Rhizobial Exopolysaccharides and Type VI Secretion Systems: A Promising Way to Improve Nitrogen Acquisition by Legumes

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At present, there are numerous examples in which symbiotic nitrogen fixation by rhizobia can totally replace the use of nitrogen fertilizers in legume crops. Over the years, there has been a great effort by research institutions to develop and select rhizobial inoculants adapted for these crops. The symbiotic process is highly dependent on the dynamic exchange of signals and molecular nutrients between partners. Our focus in this review was to discuss the two key determinants in successful symbiotic interactions of rhizobia to nodulate pulses. One of them is the production of exopolysaccharides (EPS) and the other the presence of the type VI secretion system (T6SS). EPS are extracellular polymers weakly associated with the bacterial surface and are abundantly released into acid soils facilitating, among other functions, an adaptation of rhizobia to this environment. On the other hand, different protein secretion systems, involved in symbiosis, have been described in rhizobia. This is not the case with the T6SS. The current availability of various rhizobial genomes offers the possibility of discussing its role in symbiosis. The study of these determinants will be of great utility for the selection of effective inoculants for legumes, a promising way to improve nitrogen acquisition by legumes.

Keywords: Rhizobium, root nodulating bacteria, effectors, type VI secretion systems, inoculant, exopolysaccharide, symbiosis

RHIZOBIA FOR PULSES

Legumes are of capital importance for human and animal feed and for most ecosystems. This is due, among other factors, to the fact that most species are capable of biologically fixing nitrogen (BNF) by association with soil diazotrophic bacteria called rhizobia. Most legume crops can self-supply the nitrogen they need, this being a fact of great relevance to mitigate the negative effects of nitrogen fertilizer leaching in the environment. All this has led to great interest in the selection of effective inoculants. Brazil is considered a global leader in the use of inoculants and one of the
largest producers of pulses worldwide (Santos et al., 2019). A reference is made in the following section to the most commonly selected strains (Bradyrhizobium and Rhizobium), as well as the beneficial role that rhizobial exopolysaccharides (EPS) and T6SS can have in an effective symbiosis and therefore in BNF.

**BRADYRHIZOBIUM SPECIES IN BRAZILIAN SOILS**

Reports on legume nodulation by Bradyrhizobium strains in Brazil are quite abundant, mainly in terms of legumes and bradyrhizobial isolates diversity. However, there are still a large number of rhizobial isolates waiting to be characterized. In this section, we summarize data on the history and evolution of studies on Bradyrhizobium in soybean (Glycine max).

The SEMIA Rhizobia Collection of Brazil originated in 1950 through the isolation and selection of efficient native strains for legumes of economic importance by professionals from the Agricultural Microbiology Section (SEMA) of the Agronomic Research Institute (IPAGRO today FEPAGRO). Brazilian soybean crops boosted the beginning of inoculant production in Brazil in the 1960s due to the lack of native bacteria that were able to efficiently nodulate soybean, therefore, supplied mainly with North American strains. The first record of the SEMIA Collection was in 1973. In the 1980s, studies showed enormous genetic and physiological variability between the strains of Bradyrhizobium japonicum, suggesting the subdivision of Bradyrhizobium into two species, B. japonicum and B. elkanii (Kuykendall et al., 1992).

Argentina, Brazil, and the United States are the most important soybean producers. Bradyrhizobium is the most prominent and widely studied in terms of nodulation and nitrogen fixation in soybeans. The Ministry of Agriculture, Livestock and Food (MAPA) of Brazil emitted official reports about the quality of soybean inoculants (number of viable cells, strain identification, and presence of contaminants). In Brazil and South America, today, MAPA authorizes the use of different Bradyrhizobium strains in the formulation of commercial inoculants (Supplementary Table 1), consequently settling these populations in most of the soybean Brazilian soils (Hungria et al., 2001; Delamuta et al., 2013). Since the beginning of inoculant production in the 1980s, seven species that were able to efficiently nodulate soybean have been described, B. japonicum, B. diaeoefficiens, B. elkanii, and B. liaoningense (Willems, 2006); and Mesorhizobium tianshanense (Chen et al., 1995); Sinorhizobium fredii and S. xinjiangense (Chen et al., 1988). The number of validated Bradyrhizobium species published has greatly increased in recent years, and ~35% are from South America and another 35% from other regions mainly from China.

