driver of chromosome segregation. Nat Rev Microbiol 8, 602 600-607. https://doi.org/10.1038/nrmicro2391 603 Katoh, K., Standley, D.M., 2013. MAFFT Multiple Sequence Alignment Software Version 7: 604 Improvements in Performance and Usability. Mol Biol Evol. 30, 772–780. 605 Kwong, S.M., Lim, R., LeBard, R.J., Skurray, R.A., Firth, N., 2008. Analysis of the pSK1 replicon, a 606 prototype from the staphylococcal multiresistance plasmid family. Microbiology (Reading) 607 154, 3084-3094. https://doi.org/10.1099/mic.0.2008/017418-0 608 Kwong, S.M., Skurray, R.A., Firth, N., 2004. Staphylococcus aureus multiresistance plasmid pSK41: 609 analysis of the replication region, initiator protein binding and antisense RNA regulation. Mol 610 Microbiol 51, 497–509. https://doi.org/10.1046/j.1365-2958.2003.03843.x 611 Larsen, R.A., Cusumano, C., Fujioka, A., Lim-Fong, G., Patterson, P., Pogliano, J., 2007. Treadmilling of 612 a prokaryotic tubulin-like protein, TubZ, required for plasmid stability in Bacillus 613 thuringiensis. Genes Dev 21, 1340–1352. https://doi.org/10.1101/gad.1546107 Lau, I.F., Filipe, S.R., Søballe, B., Økstad, O.-A., Barre, F.-X., Sherratt, D.J., 2003. Spatial and temporal 614 615 organization of replicating Escherichia coli chromosomes. Mol Microbiol 49, 731–743. 616 https://doi.org/10.1046/j.1365-2958.2003.03640.x 617 Lawley, T.D., Taylor, D.E., 2003. Characterization of the double-partitioning modules of R27: 618 correlating plasmid stability with plasmid localization. J Bacteriol 185, 3060–3067. 619 https://doi.org/10.1128/JB.185.10.3060-3067.2003 Le Gall, A., Cattoni, D.I., Guilhas, B., Mathieu-Demazière, C., Oudjedi, L., Fiche, J.-B., Rech, J., 620 621 Abrahamsson, S., Murray, H., Bouet, J.-Y., Nollmann, M., 2016. Bacterial partition complexes 622 segregate within the volume of the nucleoid. Nat Commun 7, 12107. 623 https://doi.org/10.1038/ncomms12107 624 Letunic, I., Bork, P., 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree 625 display and annotation. Nucleic Acids Res 49, W293–W296. 626 https://doi.org/10.1093/nar/gkab301 627 Li, Y., Austin, S., 2002. The P1 plasmid in action: time-lapse photomicroscopy reveals some 628 unexpected aspects of plasmid partition. Plasmid 48, 174–178. 629 https://doi.org/10.1016/s0147-619x(02)00104-x 630 Lim, H.C., Surovtsev, I.V., Beltran, B.G., Huang, F., Bewersdorf, J., Jacobs-Wagner, C., 2014. Evidence for a DNA-relay mechanism in ParABS-mediated chromosome segregation. Elife 3, e02758. 631 632 https://doi.org/10.7554/eLife.02758 633 Liu, M.A., Kwong, S.M., Pon, C.K., Skurray, R.A., Firth, N., 2012. Genetic requirements for replication 634 initiation of the staphylococcal multiresistance plasmid pSK41. Microbiology (Reading) 158, 635 1456-1467. https://doi.org/10.1099/mic.0.057620-0 636 Liu, Z., Capaldi, X., Zeng, L., Zhang, Y., Reyes-Lamothe, R., Reisner, W., 2022. Confinement anisotropy 637 drives polar organization of two DNA molecules interacting in a nanoscale cavity. Nat 638 Commun 13, 4358. https://doi.org/10.1038/s41467-022-31398-x 639 Llosa, M., Gomis-Rüth, F.X., Coll, M., de la Cruz Fd, F., 2002. Bacterial conjugation: a two-step 640 mechanism for DNA transport. Mol Microbiol 45, 1-8. https://doi.org/10.1046/j.1365-641 2958.2002.03014.x 642 Madej, T., Marchler-Bauer, A., Lanczycki, C., Zhang, D., Bryant, S.H., 2020. Biological Assembly 643 Comparison with VAST. Methods Mol Biol 2112, 175–186. https://doi.org/10.1007/978-1-644 0716-0270-6_13 645 Møller-Jensen, J., Borch, J., Dam, M., Jensen, R.B., Roepstorff, P., Gerdes, K., 2003. Bacterial mitosis: 646 ParM of plasmid R1 moves plasmid DNA by an actin-like insertional polymerization 647 mechanism. Mol Cell 12, 1477–1487. https://doi.org/10.1016/s1097-2765(03)00451-9 648 Møller-Jensen, J., Ringgaard, S., Mercogliano, C.P., Gerdes, K., Löwe, J., 2007. Structural analysis of 649 the ParR/parC plasmid partition complex. EMBO J 26, 4413–4422. 650 https://doi.org/10.1038/sj.emboj.7601864 651 Ni, L., Xu, W., Kumaraswami, M., Schumacher, M.A., 2010. Plasmid protein TubR uses a distinct mode 652 of HTH-DNA binding and recruits the prokaryotic tubulin homolog TubZ to effect DNA

