TITLE PAGE

Title: Multi-disciplinary approaches for studying rhizobium – legume symbioses

Running title: Techniques for studying rhizobia

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ABSTRACT

The rhizobium-legume symbiosis is a major source of fixed nitrogen (ammonia) in the biosphere. The potential for this process to increase agricultural yield while reducing the reliance on nitrogen-based fertilizers has generated interest in understanding and manipulating this process. For decades, rhizobium research has benefited from the use of leading techniques from a very broad set of fields, including population genetics, molecular genetics, genomics, and systems biology. In this review, we summarize many of the research strategies that have been employed in the study of rhizobia and the unique knowledge gained from these diverse tools, with a focus on genome and systems-level approaches. We then describe ongoing synthetic biology approaches aimed at improving existing symbioses or engineering completely new symbiotic interactions. The review concludes with our perspective of the future directions and challenges of the field, with an emphasis on how the application of a multi-disciplinary approach and the development of new methods will be necessary to ensure successful biotechnological manipulation of the symbiosis.

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SYMBIOTIC NITROGEN FIXATION: CHALLENGES AND PROSPECTS

Biological nitrogen fixation (BNF) is an agriculturally and ecologically crucial process that is performed by prokaryotes in two ecological groups: i) BNF performed by free-living cells often in close association with plants, or ii) by rhizobia that fix nitrogen during an endosymbiotic relationship with legumes. The fixation of N_2-gas into ammonia by root-nodule bacteria (rhizobia) is referred to as symbiotic nitrogen fixation (SNF), and it is a more efficient process in terms of supplying nitrogen to the plant. Phylogenetically, most rhizobia are α-proteobacteria, but some rhizobia are β-proteobacteria (Figure 1). The various genetic, biochemical, and evolutionary aspects of the symbiotic interaction have been reviewed over the years (Long 1996, Gage 2004, MacLean et al. 2007, Jones et al. 2007, Gibson et al. 2008, Oldroyd and Downie 2008, Masson-Boivin et al. 2009, Downie 2010, Oldroyd et al. 2011, Udvardi and Poole 2013, Haag et al. 2013, Remigi et al. 2016, Mus et al. 2016, Poole et al. 2018). In brief, the symbiosis is initiated following an exchange of signals between the roots of the plant and free-living rhizobia in root-proximal soil (the rhizosphere). As root nodule tissue develops (Figure 2), the rhizobia enter this specialized tissue through an extracellular infection thread; as these inwardly growing threads reach differentiated nodule cells, the bacteria become surrounded by a plant-derived membrane and taken up into the plant cytosol, where they are known as bacteroids. The nascent bacteroids then undergo major morphological and transcriptional changes, leading to active nitrogen fixation, which is the conversion of atmospheric N_2 into NH_3 for the plant.

It has been estimated that BNF contributes several teragrams of nitrogen (the equivalent of billions of USD of nitrogen based fertilizer) annually to global agricultural systems (de Vries et al., 2011; Herridge et al., 2008; Ladha et al., 2016). Rhizobial inoculants are inexpensive alternatives to industrial nitrogen fertilizers that can improve crop yields (Figure 2), resulting in

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greater profits and potentially significant impacts on the livelihood of the community, especially for the world's poorest farmers (Bloem et al. 2009, Mutuma et al. 2014). BNF also has a substantial positive impact on the environment through reducing the application of nitrogen fertilizers. These fertilizers contribute to elevated atmospheric levels of the potent greenhouse gas nitrous oxide (Park et al. 2012), to toxic nitrate-laden water that contributes to algae blooms and eutrophication (Conley et al., 2009; Randall and Mulla, 2001; Ward, 2009), and to the depletion of fossil fuel as industrial ammonia synthesis by the Haber-Bosch process accounts for 1-2% of total human energy consumption (Erisman et al. 2008). These negative consequences are ameliorated by relying more on biological solutions to nitrogen generation for agricultural systems.

The benefits of SNF can be maximized in agricultural systems by two general strategies. The first is optimizing the amount of nitrogen fixed by rhizobial bio-inoculants in extant legume symbioses. This will involve not only increasing the rate of nitrogen fixation in the nodule, but also increasing the competitiveness of inoculant strains in the soil and rhizosphere. The second strategy is to engineer completely new nitrogen fixing symbioses with non-leguminous plants. Many of the world's staple crops, such as the cereals, do not enter into a N_2-fixing symbiosis with rhizobia (Charpentier and Oldroyd 2010, Oldroyd and Dixon 2014). Engineering synthetic symbioses with these crops therefore presents a monumental and challenging opportunity for exploiting SNF.

Successful implementation of either strategies requires an intricate understanding of both symbiotic partners, in terms of abiotic interactions, responses to complex microbial communities, symbiotic communication, developmental processes, and the metabolism that underpins the nitrogen fixation process. Below, we will explore leading-edge questions in rhizobial biology, and describe how a variety of sound experimental approaches have propelled our understanding of

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106 symbiosis biology. The current synthetic biology approaches and future directions and challenges
107 for engineering BNF for agricultural gains will be described, highlighting the need for multi-level
108 studies of rhizobial biology to provide the necessary data to guide rational improvement of SNF
109 through systems biology approaches.
110

111 **RHIZOBIAL LIFE-HISTORY**

112 Nitrogen fixing rhizobia have complex life-cycles (Figure 3) (Poole et al. 2018). Rhizobia
113 are found as free-living bacteria in general soil environments and in the rhizosphere. These two
114 environments provide unique nutritional and stress conditions (Hinsinger et al. 2009) that the
115 rhizobia must effectively manage. Rhizobia must also compete against other microbial community
116 members to establish a stable population (Smalla et al. 2001, Li et al. 2016). From the rhizosphere,
117 the rhizobia can infect a compatible legume host, wherein growth conditions vary according to the
118 stage of symbiotic development, from the acidic conditions in the pocket of a curled root hair
119 (Hawkins et al. 2017), to linear growth along penetrating infections threads, to symbiotic
120 differentiation after release into host cells. Each stage of free-living and symbiotic growth requires
121 a unique set of genes and metabolic capabilities. Development of next-generation commercial
122 inoculant strains must account for these and other biological properties, including growth in
123 industrial liquid cultures and survival during desiccation (O'Callaghan 2016).

124 The rhizobial life-cycle does not occur in isolation. It is also necessary to account for social
125 interactions between rhizobia and host plants, and between rhizobia and competing microbes
126 (Checcucci et al. 2017). The rhizobia-host interaction involves multiple signal exchange events,
127 as well as the massive exchange of metabolic resources. The initial events of infection thread
128 formation and nodule primordium induction exhibit a high degree of cooperation between partners;

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yet, the culminating developmental events leading to N fixation reveal hints of conflict and aspects of an evolutionary arms race (Sachs et al. 2018). For example, plant-produced nodule-specific cysteine-rich (NCR) peptides (Mergaert et al. 2003, Van de Velde et al. 2010) and corresponding bacterial NCR peptidases (Price et al. 2015) represent a late-stage dialogue in many legumes in which bacteroids improve their fitness at the cost of the host plant by resisting symbiotic enslavement (for reviews, refer to: (Kondorosi et al. 2013, Haag et al. 2013, Alunni and Gourion 2016, Pan and Wang 2017). As multiple rhizobial strains may inhabit the same or different nodules on a single plant (Hagen and Hamrick 1996, Checcucci et al. 2016), cheating behaviours have emerged in the rhizobia (Singleton and Tavares 1986, Sachs et al. 2010, Checcucci et al. 2016, Regus et al. 2017), while plants have been observed to limit cheating through sanctions and partner discrimination (Kiers et al. 2003, Kiers and Denison 2008, Heath and Tiffin 2009, Sachs et al. 2010, Quides et al. 2017, Westhoek et al. 2017, Daubech et al. 2017).

Rhizobia also interact with a diverse soil microbial community, where they must effectively compete by antagonism and scavenging of nutrients. This can involve, for example, the production of bacteriocins that have anti-bacterial activity against closely related taxa (Hirsch 1979, Wilson et al. 1998, Twelker et al. 1999, Oresnik et al. 1999). The native microbiome may also promote symbiotic development by a rhizobial inoculant; it was observed that soil isolated *Rhizobium fabae* can promote nodulation of *R. etli* in a quorum sensing dependent mechanism (Miao et al. 2018). Rhizobia also encounter numerous bacteriophages in nature that may influence the effectiveness of a rhizobial inoculant. Phages generally are virulent to only some strains of a species, and can be used to reduce the population density of a poor symbiont to allow the better symbiont to flourish (Evans et al. 1979, Hashem and Angle 1990). However, phages can also reduce the effectiveness of rhizobial inoculants (Mendum et al. 2001).

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Nodulation efficiency and efficiency of nitrogen fixation are distinct phenotypes (Bourion et al. 2017). It is clear that understanding the diverse mechanisms underlying all stages of the rhizobial life-history, including competitive fitness in the rhizosphere and each stage of symbiotic development, will be imperative in designing and implementing improved rhizobia-based agricultural strategies. To this end, moving beyond artificial, sterile symbiotic assays in lab conditions will be necessary to generalize results to field conditions.