The soybean BNF is consolidated though many farmers use synthetic fertilizers. Bradyrhizobium inoculants are alternatives to reduce water pollution and nitrous oxide (N₂O) emission (Akiyama et al., 2016). However, Obando et al. (2019) showed the first report that pointed out the incomplete denitrification pathway in five of the most used strains for soybean.

Göttfert et al. (2001) described symbiosis-specific genes in a 410-kilobase DNA region of the B. diaeoefficiens USDA110 chromosome and in 2002, the complete genomic sequence was described (Kaneko et al., 2002). The pioneering study reporting the high diversity of Bradyrhizobium genospecies was through a DNA–DNA hybridization conducted by Willems et al. (2001). Bradyrhizobium's genomes are larger than those of other genera of rhizobia, reflecting their vast metabolic, and different life cycles. In addition, high phenotypic and/or genotypic diversity, both intra- and interspecific, between the strains of Bradyrhizobium was observed (Guimarães et al., 2012, 2015; Ruffini et al., 2014). Despite this diversity, Bradyrhizobium species have a monophyletic character with few exceptions, particularly the photosynthetic bradyrhizobia (Moulin et al., 2004). Iida et al. (2015) reported the presence of some islands of symbiosis shuffled with abundant insertion sequences in the genomes of extra-slow-growing Bradyrhizobium strains. In the symbiotic islands, there are insertion sequences and groups of symbiotic genes, such as nod, nif, fix, and rhs. nifH and nod Genes are the most used for phylogenetic reconstructions of symbiosis, both transferred vertical and horizontally between different chromosomal backgrounds (Menna and Hungria, 2011). Generally, the transfer of specific symbiotic genes is considered a fundamental mechanism that allows legumes to form symbioses with rhizobia adapted to private soils (Andrews et al., 2017).

**RHIZOBIUM TROPICI, A SPECIES RESISTANT TO ACIDIC GROWTH CONDITIONS**

Rhizobium species are widely used as inoculants. R. tropici CIAT899 establishes symbiosis with different legume species, including common beans (Phaseolus vulgaris) (Martínez-Romero et al., 1991). However, the success of this process can be limited by different environmental conditions, such as high temperature, low pH, presence of host legumes, and soil (Martínez-Romero et al., 1991; Hungria et al., 2001; Vinuesa et al., 2003; Puozza et al., 2017).

When pH is reduced by plant exudates containing protons and organic acids, it provides a limitation for the survival of microorganisms and nodulation, and BNF can be severely affected. Finding acid-tolerant plants and compatible rhizobia are of remarkable agronomic and ecological relevance. R. tropici CIAT899 is more resistant to acidic conditions than most rhizobia. This resistance is related to several factors, such as hydroxylated ornitnine lipids, which make the membrane less fluid and less permeable to protons (Vinuesa et al., 2003; Vences-Guzmán et al., 2011), Guerrero-Castro et al. (2018) identified 26 genes in R. tropici CIAT899 involved in the pH stress response, and transcriptomic analysis from cells grown under different pH conditions allowed the identification of 383 genes that are differentially expressed. The genes included response regulators and membrane transporters, enzymes involved in the metabolism of amino acids and carbohydrates, and proton extrusion.
Biosynthesis of EPS encoded in the *R. tropici* CIAT899 genome has been associated with acid tolerance, and implication of EPS in symbiosis is discussed in the next section.

**IMPORTANCE OF EXOPOLYSACCHARIDES PRODUCTION IN SYMBIOSIS**

Rhizobial EPS are biopolymers of high- and low-molecular weight, secreted in the environment both in free living and in symbiosis (Skorupska et al., 2006). EPS contribute to tolerance of rhizobia against unfavorable conditions: reactive oxygen species, detergents, salt, acidic pH, drought, antimicrobial agents, etc. (Staehelin et al., 2006; Geddes et al., 2014; Naseem et al., 2018; Sun et al., 2020). Also, they participate in biofilm formation and attachment to abiotic or plant surfaces (Russo et al., 2006; Schäper et al., 2019). In the *Rhizobium*–legume interactions, EPS affect pre-infection events with the host, such as root hair curvature (Downie, 2010; Janczarek, 2011) and suppress the defense responses of the host plant (Jones et al., 2007). EPS can be perceived by plant LysM kinase receptors impairing or facilitating symbiosis with rhizobia depending on the polysaccharide composition, in *L. japonicus*, EPR3 receptor recognizes EPS controlling bacterial infection (Kawaharada et al., 2017; Kelly et al., 2017; Wong et al., 2020). EPS can have a negative effect on symbiosis as seen in *S. fredii* HH103/Glycine max, where flavonoids activate transcription of noduleation genes but repress EPS production (Acosta-Jurado et al., 2016).