653 partition. Proc Natl Acad Sci U S A 107, 11763–11768. 654 https://doi.org/10.1073/pnas.1003817107 Nordström, K., Austin, S.J., 1989. Mechanisms that contribute to the stable segregation of plasmids. 655 656 Annu Rev Genet 23, 37–69. https://doi.org/10.1146/annurev.ge.23.120189.000345 657 Osorio-Valeriano, M., Altegoer, F., Das, C.K., Steinchen, W., Panis, G., Connolley, L., Giacomelli, G., 658 Feddersen, H., Corrales-Guerrero, L., Giammarinaro, P.I., Hanßmann, J., Bramkamp, M., 659 Viollier, P.H., Murray, S., Schäfer, L.V., Bange, G., Thanbichler, M., 2021. The CTPase activity of ParB determines the size and dynamics of prokaryotic DNA partition complexes. Mol Cell 660 661 81, 3992-4007.e10. https://doi.org/10.1016/j.molcel.2021.09.004 662 Planchenault, C., Pons, M.C., Schiavon, C., Siguier, P., Rech, J., Guynet, C., Dauverd-Girault, J., Cury, J., 663 Rocha, E.P.C., Junier, I., Cornet, F., Espéli, O., 2020. Intracellular Positioning Systems Limit the 664 Entropic Eviction of Secondary Replicons Toward the Nucleoid Edges in Bacterial Cells. J Mol 665 Biol 432, 745–761. https://doi.org/10.1016/j.jmb.2019.11.027 666 Quèbre, V., Del Campo, I., Cuevas, A., Siguier, P., Rech, J., Le, P.T.N., Ton-Hoang, B., Cornet, F., Bouet, 667 J.-Y., Moncalian, G., de la Cruz, F., Guynet, C., 2022. Characterization of the DNA Binding 668 Domain of StbA, A Key Protein of A New Type of DNA Segregation System. J Mol Biol 434, 669 167752. https://doi.org/10.1016/j.jmb.2022.167752 Reyes-Lamothe, R., Tran, T., Meas, D., Lee, L., Li, A.M., Sherratt, D.J., Tolmasky, M.E., 2014. High-copy 670 671 bacterial plasmids diffuse in the nucleoid-free space, replicate stochastically and are 672 randomly partitioned at cell division. Nucleic Acids Res 42, 1042–1051. 673 https://doi.org/10.1093/nar/gkt918 674 Ringgaard, S., van Zon, J., Howard, M., Gerdes, K., 2009. Movement and equipositioning of plasmids 675 by ParA filament disassembly. Proc Natl Acad Sci U S A 106, 19369–19374. 676 https://doi.org/10.1073/pnas.0908347106 Sau, S., Ghosh, S.K., Liu, Y.-T., Ma, C.-H., Jayaram, M., 2019. Hitchhiking on chromosomes: A 677 678 persistence strategy shared by diverse selfish DNA elements. Plasmid 102, 19-28. 679 https://doi.org/10.1016/j.plasmid.2019.01.004 680 Schumacher, M.A., Glover, T.C., Brzoska, A.J., Jensen, S.O., Dunham, T.D., Skurray, R.A., Firth, N., 681 2007. Segrosome structure revealed by a complex of ParR with centromere DNA. Nature 450, 1268–1271. https://doi.org/10.1038/nature06392 682 683 Schumacher, M.A., Lee, J., Zeng, W., 2016. Molecular insights into DNA binding and anchoring by the 684 Bacillus subtilis sporulation kinetochore-like RacA protein. Nucleic Acids Res 44, 5438–5449. 685 https://doi.org/10.1093/nar/gkw248 686 Sengupta, M., Austin, S., 2011. Prevalence and significance of plasmid maintenance functions in the 687 virulence plasmids of pathogenic bacteria. Infect Immun 79, 2502–2509. 688 https://doi.org/10.1128/IAI.00127-11 689 Shearer, J.E.S., Wireman, J., Hostetler, J., Forberger, H., Borman, J., Gill, J., Sanchez, S., Mankin, A., 690 Lamarre, J., Lindsay, J.A., Bayles, K., Nicholson, A., O'Brien, F., Jensen, S.O., Firth, N., Skurray, 691 R.A., Summers, A.O., 2011. Major families of multiresistant plasmids from geographically and 692 epidemiologically diverse staphylococci. G3 (Bethesda) 1, 581–591. 693 https://doi.org/10.1534/g3.111.000760 694 Simpson, A.E., Skurray, R.A., Firth, N., 2003. A single gene on the staphylococcal multiresistance 695 plasmid pSK1 encodes a novel partitioning system. J Bacteriol 185, 2143–2152. 696 https://doi.org/10.1128/jb.185.7.2143-2152.2003 697 Soh, Y.-M., Davidson, I.F., Zamuner, S., Basquin, J., Bock, F.P., Taschner, M., Veening, J.-W., De Los 698 Rios, P., Peters, J.-M., Gruber, S., 2019. Self-organization of parS centromeres by the ParB 699 CTP hydrolase. Science 366, 1129–1133. https://doi.org/10.1126/science.aay3965 700 Vecchiarelli, A.G., Hwang, L.C., Mizuuchi, K., 2013. Cell-free study of F plasmid partition provides 701 evidence for cargo transport by a diffusion-ratchet mechanism. Proc Natl Acad Sci U S A 110, 702 E1390-1397. https://doi.org/10.1073/pnas.1302745110