GENETIC MANIPULATIONS AND FUNCTIONAL GENOMICS

The majority of the community's knowledge of the core symbiotic processes comes from classical molecular genetic analyses involving forward- and reverse-genetic techniques, wherein single mutations are associated with defective phenotypes. Tn*5*-based transposon mutagenesis, coupled with plasmid-based complementation and DNA sequencing, played a foundational role in the discovery of the rhizobial *nod* and *nif/fix* genes (Becker et al., 1993a; 1993b; 1993c; Buikema et al., 1983; Chua et al., 1985; Glucksmann et al., 1993; Jagadish and Szalay, 1984; Long et al., 1988; 1982; Swanson et al., 1987). Both suicide plasmids and broad host-range plasmids outfitted to autonomously replicate in rhizobia have permitted studies that rely on random transposon mutagenesis, targeted gene disruption, and complementation. Such plasmid-based gene manipulation systems continue to play a prominent role in genetic studies in rhizobia (Griffitts and Long 2008, Griffitts et al. 2008, Carlyon et al. 2010, VanYperen et al. 2015), and targeted genetic studies remain essential to complement and validate observations from systems- and genome-level analyses.

High-throughput functional genomic studies have become more common for cataloguing rhizobial gene functions. *Sinorhizobium meliloti* ORFeome (Schroeder et al. 2005) and fusion

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(Cowie et al. 2006) libraries have been developed to facilitate functional and expression analyses in free-living and symbiotic states. A *S. meliloti* promoter library was developed and employed in the identification of genes involved in the early symbiotic process (Zhang and Cheng 2006). Similarly, a *Rhizobium leguminosarum* genome library was produced for use with *in vivo* expression technology (IVET), and used to identify genes expressed specifically in the legume rhizosphere (Barr et al. 2008).

Several groups have employed signature-tagged mutagenesis (STM) in the genome-scale identification of genes involved in symbiosis or competition for nodule occupancy. STM involves production insertion mutants using a library of transposons each containing a distinct tag. Mutants containing different transposons are then be pooled, passed through a selective regimen, and the relative abundance of each mutant in the output, relative to the input, can be measured using microarrays detecting the unique tags (Becker and Pobigaylo 2013). A STM library of *S. meliloti* was constructed (Pobigaylo et al. 2006), and insertions in nearly 9,600 strains were mapped to ~ 3,700 genes throughout the genome (Serrania et al. 2017). This STM library was employed in the identification of 38 new genes influencing symbiosis. (Pobigaylo et al. 2008). In further work, this library was used to show that ~ 2% of *S. meliloti* genes contribute to rhizosphere colonization (Salas et al. 2017). Screening of a *Mesorhizobium loti* STM library on host plants identified 13 Nod^- or Fix^- mutants as well as 33 mutants impaired in symbiotic competitiveness (Borjigin et al. 2011). Recently, a *Sinorhizobium fredii* STM library consisting of 25,500 mutants was reported, and an initial screen of 10% of these mutants identified four mutants that were impaired in symbiosis (Wang et al. 2016). STM has also been employed in *Rhizobium leguminosarum* bv. *viciae* (Garcia-Fraile et al. 2015), and a screen for genes relevant to rhizosphere colonization

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revealed the importance of arabinose and protocatechuate catabolism specifically during rhizosphere growth (Garcia-Fraile et al. 2015).

Although STM facilitates much higher throughput than classical analyses, the more recently developed transposon sequencing (Tn-seq), also known as insertion sequencing (INseq), allows for a further increase in throughput and quantitative assessment of gene functions. In this technique, a saturation insertion mutagenesis is performed to produce a pool of colonies each containing a single insertion, and next-generation sequencing is employed to identify the position of hundreds of thousands of insertions throughout the genome. In this way, mutations contributing to fitness in a given environment can be mapped across an entire genome with a resolution of just several nucleotides (Chao et al. 2016). Example data is shown in Figure 4, illustrating the sensitivity of this method in identifying essential or condition-specific essential genes. Multiple research groups have recently adapted Tn-seq for use in the rhizobia. A mariner transposon was used to examine the core genome of *R. leguminosarum* bv. *viciae* (Perry and Yost 2014, Perry et al. 2016), as well as to investigate the effects of carbon source and O_2 levels, a key signal during symbiosis, on the fitness of gene disruptions (Wheatley et al. 2017). Similarly, a Tn*5* derived transposon was used to interrogate the core genome of *S. meliloti*, and to inspect how chromosomal gene phenotypes differ when the extra-chromosomal replicons are removed (diCenzo et al. 2018). Additionally, a mariner transposon was used to investigate the genes contributing to sensitivity of *S. meliloti* to a NCR peptide, which plays a key role during rhizobial differentiation in the nodule (Arnold et al. 2017). Future use of this technique might prove valuable in the genome-scale elucidation of genes involved at various stages of the rhizobial life-style.

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LESSONS FROM COMPARATIVE GENOMICS

Genetic variations present in natural rhizobium strains can be powerfully leveraged to understand SNF through the comparison of genomic features, a process known as comparative genomics. This approach has become increasingly feasible due to the ever-growing number of rhizobial species and strains with whole-genome sequences available through public repositories. These genome sequences have facilitated many high-quality comparative genomic analyses of rhizobia that have contributed to our understanding of symbiosis and plant growth promotion.

Multiple comparative genomic studies have led to the perhaps surprising observation that there is significant variation between rhizobia in terms of their genomic adaptations to symbiosis (González et al. 2003, Masson-Boivin et al. 2009, Black et al. 2012). Indeed, while there are many genes that appear specific to symbiotic nitrogen fixing bacteria (Young et al. 2006, Pini et al. 2011), there appears to be no gene unique to all bacteria capable of performing SNF (Amadou et al. 2008). Even the common *nodABC* genes required for Nod factor synthesis are not 100% conserved, as they are absent in certain *Bradyrhizobium* species capable of forming nodules in a Nod factor-independent fashion (Giraud et al. 2007). Other examples of symbiotic genes showing variable presence/absence are accessory *nod* genes involved in determining host specificity, the *fixNOPQ* genes encoding a cytochrome cbb3 oxidase complex, as well as genes encoding polysaccharide biosynthesis and secretion systems (Black et al. 2012, Sugawara et al. 2013, De Meyer et al. 2016). In addition, the operon structure and genomic organization of symbiotic genes within rhizobial genomes differs among species (González et al. 2003, MacLean et al. 2007, Black et al. 2012, De Meyer et al. 2016). Overall, while many of the core symbiotic genes are highly conserved, the observed genomic variation reflects multiple mechanisms and adaptations for establishing an effective symbiosis, as discussed by (Masson-Boivin et al. 2009). Such diversity

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241 is bound to complicate our understanding of the minimal set of genes necessary for establishing a
242 symbiosis, and it suggests that strategies to optimize symbiotic interactions may be strain specific.
243 Despite the differences in the symbiotic gene clusters of distinct rhizobia, comparative
244 genomics can help identify new symbiotically relevant genes. A comparative analysis of 163
245 rhizobial genomes identified 184 protein families putatively involved in optimizing the symbiotic
246 process (Seshadri et al. 2015). In another study using a similar comparative genomic approach,
247 139 putative genes specific to symbiotic rhizobia were identified (Queiroux et al. 2012). Further
248 characterization of 13 of these genes in *S. meliloti* showed that eight were expressed more highly
249 by *S. meliloti* in the nodule than in free-living *S. meliloti* (Queiroux et al. 2012). Additionally,
250 mutation of the one gene chosen for follow-up study was shown to increase nodule occupancy
251 competitiveness (Queiroux et al. 2012). These results illustrate how comparative genomics
252 coupled with experimental validation is a powerful approach to identify new candidate genes for
253 improving rhizobial symbiotic efficiency.
254 Population genetic studies (i.e., the genetic variation present within a population) have
255 uncovered high genetic variability in rhizobia, which reflected in the large, open pangenomes of
256 many rhizobial species (González et al. 2010, Galardini et al. 2011, Tian et al. 2012, Galardini et
257 al. 2013, Sugawara et al. 2013, Porter et al. 2017). This is demonstrated in Figure 5, which displays
258 the distribution pattern of 17,494 genes found in at least one of the 20 included strains of *S. meliloti*.
259 A clearer understanding of the eco-evolutionary drivers of this genomic variation constitutes an
260 important research frontier in this field. The influence of soil properties, plant host, and other biotic
261 factors including microbial communities, bacteriophages, plasmids, and other invasive DNA
262 elements all surely play a role in the generation of rhizobial diversity (Bromfield et al. 1987,
263 Harrison et al. 1989, Carelli et al. 2000, Silva et al. 2007, Talebi et al. 2008, Toro et al. 2018).

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Understanding the relationships between these factors and the variable genome could potentially allow for the exploitation of the large genetic diversity of rhizobial species for developing elite rhizobial bioinoculants in precision agriculture (Checcucci et al. 2017).

LARGE-SCALE GENOME MANIPULATIONS

Several *Rhizobium*, *Sinorhizobium*, and *Mesorhizobium* derivatives have been produced that are 'cured' of one or more of their large extra-chromosomal replicons, revealing the influence of these replicons on numerous free-living phenotypes. These include the ability to catabolize a wide range of nutrient sources, growth and survival in soil environments (including the rhizosphere), biosynthesis of essential nutrients, production of polysaccharides, and motility (Barbour and Elkan 1989, Hynes et al. 1989, Hynes and McGregor 1990, Baldani et al. 1992, Brom et al. 1992, Moënne Loccoz 1995, Hu et al. 2007, García-de-los-Santos et al. 2008, Stasiak et al. 2014). Similarly, genome reduction studies have highlighted the importance of megaplasmids to the symbiotic process, revealing that key symbiotic genes can be located on megaplasmids, that optimal symbiotic performance often requires more than one megaplasmid, and that megaplasmids can carry loci required for effective competition for nodule occupancy (Barbour and Elkan 1989, Hynes and McGregor 1990, Baldani et al. 1992, Brom et al. 1992, Hu et al. 2007, Barreto et al. 2012, Stasiak et al. 2014). In contrast, some rhizobial plasmids impair symbiotic development. Loss of a large *Mesorhizobium* plasmid can improve both nitrogen fixation and nodulation competitiveness (Pankhurst et al. 1986, Hu et al. 2007), while several large *S. meliloti* accessory plasmids may result in legume-host incompatibility (Crook et al. 2012, Price et al. 2015).