EPS biosynthesis is unknown in most of the inoculants authorized in Brazil (Supplementary Table 1), particularly in *Bradyrhizobium* strains, however, it has been well-studied in *Sinorhizobium meliloti* and *Rhizobium leguminosarum* and required *exo*, *exs*, *exp*, and *muc* genes, which are mainly grouped and conserved in different strains (Janczarek et al., 2015). They are distributed on the chromosome or in symbiotic plasmids (Ivashina and Ksenzenko, 2012; Ormeño-Orrillo et al., 2012). EPS contain mostly D-glucose and D-galactose (Castellane et al., 2014, 2017, 2018, 2019), uronic acids and non-sugar substitutions can also be very important (López-Baena et al., 2016). Enzymes such as glycosyltransferase (PssA) and galactosyltranserase (PssJ) are responsible for assembling EPS sub-units in *R. leguminosarum* (Marczak et al., 2020).

Rhizobia are capable of producing two EPS forms, type I (sucinoglycans) involved in *S. meliloti* nodule development (Reuber et al., 1991; Becker et al., 2002; Skorupska et al., 2006) and type II (galactoglycans) required for root hair attachment and biofilm formation (Rüberg et al., 1999; Becker et al., 2002; Skorupska et al., 2006; Sorroche et al., 2010). Also, *R. leguminosarum* EPS protect against zinc stress in the symbiosis with *Trifolium* (Kopycinska et al., 2018). In the symbiosis of *S. fredii* HH103/Glycyr rhiza uralensis, EPS were not strictly necessary (Margaret-Oliver et al., 2012) but *Mesorhizobium tianhanense* non-mucoid mutants were defective in nodulation with the same legume (Wang et al., 2008). *R. tropici* CIAT899 EPS are not required for bean noduleation but it is involved in competitiveness (Milner et al., 1992). A *R. leguminosarum* pssZ mutant showed reduced EPS production and nodule formation and affected bacteroids development (Lipa et al., 2018). Different amounts of EPS do not seem to interfere with the host, but with the ability to survive in the rhizosphere (Donati et al., 2013). *S. meliloti* Rm8530 produced three times more EPS than an *expR* mutant (Primo et al., 2020), and both had the same efficiency in the symbiosis with *Medicago sativa* (Pellock et al., 2002). Despite all these studies, the role of EPS in symbiosis remains unclear (Skorupska et al., 2006; Marczak et al., 2017).

In acidic soils, rhizobia from different hosts, such as *Cicer, Phaseolus*, *Lens*, and *Leucaena* can produce a considerable quantity of EPS (Cunningham and Munns, 1984). Secreted EPS are increased under abiotic stress (Hirsch, 1996; Sorroche et al., 2018; Primo et al., 2020). However, at acid pH *R. tropici* CIAT899 showed a lower production of biopolymers (Avelar Ferreira et al., 2012), whereas EPS production was not affected in *R. favelukessi* LPU83 (Nilsson et al., 2021).

The EPS in *P. vulgaris* induce genes involved in carbon metabolism, transcriptional regulation, circadian cycle, and phytohormone production (Via et al., 2015).

**TYPE VI SECRETION SYSTEM (T6SS) IN RHIZOBIA**

The T6SS secretion system has been hardly related to symbiosis. It was described in 2006 in *Vibrio cholerae* and *Pseudomonas aeruginosa* (Mougous et al., 2006; Pukatzki et al., 2006). Since then it has been characterized in numerous bacteria, mainly Proteobacteria (Boyer et al., 2009). The T6SS is a nano-syringe that enables bacteria to inject proteins called effectors into both eukaryotic and prokaryotic cells (Basler and Mekalanos, 2012).

T6SS biosynthesis usually requires 13 core structural proteins encoded by *ts* genes and accessory names encoded *tag* (type six accessory gene) (Shalom et al., 2007; Silverman et al., 2012).