703	Vecchiarelli, A.G., Mizuuchi, K., Funnell, B.E., 2012. Surfing biological surfaces: exploiting the nucleoid
704	for partition and transport in bacteria. Mol Microbiol 86, 513–523.
705	https://doi.org/10.1111/mmi.12017
706	Vecchiarelli, A.G., Neuman, K.C., Mizuuchi, K., 2014. A propagating ATPase gradient drives transport
707	of surface-confined cellular cargo. Proc Natl Acad Sci U S A 111, 4880–4885.
708	https://doi.org/10.1073/pnas.1401025111
709	Virolle, C., Goldlust, K., Djermoun, S., Bigot, S., Lesterlin, C., 2020. Plasmid Transfer by Conjugation in
710	Gram-Negative Bacteria: From the Cellular to the Community Level. Genes (Basel) 11, E1239.
711	https://doi.org/10.3390/genes11111239
712	Vivian, J.P., Porter, C.J., Wilce, J.A., Wilce, M.C.J., 2007. An asymmetric structure of the Bacillus
713	subtilis replication terminator protein in complex with DNA. J Mol Biol 370, 481–491.
714	https://doi.org/10.1016/j.jmb.2007.02.067
715	Wang, Y., 2017. Spatial distribution of high copy number plasmids in bacteria. Plasmid, SI: ISPB
716	Plasmid 2016 91, 2–8. https://doi.org/10.1016/j.plasmid.2017.02.005
717	