A more targeted approach is the production of a library of large-scale deletion mutations that cumulatively remove all of the extra-chromosomal replicon(s). We are aware of this approach

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being employed with *S. meliloti* and *R. etli* (Landeta et al. 2011, Yurgel et al. 2013a, Milunovic et al. 2014). These libraries have been used to localize and identify novel symbiotic genes, as well as to screen for phenotypes such as carbon metabolic abilities, cytochrome c oxidase activity, soil growth, and osmotolerance (Dominguez-Ferreras et al. 2006, Landeta et al. 2011, Yurgel et al. 2013a, diCenzo et al. 2016a, Zamani et al. 2017).

In addition to genome reduction approaches, genome expansion studies have been used to evaluate the genetic basis of symbiosis. The large symbiotic plasmids of several *Rhizobium* spp. and *S. meliloti* have been transferred to related, non-symbiotic organisms such as *Agrobacterium* spp. and *Ensifer adhaerens* (Hooykaas et al. 1982, Wong et al. 1983, Truchet et al. 1984, Hirsch et al. 1984, Hynes et al. 1986, Finan et al. 1986, Martínez et al. 1987, Novikova and Safronova 1992, Abe et al. 1998, Rogel et al. 2001, Nakatsukasa et al. 2008). The primary conclusion of these studies is that poor, if any, symbiotic abilities are transferred to the recipient strain. This observation is perhaps surprising considering that natural lateral transfer of symbiotic plasmids and islands between related strains results in a gain of symbiotic abilities (Mozo et al. 1988, Laguerre 1992, Sullivan et al. 1995, Sullivan and Ronson 1998, Pérez Carrascal et al. 2016, Haskett et al. 2016). However, non-fixing outcomes after inter-species heterologous gene transfer may be due to an incomplete complement of symbiotic functions in the transconjugant strains, or due to incompatible alleles of a common gene (diCenzo et al. 2017c). To overcome these limitations, a *S. meliloti* strain lacking the pSymA and pSymB symbiotic replicons was constructed, which represents a 45% reduction of the genome (diCenzo et al. 2014). As expected, this strain is Nod$^-$ Fix$^-$, and re-introduction of all pSymA and pSymB genes fully restored symbiosis (diCenzo et al. 2016b). The chromosome-only strain, known to be permissive to SNF,

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is being used for identification of the necessary and sufficient set of symbiotic genes on these replicons.

In a particularly notable case, inter-species transfer of a symbiotic plasmid was coupled with laboratory evolution in an ongoing attempt to yield a symbiosis-competent strain. The researchers established *Ralstonia solanacearum* (a plant pathogen) carrying the symbiotic plasmid of the rhizobium species *Cupriavidus taiwanensis* as the test system (Marchetti et al. 2010, Guan et al. 2013, Marchetti et al. 2014, Remigi et al. 2014, Marchetti et al. 2017, Capela et al. 2017). Although initially unable to form nodules, adaptive mutations, such as in the type III secretion system, were identified that allowed for nodulation to occur (Marchetti et al. 2010). Sequential passage of these nodulating strains led to the recovery of derivatives with improved nodulation capabilities, although not yet nitrogen fixation (Marchetti et al. 2017). Nevertheless, this long-term study lends insight into the genomic changes required for the evolution of a new rhizobial species, such as a need to rewire transcriptional networks and to limit induction of the legume immunity response (Marchetti et al. 2014, Capela et al. 2017).

In contrast to the inter-species plasmid transfers described above, intra-species transfer of megaplasmids in *S. meliloti* has remarkably yielded strains displaying plant host cultivar specific improvements in symbiotic effectiveness (Checcucci et al. 2018), suggesting that plasmid transfer is a viable method for 'breeding' improved inoculants. Collectively, these studies underscore the importance of subtle genetic interactions between replicons in bringing about efficient symbiotic nitrogen fixation.

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TRANSCRIPTOMIC STUDIES OF RHIZOBIA

Transcriptomic studies, in the form of both microarrays and RNA deep-sequencing (RNA-seq) have been used to investigate the transcriptional responses to a broad range of genetic and environmental conditions, to elucidate the regulons of key transcriptional regulators, and to study RNA-based regulation (see Table 1 for a detailed list and description of rhizobium transcriptomic studies). Notable examples of studies of symbiotically relevant regulators include the FixL/FixJ/FixK system involved in activation of symbiotic genes in response to low oxygen (Bobik et al. 2006, Mesa et al. 2008), and NodD involved in induction of nodulation genes (Capela et al. 2005).

Transcriptomic studies have also probed early and late aspects of symbiotic development (Table 1). This has involved characterizing processes involved in rhizosphere colonization, such as growth in the presence of root exudates (Liu et al. 2017b, Klonowska et al. 2018), or during adaptation to various plant rhizosphere environments (Ramachandran et al. 2011). Several studies have examined the transcriptional consequences of flavonoids detection (Ampe et al. 2003, Barnett et al. 2004, Capela et al. 2005, Pérez-Montaño et al. 2016a, 2016b), a key initial step in the establishment of the symbiosis (reviewed by (Jiménez-Guerrero et al. 2017). In some species, flavonoid perception results in the induction of many genes (Pérez-Montaño et al. 2016b), and can influence a range of phenotypes including growth and biofilm formation (Spini et al. 2015). Differentiation within the nodule involves changes in the cell cycle, and studies have examined the transcriptional control of the cell cycle of *S. meliloti* and cell cycle dependent gene expression (De Nisco et al. 2014, Pini et al. 2015). Others have examined the transcriptional effects of plant produced NCR peptide application to free-living rhizobia, with the results revealing changes in cell cycle behaviour (Penterman et al. 2014) and changes associated with membrane depolarization

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(Tiricz et al. 2013). Moreover, the transcriptome of *S. meliloti* has been compared between cells growing in oxic and microoxic conditions (Ampe et al. 2003, Becker et al. 2004, Bobik et al. 2006, Pessi et al. 2007), as oxygen limitation is a major trigger for symbiotic gene expression. These studies have confirmed that FixJ is the major regulator of genes differentially expressed under these conditions (Bobik et al. 2006), and that not all genes induced by oxygen limitation in free-living bacteria are expressed in the nodule (Becker et al. 2004).

Transcriptomic experiments have been carried out to evaluate gene regulatory events occurring in root nodules (Table 1). Some of these studies have monitored gene expression of both symbiotic partners (for example, (Barnett et al. 2004, Heath et al. 2012, Roux et al. 2014, Mitsch et al. 2018), or have focused on comparing free-living versus symbiotic bacterial cells (Ampe et al. 2003, Becker et al. 2004, Barnett et al. 2004, Pessi et al. 2007, Karunakaran et al. 2009). A general consensus from these studies is that the bacteria undergo dramatic transcriptional changes in the symbiotic state, with possibly a thousand genes differentially expressed. The majority of differentially expressed genes are down-regulated in the nodule, consistent with a general metabolic repression during SNF. Transcriptomics has also been used to decipher the role of specific genes, regulators, or metabolic processes in the symbiotic process (Table 1), such as the genes *fixJ* (Barnett et al. 2004) and *bacA* (Karunakaran et al. 2010), and carbon import (Mitsch et al. 2018). Others have examined the transcriptome differences of bacteroids displaying distinct morphologies (Lamouche et al. 2018). Additionally, researchers have examined how genetic variations in the plant or rhizobium partner influence the nodule transcriptome (Heath et al. 2012, Burghardt et al. 2017), as well as how the bacteroid transcriptome of the same strain differs depending on the plant hosts (Ampe et al. 2003, Karunakaran et al. 2009, Li et al. 2013). These

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studies highlight how variation in symbiotic compatibility can result in significant transcriptional alterations.

Researchers have developed imaginative approaches to tease apart the various stages of symbiotic development by either isolating RNA from nodules of different ages (Ampe et al. 2003, Capela et al. 2006) or by using plant or bacterial mutants that are blocked at various stages of the developmental process (Ampe et al. 2003, Tian et al. 2006, Starker et al. 2006, Capela et al. 2006, Maunoury et al. 2010). Others have used laser-capture microdissection to separately capture each zone of the nodule to determine the spatial expression patterns within nodules (Roux et al. 2014). Such studies have clearly demonstrated a cascade of transcriptional events occurring throughout symbiotic development. This is demonstrated in Figure 6, which displays the unique gene expression patterns of ten genes across four developmental stages. These results highlight the complexity of the differentiation process and the need for detailed studies of each developmental stage in additional to whole nodule analyses.