Its structure contains three elements: a trans-membrane complex (TssMLJ), a cytoplasmic baseplate (TssEFGK), and a double tube, the outer one formed by a contractile sheath (TssBC) and the inner one by Hcp (TssD) ending with the puncturing device VgrG (Tss)-PAAR (Wang et al., 2019) (Figure 1). The PAAR domain (proline–alanine–alanine–arginine) is a sharp conical extension on the VgrG spike, is similar to DUF4150 domain, and facilitates secretion of a broad range of toxins (Shneider et al., 2013). The assembly of the inner tube and the contractile sheath requires TssA. After contraction, the T6SS tube is recycled by TssH (ClpV), an ATPase that disassembles the structure (Wang et al., 2019).

Many of the known effectors targeting bacteria have a toxic function (lipases, DNases, and peptidoglycanases) killing potential competitors (Couthurst, 2019). Toxic effector genes are adjacent to cognate immunity genes that prevent self-toxicity or toxicity of sibling cells, and they are known as effector–immunity pairs (E/I pairs) (Yang et al., 2018). It is relevant that the presence of genes coding for possible effectors not accompanied by their cognate immunity gene and called orphan genes could encode for effectors whose target was eukaryotic.
FIGURE 1 | T6SS gene cluster organization of different rhizobia. In parentheses is the host legume. Orthologous genes show the same color and pattern. The numbered Boxes 1–6 contain mainly not 
tss or 
tag genes. Box 6 corresponds to strains harboring a second cluster with the 
vgrG 
gene. Uncolored genes encode proteins with no known domains. Pseudogenes are indicated by 
thinner arrows. Dotted arrows have not been considered part of the T6SS. The numbers above the 
genes indicate the number of amino acids of the corresponding protein and boxes under the genes indicate identified domains (D: DUF, Oxa: 
oxoacyl-ACP synthases). (A) General organization in four rhizobia genera, (B) detailed gene organization of Boxes 3, 4 of Mesorhizobium and Sinorhizobium strains, (C) detailed gene 
organization of Boxes 5, 6 of Rhizobium strains, and (D) structure of a T6SS. The colors are the same as those of the genes that encode these units in (A–C). The 
effectors are symbolized by ovoid shapes.
So far the symbiotic importance of any T6SS of recommended inoculants such as those included in Supplementary Table 1 is not known. Figure 1 shows the organization of 19 T6SSs of strains representing the main genera of rhizobia that nodulate pulses. When possible the strains were chosen from Supplementary Table 1.

### GENOMIC ORGANIZATION OF RHIZOBIA T6SS CLUSTERS

Organization and arrangement of T6SS genes with structural, regulatory, or accessory functions called tss or tag genes are totally conserved in all the species of each genus included in Table 1 and Figure 1. Genes not identified as tss or tag, match mostly to hypothetical proteins and among them are potential effectors as discussed below. These genes have been studied before the description of the independent role of T6SS. Since then, a few researchers have studied the role of T6SS in symbiosis beyond mentioning its presence. In two such studies, it was found that mutations in T6SS genes do not affect symbiotic efficiency but symbiotic competitiveness (De Campos et al., 2017; Lin et al., 2018), and in another study, a positive effect on symbiosis was demonstrated (Salinero-Lanzarote et al., 2019).

cells (Trunk et al., 2018). Sometimes there is a third gene that codes a chaperone or adaptor for loading the effector onto the cognate VgrG. Adaptors contain conserved domains as DUF2169 or DUF4123 (Lien and Lai, 2017). Genes encoding effectors are often close to
tag
genes are