718 Figure legends

719 Figure 1. Mechanisms of typical partition systems. As indicated, centromere binding proteins 720 are represented as yellow circles, and NTPases as ovals (ATP-bound, light blue; ADP-bound, 721 dark blue). (A) Type I partition systems. The Brownian ratchet mechanism relies on the ParA ATPase, which binds to the nucleoid in a nonspecific and ATP-dependent manner to DNA. 722 ParB/parS partition complexes associate with the nucleoid via ParA-ATP, and then stimulate 723 724 the release of ParA from the DNA by ATP hydrolysis or conformational change. Due to the slow 725 re-binding of ParA to the nucleoid, a void of ParA is created and serves as a barrier so that the 726 partition complexes move toward opposite directions by following the ParA remaining bound 727 to the nucleoid. (B) Type II partition systems. The R1 paradigm plasmid uses ATP-dependent 728 polymerization of the actin-like ParM ATPase to push plasmids poleward. ParR/parC partition 729 complexes bind to the terminal ParM-ATP subunits at the growing end of the filament. Hydrolysis of ATP to ADP leads to destabilization of the filaments, allowing entry of the ParM-730 ATP subunits. The filaments are polar and associate antiparallel, so that the plasmids are 731 732 pushed in a bidirectional manner. (C) Type III partition systems. The pulling mechanism, 733 exemplified by pXO1, involves polymerization of the tubulin-like TubZ GTPase, which forms

polar and dynamic filaments in a treadmilling-like pattern. These grow at the plus end by addition of TubZ-GTP and disassemble at the minus end, from which the TubR/*tubC* partition complexes are pulled to the pole.

737

Figure 2. Genetic organization of *par* (A) and *stb* (B) regions. Promoters are indicated by black bent arrows, dotted black the putative promoter *Pstb* (predicted with SAPPHIRE, (Coppens and Lavigne, 2020)). Gray boxes represent –10 and –35 sequences of *Ppar* and the putative *Pstb.* As indicated, Par and StbA repress their own promoter. Direct repeats of the centromere-like sites are represented by orange and yellow (*stbDRs*) solid arrows, respectively. The origin of conjugative transfer of plasmid R388 is shown as a vertical arrow. The scale is not respected.

745

Figure 3. Distribution of Par (A) and StbA (B) segregation systems. The event plots represent
the presence of Par or StbA (blue vertical lines) in the 92 staphylococcal or in the 5820
enterobacterial replicons (red vertical lines), respectively, and which were ranked according
to their size (x-axis). The data for staphylococcal plasmids are from (Shearer et al., 2011).

750

Figure 4. Phylogenetic tree of the StbA representative sequences. Accession numbers of plasmids are indicated and the reference plasmids are in large bold type. Based on the tree topology, the sequences could be separated in four groups. As indicated: Group 1 (red) includes plasmids R46 (AY046276), pNAH7 (AB237655), R721 (NC_002525) and R388 (NC_028464) – Group 2 (green) includes pBS228 (NC_008357) – Group 3 (blue) include

plasmid pTF-FC2 (TFETFFC2) – Group 4 (orange) include plasmid pET49 (CU468131). The tree
 display was obtained with online iTOL ((Letunic and Bork, 2021), <u>https://itol.embl.de</u>).

758

Figure 5. Par and StbA DNA binding domains. (A) Schematic representing Par and StbA 759 structural domains. (B) Structure of Par DNA-binding domains bound to DNA. The two 760 761 subunits do not make contact, and each binds to a half site of the centromere. α -helices and 762 β-strands of the winged-HTH domain are labelled (PDB ID: 8CSH, (Chan et al., 2022)). (C) Model 763 of StbA DNA-binding domain bound to DNA. The StbA N-terminal domain (PDB ID: 7PC1) and 764 the replication terminator protein of *Bacillus subtilis* bound to DNA (PDB ID: 1F4K) structures were superimposed. In the resulting model, StbA assembles as a head-to-tail dimer, and the 765 766 recognition helix α 3 of each monomer inserts into the major groove of the DNA. α -helices of the HTH domain are indicated. The display of structures and analyses were performed with 767 768 UCSF Chimera (developed by the Resource for Biocomputing, Visualization, and Informatics at 769 the University of California, San Francisco, with support from NIH P41-GM103311).

770

Figure 6. Models of StbA DNA-binding domain dimerization. (A) The structure of the DNA-771 772 binding domain (StbA1-75) is from our crystal structure. The crystals showed a threefold 773 symmetry between three identical units, revealing possible interactions between monomers. One monomer interacts with helix α 3 and the C-terminal part of α 1 of another monomer via 774 775 the N-terminal part of its helix $\alpha 1$. The third monomer is not show for clarity. (B) The structure 776 of the DNA-binding domain is from modeling with AlphaFold 2. The arrangement of the two 777 monomers is head-to-tail, so that the N-terminal part of helix $\alpha 1$ of one monomer is packed 778 between helix $\alpha 1$ and $\alpha 2$ of the other monomer and vice versa.