PROTEOME LEVEL STUDIES OF RHIZOBIA

Numerous proteomic studies of rhizobia have been prepared, and a detailed list of these studies are provided in Table 1. Large-scale proteomic studies have been used for purposes such as improving genome annotation, identifying the phosphorylated proteome, characterizing the functions of specific proteins and non-coding RNAs, and evaluating the cellular response to varying environmental conditions (Table 1). Additionally, comparative proteomic studies have been used in the identification of putative symbiotic genes (Gomes et al. 2012b). Several processes of more direct relevance to SNF have also been studied using proteomics (Table 1), such as the proteomic response to flavonoids (Guerreiro et al. 1997, Chen et al. 2000b, Hempel et al. 2009, da

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Silva Batista and Hungria 2012, Tolin et al. 2013, Arrigoni et al. 2013, Gao et al. 2015, Meneses et al. 2017), and to micro-aerobic or anaerobic conditions (Dainese-Hatt et al. 1999). The secretome of rhizobia, with a particular focus on the proteins exported by type III secretion systems, has also been investigated (Saad et al. 2005, Rodrigues et al. 2007, Hempel et al. 2009, Okazaki et al. 2009).

Symbiotic nodule-associated rhizobia have also been the subject of proteomic studies (Table 1). Comparison of the proteomes of nitrogen-fixing rhizobia to that of their free-living counterparts have helped identify biochemical processes active during SNF (Djordjevic 2004, Sarma and Emerich 2005, 2006, Djordjevic et al. 2007, Delmotte et al. 2010, Resendis-Antonio et al. 2011, Tatsukami et al. 2013). Similarly, comparing the proteomes of bacteroids isolated from naturally effective and ineffective symbioses suggested pathways contributing to symbiotic efficiency (Cooper et al. 2018). The proteome of the peribacteroid space and peribacteroid membrane (Saalbach et al. 2002) as well as the bacteroid periplasm (Strodtman et al. 2017) have been determined, and the results suggest strong partitioning of proteins among these functionally distinct compartments (Strodtman et al. 2017). Furthermore, nodule proteomics has been used to examine the proteome during field growth conditions (Delmotte et al. 2010), differences between root and stem nodules (Delmotte et al. 2014), and NifA regulated proteins (Salazar et al. 2010). Studies have compared the proteomes of nodules of different maturities/ages (Delmotte et al. 2014, Nambu et al. 2015, Marx et al. 2016), and recently, proteomes of three zones from the same nodules were independently isolated and characterized (Ogden et al. 2017). These studies have revealed how the proteome changes during symbiotic development, and they complement and extend conclusions generated using transcriptomics. A more detailed review on the use of proteomics to characterize the symbiosis was recently published (Larrainzar and Wienkoop 2017).

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METABOLITE ANALYSES

Metabolomics, the large-scale study of the metabolites of the cell or its environment, has been used to characterize several rhizobial processes. Several studies have examined how the intra-cellular metabolome of free-living rhizobia differs depending on nutritional or stress conditions, or as a result of genetic manipulations (see Table 1 for a detailed list of relevant studies). Additionally, lipidomics has been used to characterize the membrane lipid composition and the effects of phosphate or acid stress, as well as to identify proteins involved in membrane lipid metabolism (Table 1). A couple of studies have also used a fluxomic approach (the study of the rates of reaction fluxes) to characterize central carbon metabolism during growth with either glucose (Fuhrer et al. 2005) or succinate (Terpolilli et al. 2016) as the sole source of carbon.

Metabolomics has been employed to characterize the metabolic shifts occurring during SNF (Table 1). Studies have compared the metabolomes of bacteroids to free-living rhizobia to identify biochemical processes that differ between these physiologically distinct cell populations (Resendis-Antonio et al. 2012, Vauclare et al. 2013), such as the role of lipogenesis in nitrogen fixing bacteroids (Terpolilli et al. 2016). Others have compared the metabolite profile of effective and non-effective nodules to identify metabolic processes specifically active during SNF (Ye et al. 2013, Gemperline et al. 2015). As with transcriptomics and proteomics, metabolomics of nodules of different age/maturity has been performed to examine the developmental progression (Lardi et al. 2016), and zone-specific metabolite analyses have been performed (Ogden et al. 2017). Metabolomics has further been employed to compare the metabolome of nodules from different plants containing the same rhizobium (Lardi et al. 2016). Furthermore, metabolomics has been used to characterize metabolism in root hairs during the early stages of symbiosis (Brechenmacher

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et al. 2010). Overall, such studies provide insights into the metabolic processes occurring during SNF, and how these processes differ depending on the partners involved.

IN SILICO METABOLIC MODELLING

Genome-scale metabolic network reconstruction refers to the process of building an *in silico* representation of the complete metabolism of the cell (Thiele and Palsson 2010). Reconstructions link metabolism to genetics by listing all reactions expected to be present in the cell together with the corresponding genes encoding the enzymes that catalyze the reaction; they can therefore serve as a valuable knowledge base of the metabolic properties of an organism. The reconstructions can then be used to simulate the metabolism of cells under defined environmental conditions, using mathematical approaches such as flux balance analysis (FBA) (Schellenberger et al. 2011). This approach can predict the metabolic fluxes through the reactions in a given nutritional condition, can simulate how environmental perturbations change the flux distributions, and can predict how deletion of one or more genes influences growth rate and flux distribution. As an example, Figures 7 and S1 display the predicted distribution of flux through the central carbon metabolic pathways of *S. meliloti* when grown with glucose or succinate as a carbon source.

Metabolic modelling is a relatively new method in the study of rhizobia. The first rhizobial reconstruction was reported in 2007 for *R. etli* (Resendis-Antonio et al. 2007) followed by several refinements (Resendis-Antonio et al. 2011, 2012). In 2012, the first reconstruction for *S. meliloti* was reported (Zhao et al. 2012). These reconstructions were relatively small and were designed solely for the purpose of representing bacteroid metabolism. Simulations of flux distribution and analyses of genes essentiality during SNF generally displayed good correlation with experimental data (Resendis-Antonio et al. 2007, Zhao et al. 2012). Recently, genome-scale metabolic network

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reconstructions capable of representing both free-living and symbiotic metabolism were reported for *S. meliloti* (diCenzo et al. 2016a) and *Bradyrhizobium diazoefficiens* (Yang et al. 2017c). Simulations with both of these models were consistent with transcriptomic data that indicate a major metabolic shift and general down-regulation, particularly in biosynthesis of cellular components, during symbiosis compared to free-living bacteria (diCenzo et al. 2016a, Yang et al. 2017c). A metabolic reconstruction for the legume *Medicago truncatula* has also been developed (Pfau et al. 2016).

We expect that metabolic reconstruction and constraint-based modelling will serve as a useful tool in the field of SNF, and it will provide an *in silico* method for examining the progression of symbiotic development, which can be experimentally difficult to dissect. Results of the simulations may then be used to guide experimental follow-up, and they may contribute to the identification of putative targets for biotechnological manipulation and experimental validation. The use of metabolic modelling will be facilitated by the wealth of systems-level data available for rhizobia, as summarized above. Several methods exist for the integration of transcriptomics, metabolomics, proteomics, and functional genomics (Tn-seq) data into modelling procedures (Fondi and Liò 2015, diCenzo et al. 2017a), and rhizobial models have been combined with each of these data sets (Resendis-Antonio et al. 2011, 2012, Yang et al. 2017c, diCenzo et al. 2018). This process results in the creation of high-quality, context-specific representations of cellular metabolism. However, current incomplete knowledge of the metabolic exchange between the plant and bacterium may still limit attempts to accurately represent SNF through a metabolic modelling approach.

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SYNTHETIC BIOLOGY APPROACHES TO ENGINEERING THE SYMBIOSIS

Developing improved rhizobium bio-inoculants has been the topic of a few recent reviews and book chapters (Lupwayi et al. 2006, Archana 2010, Dwivedi et al. 2015, Checcucci et al. 2017), and has been of interest to the scientific community for decades (Paau 1991). In order for a rhizobial strain to serve as an effective agricultural inoculant, it must be able to i) fix large amounts of nitrogen, and ii) effectively outcompete the indigenous rhizobial population for nodule occupancy. Unfortunately, natural rhizobial strains employed in agricultural settings are often effective in only one of these two abilities; and yet, despite the many genetic and systems-level studies that shed light on this symbiosis, genetically engineered rhizobial inoculants are not available. Studies such as those described in this review provide a solid background for targeted attempts at improving the rate of nitrogen fixation and exchange of nitrogen during the symbiosis. Indeed, there are several reports in the literature supporting the feasibility of doing so (Paau 1991, Ramírez et al. 1999, van Dillewijn et al. 2001, Orikasa et al. 2010).

However, a strain capable of high rates of nitrogen fixation is of little use if it is unable to effectively establish a population in the soil microbiome and outcompete native rhizobia for nodule occupancy. The native rhizobial community that is often the most competitive for nodule occupancy tends to exhibit low nitrogen fixation rates (see for example (Cardoso et al. 2017a, Chibeba et al. 2017). Indeed, for decades the failure of rhizobia to improve crop yield has often been attributed to the poor competitive abilities of the inoculant and not due to poor nitrogen fixation abilities (Triplett and Sadowsky 1992, Streeter 1994, Ndungu et al. 2018). Improving this aspect of the rhizobial life-cycle may be more difficult as it is likely that the traits required for effective competition will vary across the geographical landscape (Checcucci et al. 2017). A genomic-based approach comparing the genomes of nodule isolated rhizobia with the soil

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509 rhizobium population, coupled with Tn-seq studies of highly competitive strains in non-sterile
510 environments, may assist in the identification of genes contributing to high competitiveness for
511 nodule occupancy.