### TABLE 1 Proteins encoded by different rhizobial T6SS genes (no tss or tag genes).

| Host | Strains | Origin | Number of aa of proteins from not tss nor tag genes | References and NCBI sequence |
|------|---------|--------|--------------------------------------------------|-----------------------------|
| Arachis hypogaea | B. nanningense CCBAU 53390 China | 187, 528 (Figure 1A, Boxes 1, 2) | DS* NZ_LJYJ010000000 |
| Lupinus albus | B. elkanii SEMIA 938 Brazil | 187, 533 (Figure 1A, Boxes 1, 2) | Hungria et al., 2019, NZ_SZZP010000000 |
| Vigna unguiculata | B. pachyrhizus BR3262 Brazil | 187, 528 (Figure 1A, Boxes 1, 2) | Simões-Araújo et al., 2016 LJVE000000000 |
| Glycine max | B. elkanii SEMIA 587 Brazil | 187, 528 (Figure 1A, Boxes 1, 2) | De Souza et al., 2012 NZ_SWAO010000000 |
| | B. elkanii SEMIA 5019 Brazil | 187, 533 (Figure 1A, Boxes 1, 2) | DS* NZ_SWAP010000000 |
| | B. japonicum SEMIA 5079 Brazil | 116, 533 (Figure 1A, Boxes 1, 2) | Siqueira et al., 2014 NZ_CP007566.1 |
| | B. diazoefficmiss SEMIA 5080 Brazil China | 187, 533 (Figure 1A, Boxes 1, 2) | Siqueira et al., 2014 ADU000000000 |
| | S. fredii HH103 (USDA207) China | 115, 319, 324, 343, 366 (Figures 1A,B, Box 4) | Sugawara et al., 2013 NZ_WITA01000128 |
| Cicer arietinum | M. ciceri CC1192 Israel | 776, 131, 361, 265, 189, 253, 351, 353 (Figures 1A,B, Boxes 3, 4) | Haskett et al., 2016 NZ_CP015063 |
| | M. mediterraneae USDA3392 France | 551, 777, 131, 387, 347, 362, 162, 137, 300 (Figures 1A,B, Boxes 3, 4) | DS* NZ_NPK010000026 |
| Pisum sativum | Mesorhizobium SEMIA 3007 Mexico | 441, 807, 166, 115, 299, 368, 341, 367 (Figures 1A,B, Boxes 3, 4) | DS* NZ_MDLO010000000 |
| | R. ruizarguesonis UPM1132 Italy | 197; 369, 508, 349, 250, 132, 391, 370 (Figures 1A,C, Box 5) | Jorrin et al., 2020, NZ_PQIO010000000 |
| | R. ruizarguesonis UPM1133 Italy | 360, 296, 365, 508, 349 (Figures 1A,C, Box 5) | Jorrin et al., 2020, NZ_PQIO010000000 |
| | R. ruizarguesonis UPM1134 Italy | 197; 369, 508, 349, 250, 114, 391, 370 (Figures 1A,C, Box 5) | Jorrin et al., 2020, NZ_PQIO010000000 |
| Lens culinaris | R. laguerreae OL29 Algeria | 356, 274, 192, 229, 89, 224, 100, 221, 199, 242, 392 (Figures 1A,C, Box 5) | DS* NZ_WIFJ010000000 |
| | R. leguminosarum L145 France | 358, 298, 369, 499, 330, 128, 500, 370, 221, 150, 101, 199, 303, 329, 250, 198 (Figures 1A,C, Box 5) | DS* NZ_WIED000000000 |
| Phaseolus vulgaris | Rhizobium sp. SEMIA 4032 Brazil | 129, 166, 371, 366, 402, 196, 169, 1458, 283 (Figures 1A,C, Box 5) | DS* NZ_QERH010000000 |
| | Rhizobium sp. SEMIA 439 Argentina | 129, 166, 371, 366, 101, 354, 282, 175, 202, 176, 238 (Figure 1C, Boxes 5, 6) | DS* NZ_QERG010000000 |
| Vicia faba | R. leguminosarum CCBAU 03058 China | 358, 298, 250, 392, 369, 508, 305, 349 (Figures 1A,C, Box 5) | DS* NZ_WIEO010000000 |

*DS, Direct submission.*
marked in Figure 1 by boxes numbered 1–6 and they are not as conserved as the previous ones, especially when different genera are compared. The T6SSs of all the *Bradyrhizobium* strains in Table 1 have identical gene organization (Figure 1A). The three *Mesorhizobium* T6SSs presented slight differences among them as shown in Boxes 3, 4 of Figure 1. *Sinorhizobium* T6SS is similar to that of *Mesorhizobium*. The T6SSs of the *Rhizobium* strains show the greatest diversity in genes of Boxes 5, 6. Genomes of *R. SEMIA* 439 and *R. SEMIA* 4032 have an extra copy of gene vgrG (Figure 1C, Box 6).