779

780 Figure 7. Models for plasmid segregation mediated by single-protein systems. Symbols are indicated in the legend of the figure (left panel). (A)-(D) Schematics showing the sub-cellular 781 782 positioning of pSK1 minireplicons and R388 plasmids in *S. aureus* and *E. coli* cells, respectively. pSK1 minireplicons are confined in restricted areas in the presence of Par and separate into 783 784 two or more foci in dividing cells (A), whereas in the absence of Par they are highly mobile 785 (shown as a grey dotted line) and do not separate (C). Plasmids R388 are evenly distributed 786 in the nucleoid area in the presence of StbA (B), whereas they are clustered and excluded from 787 the nucleoid in the absence of StbA (D). (E) Proposed models for positioning and segregation mediated with a single-protein system. (i) and (ii) represent a hitchhiking mechanism, in which 788 789 plasmid molecules are attached on the bacterial nucleoid either through direct interactions 790 between the segregation protein and the chromosomal DNA (i), or through interactions with 791 one or more host proteins that bind to the nucleoid (ii). The plasmids thus take advantage of 792 the segregation of the chromosomes to distribute themselves between the two daughter cells 793 during bacterial division. In (iii), the partition complexes would interact with each other rather 794 than with the nucleoid to partition plasmids into the nucleoid space and ensure daughter cells 795 to receive at least one copy of the plasmid.

796

797 Supplementary material

Figure S1. (A) Structure of the replication terminator protein of *Bacillus subtilis* bound to DNA (PDB ID: 1F4K). **(B)** Structural superposition of the replication terminator protein of *B. subtilis* (green) and StbA₁₋₇₅ (crimson and pink) dimers. Part of the α -helix located C-terminal to the winge is not shown for clarity.

- 802 **Table S1.** List of plasmids used to build the phylogenetic tree. For each, the accession number,
- 803 the name, the size, the host and the group of StbA proteins (group 1 in red, group 2 in green,
- group 3 in blue and group 4 in orange) are indicated. The reference plasmids are in bold type,
- and the 18 additional sequences are highlighted in blue.
- 806