512 Many studies have highlighted strong host-strain compatibility properties in SNF (see
513 references below). Consequently, a "personalized" (i.e. cultivar-specific, environment-specific)
514 selection of elite inoculant rhizobia should be considered. However, additional studies are
515 necessary to identify the G (plant genotype) x E (environment) x M (root and soil microbiota) x R
516 (rhizobium) interactions, and to sort out the relevant rhizobium functions f this multi-component
517 interaction (Busby et al. 2017, Finkel et al. 2017, Pahua et al. 2018). For example, *M. truncatula*
518 cultivar-specific NCR peptides can block symbiosis in a bacterial strain-specific manner (Wang et
519 al. 2017, Yang et al. 2017b); many other examples of plant genes restricting symbiosis in a rhizobia
520 strain dependent fashion exist in the literature (for example, (Fan et al. 2017, Yamaya-Ito et al.
521 2018). Others have shown that relatively small differences between rhizobial genomes can result
522 in significant variations in symbiotic ability (Jozefkowicz et al. 2017). Additionally, studies have
523 highlighted rhizobial lineage-specific adaptations to symbiosis (Tian et al. 2012, Liu et al. 2017a),
524 and have identified rhizobial genes blocking symbiosis in a plant cultivar specific manner (Crook
525 et al. 2012, Price et al. 2015, Nguyen et al. 2017). Moreover, studies have demonstrated that both
526 the environment (Ji et al. 2017) and microbiome (Jozefkowicz et al. 2017) influence the symbiotic
527 properties of rhizobia.

528 A major factor limiting our ability to make the most of SNF in agriculture is the inability
529 of many stable crops, such as the cereals, of entering into a SNF relationship. As such, there is
530 significant interest in engineering synthetic symbioses between these plants and rhizobia, and there
531 have been a few recent reviews on this topic (Beatty and Good 2011, Rogers and Oldroyd 2014,

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Mus et al. 2016, Burén and Rubio 2018). As one can imagine, engineering new inter-kingdom interactions is daunting (Mus et al. 2016), and will require engineering a complex developmental pathway into the plant genome. Fortunately, legume-rhizobium symbioses makes use of the common symbiosis signalling pathway that is conserved in plants (including cereals) that participate in mycorrhizal interactions (Oldroyd 2013). In the short term, however, a simpler option would be to improve the beneficial interaction between free-living "associative" bacterial nitrogen fixing bacteria and these crop plants (Geddes et al. 2015), even if these looser symbiotic arrangements transfer nitrogen to the plant less efficiently (Beatty and Good 2011).

An alternative to achieving effective nitrogen fixation in plants is to bypass the bacterial component altogether, and directly engineer plants to perform their own nitrogen fixation. Several review and perspective articles discuss this idea and its associated challenges (Beatty and Good 2011, Oldroyd and Dixon 2014, Rogers and Oldroyd 2014, Curatti and Rubio 2014). The main objective is to engineer plants that express a functional nitrogenase inside the mitochondrion or plastid, as these organelles may have the necessary conditions to support this oxygen-sensitive enzyme. Several recent advances have been made in this area that moves the community closer to reaching this goal. Using *Saccharomyces cerevisiae* as a model eukaryotic organism, methods have been developed to express functional NifB protein (Burén et al. 2017a), NifDK tetramers (Burén et al. 2017b), and a functional version of the oxygen labile NifH protein in mitochondria (López-Torrejón et al. 2016). Other groups were able to individually express each of the *Klebsiella pneumoniae* Nif proteins within the mitochondria of a plant species (Allen et al. 2017), as well as to express active NifH in a plant plastid (Ivleva et al. 2016). Separately, others have managed to re-wire the transcriptional regulatory network of the *nif* genes (Wang et al. 2013), and identify a minimal nitrogenase gene cluster (Yang et al. 2014). Additionally, it has been shown that the

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555 functioning of nitrogenase in free-living bacterial diazotrophs can be supported by the electron-
556 transport chain proteins of plant organelles (Yang et al. 2017a). Each of these studies demonstrates
557 the feasibility of this ambitious research avenue.
558
559 **CONCLUSTIONS AND PERSPECTIVE**
560 Leading-edge techniques from an extensive range of disciplines have been applied to the
561 study of rhizobia, facilitating the development of a strong multi-disciplinary understanding of their
562 interactions with leguminous plant hosts (Table 2). Going forward, it will be valuable to
563 integratively employ these methods in orchestrated multidisciplinary studies. A major challenge
564 of the future will be the development of tools to study the spatio-temporal function of genes in the
565 nodule and during earlier stages of symbiotic development. While transcriptomic, proteomic, and
566 metabolic approaches can help provide insights into the unique developmental stages, genetic and
567 cell biology tools are required to validate conclusions reached from these systems-level analyses.
568 The inducible Cre/*lox* system by (Harrison et al. 2011) is a step in this direction, as are the *lux*
569 bioreporters for the *in vivo* analysis of the spatial and temporal presence of plant metabolites (Pini
570 et al. 2017). Similarly, the *S. meliloti* multiple gene-expression reporter strain for examining the
571 role of plant genes in nodule development helps address this problem (Lang et al. 2018). Moreover,
572 tools and experimental settings for the fast analysis of host preference in a GxExMxR interaction
573 framework are necessary, such as the recently described select and re-sequence approach
574 (Burghardt et al. 2018). The development of additional tools and their application to studying SNF
575 are a necessity to develop the required knowledge to be able to effectively manipulate this process.
576 Our understanding of the intricacies of SNF has rapidly advanced over the last few decades,
577 and the emergence of synthetic biology in recent years presents an unprecedented opportunity to

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engineer improved rhizobia and SNF relationships. However, despite the progress reviewed here, there is still a long road ahead to achieve the ambitious goal of completely replacing nitrogen fertilizer with BNF, and we should continue to balance discovery-oriented and applied research efforts.

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SUPPLEMENTARY FILE DESCRIPTIONS

File S1. Contains Figure S1, and the methods for generating the data underlying Figures 1, 5, 7, and S1.

Figure S1. *In silico* predicted flux distributions and reaction essentialities. Example data obtained through metabolic modelling. Results from *in silico* metabolic modelling of *S. meliloti* metabolism using the iGD726 metabolic reconstruction are shown (diCenzo et al. 2018). Growth was simulated using (**A**) glucose or (**B**) succinate as the sole carbon source. The pathways represent central carbon metabolism, with the exception of the pentose phosphate pathway (for simplicity). The width of the line represents the amount of flux through the reaction; a doubling in the width corresponds to a ten-fold flux increase. Arrows indicate the direction of flux. Colours indicate if the reaction is essential (red) or non-essential (blue). Genes associated with reactions are based on the information present in iGD726, but

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they are not necessarily comprehensive. See the Supplementary Materials for details on the modelling procedures used. A version without gene names is provided as Figure 8 in manuscript.

File S2. This file contains: i) an enlarged version of the phylogeny in Figure 1 that contains all taxa names, ii) a Newick formatted file of the phylogeny, iii) the corresponding annotation file, and iv) a README file to describe the contents of the archive.

REFERENCES

Abe, M., Kawamura, R., Higashi, S., Mori, S., Shibata, M., and Uchiumi, T. 1998. Transfer of the symbiotic plasmid from *Rhizobium leguminosarum* biovar *trifolii* to *Agrobacterium tumefaciens*. J. Gen. Appl. Microbiol. **44**(1): 65–74. doi:10.2323/jgam.44.65.

Alexandre, A., Laranjo, M., and Oliveira, S. 2014. Global transcriptional response to heat shock of the legume symbiont *Mesorhizobium loti* MAFF303099 comprises extensive gene downregulation. DNA Res. **21**(2): 195–206. doi:10.1093/dnares/dst050.

Allen, R.S., Tilbrook, K., Warden, A.C., Campbell, P.C., Rolland, V., Singh, S.P., and Wood, C.C. 2017. Expression of 16 nitrogenase proteins within the plant mitochondrial matrix. Front. Plant. Sci **8**: 287. doi:10.3389/fpls.2017.00287.

Alunni, B., and Gourion, B. 2016. Terminal bacteroid differentiation in the legume-rhizobium symbiosis: nodule-specific cysteine-rich peptides and beyond. New Phytol. **211**(2): 411–417. doi:10.1111/nph.14025.

Amadou, C., Pascal, G., Mangenot, S., Glew, M., Bontemps, C., Capela, D., Carrère, S., Cruveiller, S., Dossat, C., Lajus, A., Marchetti, M., Poinsot, V., Rouy, Z., Servin, B., Saad, M., Schenowitz, C., Barbe, V., Batut, J., Médigue, C., and Masson-Boivin, C. 2008. Genome

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

sequence of the β-rhizobium *Cupriavidus taiwanensis* and comparative genomics of rhizobia. Genome Res. **18**(9): 1472–1483. doi:10.1101/gr.076448.108.

Ampe, F., Kiss, E., Sabourdy, F., and Batut, J. 2003. Transcriptome analysis of *Sinorhizobium meliloti* during symbiosis. Genome Biol. **4**(2): R15. doi:10.1186/gb-2003-4-2-r15.

Archana, G. 2010. Engineering Nodulation Competitiveness of Rhizobial Bioinoculants in Soils. *In* Microbes for Legume Improvement. Springer Vienna, Vienna. pp. 157–194. doi:10.1007/978-3-211-99753-6_8.