**PUTATIVE EFFECTORS IN RHIZOBIA T6SS**

Numerous effectors are found in the vicinity of the structural genes vgrG and hcp, and it is common to find three genes in tandem so that the first corresponds to an adaptor with DUF2169 or DUF 4123 domains, the second to a toxic effector, and the third to an immunity protein. Other possibilities are E/I pair or orphan effectors (Ma et al., 2014; Bondage et al., 2016; Trunk et al., 2018; Yang et al., 2018). Many effectors have an N-terminal DUF4150 or PAAR domain followed by a C-terminal region of variable function and some immunity proteins have a similar structure to KNR4 proteins or contain DUF1851 domains (Zhang et al., 2011; Ma et al., 2014). Possible effectors are discussed below based on their location in T6SS or on domains noted in Pfam protein families database (El-Gebali et al., 2019) (Supplementary Table 2).

Two conserved genes in *Bradyrhizobium* T6SS could encode orphan effectors (Figure 1, Boxes 1, 2). Proteins from these genes were designated B.187 and B.528. Their number of amino acids depends on the strain (Table 1). No functional domain has been identified in B.187. The other, B.528, has a methyltransferase domain. The role of these proteins is unknown. Other orphan effector (197 aa) could be encoded by the gene between hcp and vgrG in *R. laguerrae* and *R. ruizarguesonis* UPM1132 and UPM 1134 (Figure 1C).

Box 3 in Figures 1A,B contains genes for three types of *Mesorhizobium* proteins, Ms166 has the DUF1036 domain mainly present in membrane proteins of alphaproteobacteria. Ms808 is homologous to M23 metalloproteases that lyse bacterial cell wall peptidoglycans (Lewis et al., 2019). And the third, Ms441, not present in *M. ciceri*, presents a caspase domain. Caspases (cysteine–aspartic proteases) participate in programmed cell death in animal tissues; however, the function of most bacterial caspase homologs are unknown (Asplund-Samuelsson, 2015). Box 4 of Figures 1A,B includes genes encoding proteins with DUF2169, DUF4150, or oxocycl-ACP synthases domains, but their roles are unknown.

Pairs E/I are present in *R. sp. SEMIA* 4032 and SEMIA 439 as Rap1/Tae4 pair (Figure 1C). Rap1 is homologous to an immunity protein that neutralizes the amidase Tae4 able to cleave muropeptides of peptidoglycans (English et al., 2012; Srikanthasan et al., 2013; Zhang et al., 2013). Other E/I pair could correspond to the genes between hcp and vgrG in the *Rhizobium* strains UPM1133, CCBAU3058, and L145, although they do not have similarity with any E/I pair described.

Proteins with N-terminal PAAR-like/DUF4150 domain followed by a C-terminal region that contains a putative effector domain (Bondage et al., 2016) are present in Boxes 4–6 of Figure 1. No function for these proteins has been identified with the exception of *M. ciceri* Mc253 that contains a DNase_NucA_NucB domain (Figure 1B, Box 4). Rse.1458 from *R. sp. SEMIA*4032, Figure 1 Box 6, has two toxic RhsA domains often present in effectors secreted by T6SS (Pei et al., 2020).

**CONCLUSIONS**

It is desirable that collections of rhizobia, such as the Brazilian SEMIA, have and provide well-characterized rhizobia capable of maximizing nitrogen fixation in legumes.

Rhizobial EPS role in plant signaling and in bacterial protection against different stresses should be elucidated because they play a crucial role in symbiosis. EPS can promote competitiveness, the development of nodules, and therefore influence the effectiveness of inoculants. The connection between different symbiotic signals and regulation of EPS and other relevant surface polysaccharide expression in different rhizobia should also be considered.

The presence of T6SS in inoculants with high nitrogen-fixing capacity can lead to (i) better competitiveness against native soil endosymbionts able to nodulate the same host legume; (ii) biocontrol against pathogens/microorganisms in the rhizosphere as demonstrated by *Pseudomonas* preventing phytopathogenic bacteria (Bernal et al., 2017, 2018) and by the antifungal activity of *Serratia marcescens* (Trunk et al., 2018); and (iii) targeting effectors to host legumes as revealed by some nodulation external proteins (Nops). Nops are type III secretion system–dependent effectors with a positive, negative, or neutral effect on symbiosis (Deakin and Broughton, 2009; Miwa and Okazaki, 2017; Kusakabe et al., 2020). It has been identified that T6SS anti-eukaryotic effectors enable infectious bacteria to survive against the immune response of their hosts (Monjarás- Feria and Valvano, 2020).

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SYNTHETIC MATERIAL

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

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