Figure 3



Α





Figure 6







Figure S1

В



		0	`		, sile	sterkbingonny
	eroup plasmi	HOST	Plasmio	plas	nio Ho	HOST AND
1	CP015374	Pandoraea promenusa strain MCB032	plasmid unnamed 3	29016	Bacteria;	Y risy Pseudomonadota; Betaprotechacteria; Burkholderiales; Burkholderiales; Pandorses
1	F0681496	Xanthomonas fuscans subsp. fuscans str. 4834-R	plb	19514	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas
1	LT853884 CP000060	Xanthomonas fragariae strain PD885 Pseudomonas savastanoi pv. phaseolicola 1448A	pPD885-27 small	27106 51711	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas
1	NC_019292	Pseudomonas savastanoi NCPPB 3335 Pseudomonas saruginosa strain 14057	pPsv48C	42103	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas
1	KC170282	Uncultured bacterium	pMBUI6	47999	Bacteria;	ria, resolucinadota, Galmaproteobacteria, resolucinonadales, resolucinonadaceae, resolucinonas ria; environmental samples
1	AXBS02000019 NC 014385	Xylella fastidiosa 6c (whole genome shotgun sequence) Escherichia coli	pXF6c pEC L46	39572 144871	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xylella ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia
1	KX518744	Escherichia coli strain HYEC7	pHYEC7-110	110226	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia
1	NC_007182	Sodalis glossinidius	p3 pSG1	81553	Bacteria; Bacteria;	ria; rseudomonadota; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Kiebsiella/Kabultella group; Kiebsiella. ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Bruguierivoracaceae; Sodalis
1	CP011646 AY046276	Klebsiella pneumoniae strain CAV1596 Salmonella enterica subsp. enterica serovar Typhimurium	pKPC_CAV1596-97 R46	96702 50969	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Klebsiella/Raoultella group; Klebsiella ria: Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Salmonella
1	NZ_CP015505	Klebsiella pneumoniae strain SKGH01	unnamed 5	34611	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Klebsiella/Raoultella group; Klebsiella.
1	NC_011148	Methylophaga nitratireducenticrescens strain GP59 Salmonella enterica subsp. enterica serovar Agona str. SL483	pGP59-34 unnamed	33560 37978	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Thiotrichales; Piscirickettsiaceae; Methylophaga ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Salmonella.
1	NC_010696	Erwinia tasmaniensis Et1/99 Escherichia coli Xuzhou21	pET35 p0157_Sal	35494	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Erwiniaceae; Erwinia. ria: Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia
1	CP018118	Escherichia coli strain MRSN346638	pMRSN346638_64.5	64467	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia
1	NC_002525 NC 004999	Escherichia coli K-12 Pseudomonas putida NCIB 9816-4	R721 pDTG1	75582 83042	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas
1	AB237655	Pseudomonas putida	pNAH7	82232	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas
1	NC_003350	Pseudomonas thivervalensis strain Plui Pseudomonas putida	PWW0	116580	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas
1	CP021134 CP017011	Pseudomonas fragi strain NMC25 Pseudomonas svringae pv. actinidiae strain NZ-47	unnamed2 pPsa22180b	54359 55944	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas ria: Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas svringae
1	CP003092	Burkholderia sp. YI23	byi_3p	115232	Bacteria;	ria; Pseudomonadota; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Burkholderia
1	LJGA01000018 CP013007	Xanthomonas citri pv. citri strain NIGEB-88 (whole genome shotgun sequence) Xanthomonas citri pv. malvacearum strain XcmH1005	pXCC_55 pXcmN	55677 59644	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas
1	CP011518	Pandoraea oxalativorans strain DSM 23570 Pandoraea oxalativorana strain DSM 23570	pP070_1	633357	Bacteria;	ria; Pseudomonadota; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Pandoraea
1	CP021022	Xanthomonas citri pv. phaseoli var. fuscans strain CFBP6167	pG	42380	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas
1	CP022269 CP011808	Xanthomonas citri pv. vignicola strain CFBP7112 Pandoraea faecigallinarum strain DSM 23572	p1B pPF72-1	42275 386625	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas ria; Pseudomonadota; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Pandoraea
1	FP885893	Ralstonia solanacearum CMR15	pRSC35	35008	Bacteria;	ria; Pseudomonadota; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Ralstonia
1	KC170278	Collimonas arenae strain Cal35 Uncultured bacterium	pMBUI4	41440 37247	Bacteria; Bacteria;	ria; Pseudomonadota; Betaproteobacteria; Burkholderiales; Oxalobacteraceae; Collimonas ria; environmental samples