Arnold, M.F.F., Shabab, M., Penterman, J., Boehme, K.L., Griffitts, J.S., and Walker, G.C. 2017. Genome-wide sensitivity analysis of the microsymbiont *Sinorhizobium meliloti* to symbiotically important, defensin-like host peptides. MBio **8**(4): e01060–17. doi:10.1128/mBio.01060-17.

Arrigoni, G., Tolin, S., Moscatiello, R., Masi, A., Navazio, L., and Squartini, A. 2013. Calcium-dependent regulation of genes for plant nodulation in *Rhizobium leguminosarum* detected by iTRAQ quantitative proteomic analysis. J. Proteome Res. **12**(11): 5323–5330. doi:10.1021/pr400656g.

Baldani, J.I., Weaver, R.W., Hynes, M.F., and Eardly, B.D. 1992. Utilization of carbon substrates, electrophoretic enzyme patterns, and symbiotic performance of plasmid-cured clover rhizobia. Appl. Environ. Microbiol. **58**(7): 2308–2314.

Barbour, W.M., and Elkan, G.H. 1989. Relationship of the presence and copy number of plasmids to exopolysaccharide production and symbiotic effectiveness in *Rhizobium fredii* USDA 206. Appl. Environ. Microbiol. **55**(4): 813–818.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

645 Barnett, M.J., and Long, S.R. 2015. The *Sinorhizobium meliloti* SyrM regulon: effects on global
646 gene expression are mediated by *syrA* and *nodD3*. J. Bacteriol. **197**(10): 1792–1806.
647 doi:10.1128/JB.02626-14.
648 Barnett, M.J., Toman, C.J., Fisher, R.F., and Long, S.R. 2004. A dual-genome Symbiosis Chip for
649 coordinate study of signal exchange and development in a prokaryote–host interaction. Proc.
650 Natl. Acad. Sci. U.S.A. **101**(47): 16636–16641. doi:10.1073/pnas.0407269101.
651 Barr, M., East, A.K., Leonard, M., Mauchline, T.H., and Poole, P.S. 2008. *In vivo* expression
652 technology (IVET) selection of genes of *Rhizobium leguminosarum* biovar *viciae* A34
653 expressed in the rhizosphere. FEMS Microbiol. Lett. **282**(2): 219–227. doi:10.1111/j.1574-
654 6968.2008.01131.x.
655 Barra-Bily, L., Fontenelle, C., Jan, G., Flechard, M., Trautwetter, A., Pandey, S.P., Walker, G.C.,
656 and Blanco, C. 2010. Proteomic alterations explain phenotypic changes in *Sinorhizobium*
657 *meliloti* lacking the RNA chaperone Hfq. J. Bacteriol. **192**(6): 1719–1729.
658 doi:10.1128/JB.01429-09.
659 Barreto, E.F., Straliotto, R., and Baldani, J.I. 2012. Curing of a non-symbiotic plasmid of the
660 *Rhizobium tropici* strain CIAT 899 affected nodule occupancy and competitiveness of the
661 bacteria in symbiosis with common beans. Eur. J. Soil Biol. **50**(C): 91–96.
662 doi:10.1016/j.ejsobi.2011.12.003.
663 Barsch, A., Patschkowski, T., and Niehaus, K. 2004. Comprehensive metabolite profiling of
664 *Sinorhizobium meliloti* using gas chromatography-mass spectrometry. Funct. Integr. Genomic
665 **4**(4): 219–230. doi:10.1007/s10142-004-0117-y.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

666 Basconcillo, L.S., Zaheer, R., Finan, T.M., and McCarry, B.E. 2009a. Cyclopropane fatty acyl
667 synthase in *Sinorhizobium meliloti*. Microbiology **155**(Pt 2): 373–385.
668 doi:10.1099/mic.0.022608-0.
669 Basconcillo, L.S., Zaheer, R., Finan, T.M., and McCarry, B.E. 2009b. A shotgun lipidomics
670 approach in *Sinorhizobium meliloti* as a tool in functional genomics. J. Lipid Res. **50**(6): 1120–
671 1132. doi:10.1194/jlr.M800443-JLR200.
672 Basconcillo, L.S., Zaheer, R., Finan, T.M., and McCarry, B.E. 2009c. A shotgun lipidomics study
673 of a putative lysophosphatidic acid acyl transferase (PlsC) in *Sinorhizobium meliloti*. J.
674 Chromatogr. B. **877**(26): 2873–2882. doi:10.1016/j.jchromb.2009.05.014.
675 Beatty, P.H., and Good, A.G. 2011. Future prospects for cereals that fix nitrogen. Science
676 **333**(6041): 416–417. doi:10.1126/science.1209467.
677 Becker, A., and Pobigaylo, N. 2013. Construction of signature-tagged mutant libraries and its
678 application to plant-symbiotic bacteria. *In* Molecular Microbial Ecology of the Rhizosphere.
679 *Edited by* F.J. de Bruijn. John Wiley & Sons, Inc., Hoboken, NJ, USA. pp. 921–928.
680 doi:10.1002/9781118297674.ch86.
681 Becker, A., Bergès, H., Krol, E., Bruand, C., Rüberg, S., Capela, D., Lauber, E., Meilhoc, E.,
682 Ampe, F., de Bruijn, F.J., Fourment, J., Francez-Charlot, A., Kahn, D., Küster, H., Liebe, C.,
683 Pühler, A., Weidner, S., and Batut, J. 2004. Global changes in gene expression in
684 *Sinorhizobium meliloti* 1021 under microoxic and symbiotic conditions. Mol. Plant Microbe
685 Interact. **17**(3): 292–303. doi:10.1094/MPMI.2004.17.3.292.
686 Berrabah, F., Balliau, T., Aït-Salem, E.H., George, J., Zivy, M., Ratet, P., Gourion, B. 2018.
687 Control of the ethylene signaling pathway prevents plant defenses during intracellular
688 accommodation of the rhizobia. New Phytol. **219**(1): 310-323. doi:10.1111/nph.15142.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

689 Bhat, S.V., Booth, S.C., McGrath, S.G.K., and Dahms, T.E.S. 2014. *Rhizobium leguminosarum*
690 bv. *viciae* 3841 adapts to 2,4-dichlorophenoxyacetic acid with "auxin-like" morphological
691 changes, cell envelope remodeling and upregulation of central metabolic pathways. PLOS One
692 **10**(4): e0123813. doi:10.1371/journal.pone.0123813.
693 Black, M., Moolhuijzen, P., Chapman, B., Barrero, R., Howieson, J., Hungria, M., and Bellgard,
694 M. 2012. The genetics of symbiotic nitrogen fixation: comparative genomics of 14 rhizobia
695 strains by resolution of protein clusters. Genes **3**(1): 138–166. doi:10.3390/genes3010138.
696 Bloem, J.F., Trytsman, G., and Smith, H.J. 2009. Biological nitrogen fixation in resource-poor
697 agriculture in South Africa. Symbiosis **48**(1-3): 18–24. doi:10.1007/BF03179981.
698 Bobik, C., Meilhoc, E., and Batut, J. 2006. FixJ: a major regulator of the oxygen limitation
699 response and late symbiotic functions of *Sinorhizobium meliloti*. J. Bacteriol. **188**(13): 4890–
700 4902. doi:10.1128/JB.00251-06.
701 Borjigin, N., Furukawa, K., Shimoda, Y., Tabata, S., Sato, S., Eda, S., Minamisawa, K., and
702 Mitsui, H. 2011. Identification of *Mesorhizobium loti* genes relevant to symbiosis by using
703 signature-tagged mutants. Microbes Environ. **26**(2): 165–171. doi:10.1264/jsme2.ME10213.
704 Bourion, V., Heulin-Gotty, K., Aubert, V., Tisseyre, P., Chabert-Martinello, M., Pervent, M.,
705 Delaitre, C., Vile, D., Siol, M., Duc, G., Brunel, B., Burstin, J., and Lepetit, M. 2017. Co-
706 inoculation of a pea core-collection with diverse rhizobial strains shows competitiveness for
707 nodulation and efficiency of nitrogen fixation are distinct traits in the interaction. Front. Plant
708 Sci **8**: 2249. doi:10.3389/fpls.2017.02249.
709 Brechenmacher, L., Lei, Z., Libault, M., Findley, S., Sugawara, M., Sadowsky, M.J., Sumner,
710 L.W., and Stacey, G. 2010. Soybean metabolites regulated in root hairs in response to the

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

symbiotic bacterium *Bradyrhizobium japonicum*. Plant Physiol. **153**(4): 1808–1822. doi:10.1104/pp.110.157800.

Brom, S., García-de los Santos, A., Stepkowsky, T., Flores, M., Davila, G., Romero, D., and Palacios, R. 1992. Different plasmids of *Rhizobium leguminosarum* bv. *phaseoli* are required for optimal symbiotic performance. J. Bacteriol. **174**(16): 5183–5189. doi:10.1128/jb.174.16.5183-5189.1992.

Bromfield, E.S.P., Thurman, N.P., Whitwill, S.T., and Barran, L.R. 1987. Plasmids and symbiotic effectiveness of representative phage types from two indigenous populations of *Rhizobium meliloti*. Microbiology **133**(12): 3457–3466. doi:10.1099/00221287-133-12-3457.

Burén, S., Jiang, X., López-Torrejón, G., Echavarri-Erasun, C., and Rubio, L.M. 2017a. Purification and *in vitro* activity of mitochondria targeted nitrogenase cofactor maturase NifB. Front. Plant Sci. **8**: 1567. doi:10.3389/fpls.2017.01567.

Burén, S., Young, E.M., Sweeny, E.A., López-Torrejón, G., Veldhuizen, M., Voigt, C.A., and Rubio, L.M. 2017b. Formation of nitrogenase NifDK tetramers in the mitochondria of *Saccharomyces cerevisiae*. ACS Synth. Biol. **6**(6): 1043–1055. doi:10.1021/acssynbio.6b00371.

Burghardt, L.T., Epstein, B., Guhlin, J., Nelson, M.S., Taylor, M.R., Young, N.D., Sadowsky, M.J., and Tiffin, P. 2018. Select and resequence reveals relative fitness of bacteria in symbiotic and free-living environments. Proc. Natl. Acad. Sci. U.S.A. **115**(10): 2425–2430. doi:10.1073/pnas.1714246115.

Burghardt, L.T., Guhlin, J., Chun, C.L., Liu, J., Sadowsky, M.J., Stupar, R.M., Young, N.D., and Tiffin, P. 2017. Transcriptomic basis of genome by genome variation in a legume-rhizobia mutualism. Mol. Ecol. **25**(2): 4946. doi:10.1111/mec.14285.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

734 Busby, P.E., Soman, C., Wagner, M.R., Friesen, M.L., Kremer, J., Bennett, A., Morsy, M., Eisen,
735 J.A., Leach, J.E., and Dangl, J.L. 2017. Research priorities for harnessing plant microbiomes
736 in sustainable agriculture. PLOS Biol. **15**(3): e2001793. doi:10.1371/journal.pbio.2001793.
737 Calatrava-Morales, N., Nogales, J., Ameztoy, K., van Steenbergen, B., and Soto, M.J. 2017. The
738 NtrY/NtrX system of *Sinorhizobium meliloti* GR4 regulates motility, EPS I production, and
739 nitrogen metabolism but Is dispensable for symbiotic nitrogen fixation. Mol. Plant Microbe
740 Interact **30**(7): 566–577. Mol. Plant Microbe Interact. doi:10.1094/MPMI-01-17-0021-R.
741 Cantero, L., Palacios, J.M., Ruiz-Argüeso, T., and Imperial, J. 2006. Proteomic analysis of quorum
742 sensing in *Rhizobium leguminosarum* biovar *viciae* UPM791. Proteomics **6**(S1): S97–106.
743 doi:10.1002/pmic.200500312.
744 Capela, D., Carrère, S., and Batut, J. 2005. Transcriptome-based identification of the
745 *Sinorhizobium meliloti* NodD1 regulon. Appl. Environ. Microbiol. **71**(8): 4910–4913.
746 doi:10.1128/AEM.71.8.4910-4913.2005.
747 Capela, D., Filipe, C., Bobik, C., Batut, J., and Bruand, C. 2006. *Sinorhizobium meliloti*
748 differentiation during symbiosis with alfalfa: a transcriptomic dissection. Mol. Plant Microbe
749 Interact. **19**(4): 363–372. doi:10.1094/MPMI-19-0363.
750 Capela, D., Marchetti, M., Clerissi, C., Perrier, A., Guetta, D., Gris, C., Valls, M., Jauneau, A.,
751 Cruveiller, S., Rocha, E.P.C., and Masson-Boivin, C. 2017. Recruitment of a lineage-specific
752 virulence regulatory pathway promotes intracellular infection by a plant pathogen
753 experimentally evolved into a legume symbiont. Mol. Biol. Evol. **34**(10): 2503–2521.
754 doi:10.1093/molbev/msx165.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

Cardoso, A.A., Andraus, M. de P., Borba, T.C. de O., Martin-Didonet, C.C.G., and Ferreira, E.P. de B. 2017a. Characterization of rhizobia isolates obtained from nodules of wild genotypes of common bean. Braz. J. Microbiol. **48**(1): 43–50. doi:10.1016/j.bjm.2016.09.002.

Cardoso, P., Santos, M., Freitas, R., Rocha, S.M., and Figueira, E. 2017b. Response of *Rhizobium* to Cd exposure: A volatile perspective. Environ. Pollut. **231**(Pt 1): 802–811. doi:10.1016/j.envpol.2017.08.067.

Carelli, M., Gnocchi, S., Fancelli, S., Mengoni, A., Paffetti, D., Scotti, C., and Bazzicalupo, M. 2000. Genetic diversity and dynamics of *Sinorhizobium meliloti* populations nodulating different alfalfa cultivars in Italian soils. Appl. Environ. Microbiol. **66**(11): 4785–4789. doi:10.1128/AEM.66.11.4785-4789.2000.

Carlyon, R.E., Ryther, J.L., VanYperen, R.D., and Griffitts, J.S. 2010. FeuN, a novel modulator of two-component signalling identified in *Sinorhizobium meliloti*. Mol. Microbiol. **77**(1): 170–182. doi:10.1111/j.1365-2958.2010.07198.x.

Chao, M.C., Abel, S., Davis, B.M., and Waldor, M.K. 2016. The design and analysis of transposon insertion sequencing experiments. Nat. Rev. Microbiol. **14**(2): 119–128. doi:10.1038/nrmicro.2015.7.

Chao, T.-C., Buhrmester, J., Hansmeier, N., Pühler, A., and Weidner, S. 2005. Role of the regulatory gene *rirA* in the transcriptional response of *Sinorhizobium meliloti* to iron limitation. Appl. Environ. Microbiol. **71**(10): 5969–5982. doi:10.1128/AEM.71.10.5969-5982.2005.

Charpentier, M., and Oldroyd, G. 2010. How close are we to nitrogen-fixing cereals? Curr. Opin. Plant Biol. **13**(5): 556–564. doi:10.1016/j.pbi.2010.08.003.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

777 Checcucci, A., Azzarello, E., Bazzicalupo, M., Galardini, M., Lagomarsino, A., Mancuso, S.,
778 Marti, L., Marzano, M.C., Mocali, S., Squartini, A., Zanardo, M., and Mengoni, A. 2016.
779 Mixed nodule infection in *Sinorhizobium meliloti-Medicago sativa* symbiosis suggest the
780 presence of cheating behavior. Front. Plant Sci. **7**: 835. doi:10.3389/fpls.2016.00835.
781 Checcucci, A., diCenzo, G.C., Bazzicalupo, M., and Mengoni, A. 2017. Trade, diplomacy, and
782 warfare: the quest for elite rhizobia inoculant strains. Front. Microbiol. **8**: 2207.
783 doi:10.3389/fmicb.2017.02207.
784 Checcucci, A., diCenzo, G.C., Ghini, V., Bazzicalupo, M., Beker, A., Decorosi, F., Döhlemann,
785 J., Fagorzi, C., Finan, T.M., Fondi, M., Luchinat, C., Turano, P., Vignolini, T., Viti, C., and
786 Mengoni, A. 2018. Creation and multi-omics characterization of a genomically hybrid strain
787 in the nitrogen-fixing symbiotic bacterium *Sinorhizobium meliloti*. bioRxiv **296483**.
788 doi:10.1101/296483.
789 Chen, H., Gao, K., Kondorosi, E., Kondorosi, A., and Rolfe, B.G. 2005. Functional genomic
790 analysis of global regulator NolR in *Sinorhizobium meliloti*. Mol. Plant Microbe Interact.
791 **18**(12): 1340–1352. doi:10.1094/MPMI-18-1340.
792 Chen, H., Higgins, J., Kondorosi, E., Kondorosi, A., Djordjevic, M.A., Weinman, J.J., and Rolfe,
793 B.G. 2000a. Identification of *nolR*-regulated proteins in *Sinorhizobium meliloti* using
794 proteome analysis. Electrophoresis **21**(17): 3823–3832. doi:10.1002/1522-
795 2683(200011)21:17<3823::AID-ELPS3823>3.0.CO;2-K.
796 Chen, H., Higgins, J., Oresnik, I.J., Hynes, M.F., Natera, S., Djordjevic, M.A., Weinman, J.J., and
797 Rolfe, B.G. 2000b. Proteome analysis demonstrates complex replicon and luteolin interactions
798 in pSyma-cured derivatives of *Sinorhizobium meliloti* strain 2011. Electrophoresis **21**(17):
799 3833–3842. doi:10.1002/1522-2683(200011)21:17<3833::AID-ELPS3833>3.0.CO;2-I.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