1	FP340278 F0681497	Xanthomonas albilineans str. GPE PC73 Xanthomonas fuscans subsp. fuscans str. 4834-R	plasmII plc	31555 41950	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas
1	NC_028464	Escherichia coli	R388	33913	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia
2	CP006601 CP001979	Cycloclasticus zancles 78-ME Marinobacter adhaerens HP15	p7ME01 pHP-42	42347 42349	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Thiotrichales; Piscirickettsiaceae; Cycloclasticus ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Marinobacteraceae; Marinobacter
2	CP003381	Methylophaga frappieri strain JAM7 Photobactarium damaalaa suban, piscicida	plasmid	47825	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Thiotrichales; Piscirickettsiaceae; Methylophaga ria: Pseudomonadota: Gammaproteobacteria: Vibrionales: Vibrionaceae: Photobacterium
2	NC_025029	Uncultured bacterium pAKD4	pAKD4	56803	Bacteria;	is, environmental samples
2	NC_025028 CP018471	Uncultured bacterium pMCBF6 Xanthomonas vesicatoria strain LM159	pMCBF6 pLM159.2	66615 62784	Bacteria; Bacteria;	ria; environmental samples ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas
2	NC_008459	Bordetella pertussis	pBP136	41268	Bacteria;	ria; Pseudomonadota; Betaproteobacteria; Burkholderiales; Alcaligenaceae; Bordetella
2	CP023440	Thauera sp. Kll	pTX1	140963	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonad ria; Pseudomonadota; Betaproteobacteria; Rhodocyclales; Zoogloeaceae; Thauera
2	CR555308	Azoarcus sp. EbN1 Commonse testosteroni CNR_1	plasmid 2	223670	Bacteria;	ria; Pseudomonadota; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Aromatoleum ria: Pseudomonadota: Bataproteobacteria: Burkholderiales: Comercoadoceae: Comercoadoceae;
2	NC_019369	Burkholderia cepacia	pYS1	82988	Bacteria;	ria; Pseudomonadota; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Burkholderia; Burkholderia cepacia complex
2	PKU001000109 CP014059	Sedimenticola sp. isolate BM503 sc_pri(whole genome shotgun sequence) Achromobacter xylosoxidans strain FDAARGOS 147	plasmid	70620 21970	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Sedimenticola. ria; Pseudomonadota; Betaproteobacteria; Burkholderiales; Alcaligenaceae; Achromobacter
2	NC_008357	Pseudomonas aeruginosa	pBS228	89147	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas
2	LFKC01000048	Hydrogenophaga sp. 14 (whole genome shotgun sequence) Escherichia coli strain HMLN-1 (whole genome shotgun sequence)	unnamed	55844	Bacteria; Bacteria;	ria; Pseudomonadota; Betaproteobacteria; Burknolderiales; Comamonadaceae; Hydrogenopnaga. ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia
2	KC170283 NC 022650	Uncultured bacterium Escherichia coli JJ1886	pDS1 plasmid pJJ1886 4	40596 55956	Bacteria; Bacteria;	ria; environmental samples ria: Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia
2	CP003886	Legionella pneumophila subsp. pneumophila LPE509	plasmid	73490	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Legionellales; Legionellaceae; Legionella
2	CP001716	Methylomicrobium alcaliphilum str. 202 Candidatus Accumulibacter phosphatis clade IIA str. UW-1	MEALZ_P pAph01	128415	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Methylococcales; Methylococcaceae; Methylotuvimicrobium ria; Proteobacteria; Betaproteobacteria; Candidatus Accumulibacter
2	CP014330	Xylella fastidiosa strain FB7 Versinia neudotuberculosis IP 31758	plasmid p.59kb	39408 58679	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xylella ria: Pseudomonadota; Gammaproteobacteria; Enterobacteria]es; Versiniaceae; Versinia
2	CP028340	Thauera aromatica K172	pKJK172	53761	Bacteria;	ria; Pseudomonadota; Betaproteobacteria; Rhodocyclales; Zoogloeaceae; Thauera
2	N2_CP012834 CP041251	Salmonella enterica subsp. enterica serovar Cerro str. CFSAN001588 Raoultella electrica strain DSM 102253	pCFSAN001588_001 unnamed4	53952 35253	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Salmonella. ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Klebsiella/Raoultella group; Raoultella
2	NZ_CP014764	Klebsiella pneumoniae strain KPNIH39 Vibrio campbellii strain IMD29	pKPN-704	36707	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Klebsiella/Raoultella group; Klebsiella.
4	CP000791	Vibrio campbellii ATCC BAA-1116	pVIBHAR	89008	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio
4	CP009359 NZ CP011294	Vibrio tubiashii ATCC 19109 Salmonella enterica subsp. diarizonae strain 11-01854	p48 unnamed2	47973 57955	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio; Vibrio oreintalis group ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Salmonella.
4	NZ_CP015845 CP017586	Escherichia coli 0157:H7 strain FRIK2455 Pantoea stewartii subsp. stewartii DC283	p35K	34689	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia. ria: Pseudomonadota: Gammaproteobacteria: Enterobacterales: Erviniscane: Pantona
4	CU468131	Erwinia tasmaniensis strain ET1/99	pET49	48751	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Erwiniaceae; Erwinia
3	CP026564 LT963396	Pseudomonas avellanae strain R21eaf Pseudomonas cerasi isolate PL963 genome assembly	p2_tig5 PP1	102862 127474	Bacteria; Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas
3	KU950310	Pseudomonas syringae pv. actinidiae strain SR198	pMG2_SR198	111158	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas syringae
3 3	NC_015855	Pseudomonas putida strain 12969 Pseudomonas putida	p12969-2 pGRT1	109708	Bacteria; Bacteria;	ria; rseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas
3	NC_010876 MG869622	Xanthomonas axonopodis pv. glycines strain 8ra Polaromonas sp. H8N	pXAG81 pH8NP2	26721	Bacteria; Bacteria	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas ria: Pseudomonadota; Betaproteobacteria; Burkholderiales; Comamonadaceae; Polaromonas
3	CP009886	Xylella fastidiosa strain Hib4	pXF64-HB	64251	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xylella
3	KX912255	Enterobacter cloacae strain H140960786	pJF-786	25354	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Enterobacter; Enterobacter cloacae complex
3	TFETFFC2	Thiobacillus ferrooxidans (incomplete)	pTF-FC2	4001.0	Bacteria;	ria; Pseudomonadota; Acidithiobacillia; Acidithiobacillales; Acidithiobacillaceae; Acidithiobacillus
3	CP019872	Pseudomonas syringae pv. tomato strain B13-200	pB13-200A	125801	Bacteria;	ran, rocumentatora, ectopiotecunacteria, meisseriates, meisseriatese; vitreosciila ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas syringae
3	CP014511 KR014105	Burkholderia sp. PAMC 28687 strain PAMC28687	plasmid 4 nGES5	20973	Bacteria; Bacteria;	ria; Pseudomonadota; Betaproteobacteria; Burkholderiales; Burkholderia ria: Pseudomonadota; Gomannotebacteria; Auromonadales; Aeromonadoceae; Aeromonas
3	CP003602	Chamaesiphon minutus PCC 6605	pCHA6605.02	31019	Bacteria;	ria; Cyanobacteria; Synechococcales; Chamaesiphonaceae; Chamaesiphon 19. Denudicateria; Synechococcales; Chamaesiphonaceae; Chamaesiphon
3	NZ CP007231	Yersinia similis strain 228	plasmid	60687	Bacteria; Bacteria;	rra, reculumunauta, uammaproteobacteria; Enterobacterares; Enterobacteriaceae; Escherichia. ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Yersiniaceae; Yersinia.
3	NZ CP013915 CP019872	Serratia fonticola strain GS2 Pseudomonas syringae pv. tomato strain B13-200	pSF002 pB13-200A	93737 125801	Bacteria; Bacteria:	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Yersiniaceae; Serratia. ria; Pseudomonadota; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas svringse
3	NC_013973	Erwinia amylovora ATCC 49946	plasmid 2	71487	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Erwiniaceae; Erwinia.
3	NZ_CP010383	Enterobacter hormaechei subsp. steigerwaltii strain 34998	p34998-106.409kb	106410	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Enterobacter; Enterobacter cloacae complex.
3	NC_011419	Escherichia coli SEll Klabeialla en ITCDAR_SE	pSE11-1	100021	Bacteria;	ria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia. ris: Pseudomonadota; Gammaproteobacteria; Enterobacterales; Esterobacteriaceae; Michaella/Devolutile.com, Michaella
3	NZ_CP016927	Klebsiella pneumoniae isolate 23	pincL_M_DHQP1400954	72093	Bacteria;	ras, iscussionadota, Gammaproteobacteria, Enterobacteriaes, Enterobacteriaceae; Kiebsielia/Kaouiteria group; Kiebsielia. ria; Pseudomonadota; Gammaproteobacteria; Enterobacteriaes; Enterobacteriaceae; Kiebsielia/Raouiteria group; Kiebsielia.
3	CP003780 AP018193	Xanthomonas citri subsp. citri Aw12879 Nostoc sp. NIES-2111	pXcaw58 plasmid9	58317 38211	Bacteria; Bacteria	ria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas ria: Cvanobacteria: Nostocales: Nostocaceae: Nostoc
3	CP026689	Nostoc sp. 'Peltigera membranacea cyanobiont N6' strain N6	pNPM7	29551	Bacteria;	ria; Cyanobacteria; Nostocales; Nostocaceae; Nostoc
3	AP018331 AP018212	NOSTOC COmmune HK-UZ Calothrix brevissima NIES-22	plasmid5 plasmid5	34879 57802	Bacteria; Bacteria;	ria; Lyanobacteria; Wöstöcales; Nostocaceae; Nostoc ria; Cyanobacteria; Nostocales; Calotrichaceae; Calothrix
3	AP018325 CP016619	Nostoc sp. HK-01 Microvirga sp. V5/3M	plasmid7 unnamed2	29094 977332	Bacteria; Bacteria	ris; Cyanobacteris; Nostocales; Nostocaceae; Nostoc ris: Pseudomonadota; Alphaproteobacteris; Hyphomicrobiales; Methylobacteriaceae; Microvinga
- 3			and the full state of the last of the	1.1332	-unestid;	,

Table S1