800 Chen, H., Teplitski, M., Robinson, J.B., Rolfe, B.G., and Bauer, W.D. 2003. Proteomic analysis
801 of wild-type *Sinorhizobium meliloti* responses to N-acyl homoserine lactone quorum-sensing
802 signals and the transition to stationary phase. J. Bacteriol. **185**(17): 5029–5036.
803 doi:10.1128/JB.185.17.5029-5036.2003.
804 Chibeba, A.M., Kyei-Boahen, S., Guimarães, M. de F., Nogueira, M.A., and Hungria, M. 2017.
805 Isolation, characterization and selection of indigenous *Bradyrhizobium* strains with
806 outstanding symbiotic performance to increase soybean yields in Mozambique. Agric.
807 Ecosyst. Environ. **246**: 291–305. doi:10.1016/j.agee.2017.06.017.
808 Cooper, B., Campbell, K.B., Beard, H.S., Garrett, W.M., Mowery, J., Bauchan, G.R., and Elia, P.
809 2018. A proteomic network for symbiotic nitrogen fixation efficiency in *Bradyrhizobium*
810 *elkanii*. Mol. Plant Microbe Interact. **31**(3): 334–343. doi:10.1094/MPMI-10-17-0243-R.
811 Cowie, A., Cheng, J., Sibley, C.D., Fong, Y., Zaheer, R., Patten, C.L., Morton, R.M., Golding,
812 G.B., and Finan, T.M. 2006. An integrated approach to functional genomics: construction of
813 a novel reporter gene fusion library for *Sinorhizobium meliloti*. Appl. Environ. Microbiol.
814 **72**(11): 7156–7167. doi:10.1128/AEM.01397-06.
815 Crook, M.B., Lindsay, D.P., Biggs, M.B., Bentley, J.S., Price, J.C., Clement, S.C., Clement, M.J.,
816 Long, S.R., and Griffitts, J.S. 2012. Rhizobial plasmids that cause impaired symbiotic nitrogen
817 fixation and enhanced host invasion. Mol. Plant Microbe Interact. **25**(8): 1026–1033.
818 doi:10.1094/MPMI-02-12-0052-R.
819 Curatti, L., and Rubio, L.M. 2014. Challenges to develop nitrogen-fixing cereals by direct *nif*-gene
820 transfer. Plant Sci. **225**: 130–137. doi:10.1016/j.plantsci.2014.06.003.
821 Cytryn, E.J., Sangurdekar, D.P., Streeter, J.G., Franck, W.L., Chang, W.-S., Stacey, G., Emerich,
822 D.W., Joshi, T., Xu, D., and Sadowsky, M.J. 2007. Transcriptional and physiological

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

responses of *Bradyrhizobium japonicum* to desiccation-induced stress. J. Bacteriol. **189**(19): 6751–6762. doi:10.1128/JB.00533-07.

D'Alessio, M., Nordeste, R., Doxey, A.C., and Charles, T.C. 2017. Transcriptome analysis of polyhydroxybutyrate cycle mutants reveals discrete loci connecting nitrogen utilization and carbon storage in *Sinorhizobium meliloti*. mSystems **2**(5): e00035–17. doi:10.1128/mSystems.00035-17.

da Silva Batista, J.S., Torres, A.R., and Hungria, M. 2010. Towards a two-dimensional proteomic reference map of *Bradyrhizobium japonicum* CPAC 15: spotlighting "hypothetical proteins". Proteomics **10**(17): 3176–3189. doi:10.1002/pmic.201000092.

da Silva Batista, J.S., and Hungria, M. 2012. Proteomics reveals differential expression of proteins related to a variety of metabolic pathways by genistein-induced *Bradyrhizobium japonicum* strains. J. Proteom. **75**(4): 1211–1219. doi:10.1016/j.jprot.2011.10.032.

Dainese-Hatt, P., Fischer, H.M., Hennecke, H., and James, P. 1999. Classifying symbiotic proteins from *Bradyrhizobium japonicum* into functional groups by proteome analysis of altered gene expression levels. Electrophoresis **20**(18): 3514–3520. doi:10.1002/(SICI)1522-2683(19991201)20:18<3514::AID-ELPS3514>3.0.CO;2-T.

Daubech, B., Remigi, P., Doin de Moura, G., Marchetti, M., Pouzet, C., Auriac, M.-C., Gokhale, C.S., Masson-Boivin, C., and Capela, D. 2017. Spatio-temporal control of mutualism in legumes helps spread symbiotic nitrogen fixation. Elife **6**: 57. doi:10.7554/eLife.28683.

De Meyer, S.E., Briscoe, L., Martínez-Hidalgo, P., Agapakis, C.M., de-Los Santos, P.E., Seshadri, R., Reeve, W., Weinstock, G., OHara, G., Howieson, J.G., and Hirsch, A.M. 2016. Symbiotic *Burkholderia* species show diverse arrangements of *nif/fix* and *nod* genes and lack typical

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

high-affinity cytochrome *cbb3* oxidase genes. Mol. Plant Microbe Interact. **29**(8): 609–619. doi:10.1094/MPMI-05-16-0091-R.

De Nisco, N.J., Abo, R.P., Wu, C.M., Penterman, J., and Walker, G.C. 2014. Global analysis of cell cycle gene expression of the legume symbiont *Sinorhizobium meliloti.* Proc. Natl. Acad. Sci. U.S.A. **111**(9): 3217–3224. doi:10.1073/pnas.1400421111.

de Rudder, K.E., Lopéz-Lara, I.M., and Geiger, O. 2000. Inactivation of the gene for phospholipid N-methyltransferase in *Sinorhizobium meliloti*: phosphatidylcholine is required for normal growth. Mol. Microbiol. **37**(4): 763–772. doi:10.1046/j.1365-2958.2000.02032.x.

de Rudder, K.E.E., Sohlenkamp, C., and Geiger, O. 1999. Plant-exuded choline is used for rhizobial membrane lipid biosynthesis by phosphatidylcholine synthase. J. Biol. Chem. **274**(28): 20011–20016. doi:10.1074/jbc.274.28.20011.

Delmotte, N., Ahrens, C.H., Knief, C., Qeli, E., Koch, M., Fischer, H.-M., Vorholt, J.A., Hennecke, H., and Pessi, G. 2010. An integrated proteomics and transcriptomics reference data set provides new insights into the *Bradyrhizobium japonicum* bacteroid metabolism in soybean root nodules. Proteomics **10**(7): 1391–1400. doi:10.1002/pmic.200900710.

Delmotte, N., Mondy, S., Alunni, B., Fardoux, J., Chaintreuil, C., Vorholt, J., Giraud, E., and Gourion, B. 2014. A proteomic approach of *Bradyrhizobium*/*Aeschynomene* root and stem symbioses reveals the importance of the *fixA* locus for symbiosis. Int. J. Mol. Sci. **15**(3): 3660–3670. doi:10.3390/ijms15033660.

diCenzo, G.C., Benedict, A.B., Fondi, M., Walker, G.C., Finan, T.M., Mengoni, A., and Griffitts, J.S. 2018. Robustness encoded across essential and accessory replicons in an ecologically versatile bacterium. PLOS Genet. **14**(4): e1007357. doi:10.1101/209916.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

867 diCenzo, G.C., Checcucci, A., Bazzicalupo, M., Mengoni, A., Viti, C., Dziewit, L., Finan, T.M.,
868 Galardini, M., and Fondi, M. 2016a. Metabolic modelling reveals the specialization of
869 secondary replicons for niche adaptation in *Sinorhizobium meliloti*. Nat. Commun. **7**: 12219.
870 doi:10.1038/ncomms12219.
871 diCenzo, G.C., MacLean, A.M., Milunovic, B., Golding, G.B., and Finan, T.M. 2014. Examination
872 of prokaryotic multipartite genome evolution through experimental genome reduction. PLOS
873 Genet. **10**(10): e1004742. doi:10.1371/journal.pgen.1004742.
874 diCenzo, G.C., Mengoni, A., and Fondi, M. 2017a. Tn-Core: context-specific reconstruction of
875 core metabolic models using Tn-seq data. bioRxiv. **221325**: doi:10.1101/221325.
876 diCenzo, G.C., Muhammed, Z., Østerås, M., O'Brien, S.A.P., and Finan, T.M. 2017b. A key
877 regulator of the glycolytic and gluconeogenic central metabolic pathways in *Sinorhizobium*
878 *meliloti*. Genetics **207**(3): 961–974. doi:10.1534/genetics.117.300212.
879 diCenzo, G.C., Zamani, M., Ludwig, H.N., and Finan, T.M. 2017c. Heterologous
880 complementation reveals a specialized activity for BacA in the *Medicago-Sinorhizobium*
881 *meliloti* symbiosis. Mol. Plant Microbe Interact. **30**(4): 312–324. doi:10.1094/MPMI-02-17-
882 0030-R.
883 diCenzo, G.C., Zamani, M., Milunovic, B., and Finan, T.M. 2016b. Genomic resources for
884 identification of the minimal N_2-fixing symbiotic genome. Environ. Microbiol. **18**(8): 2534–
885 2547. doi:10.1111/1462-2920.13221.
886 Djordjevic, M.A. 2004. *Sinorhizobium meliloti* metabolism in the root nodule: A proteomic
887 perspective. Proteomics **4**(7): 1859–1872. doi:10.1002/pmic.200300802.
888 Djordjevic, M.A., Chen, H.C., Natera, S., Van Noorden, G., Menzel, C., Taylor, S., Renard, C.,
889 Geiger, O., and Weiller, G.F. 2007. A global analysis of protein expression profiles in

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

Sinorhizobium meliloti: discovery of new genes for nodule occupancy and stress adaptation. Mol. Plant Microbe Interact. **16**(6): 508–524. . doi:10.1094/MPMI.2003.16.6.508.

Dominguez-Ferreras, A., Perez-Arnedo, R., Becker, A., Olivares, J., Soto, M.J., and Sanjuán, J. 2006. Transcriptome profiling reveals the importance of plasmid pSymB for osmoadaptation of *Sinorhizobium meliloti*. J. Bacteriol. **188**(21): 7617–7625. doi:10.1128/JB.00719-06.

Donati, A.J., Jeon, J.-M., Sangurdekar, D., So, J.-S., and Chang, W.-S. 2011. Genome-wide transcriptional and physiological responses of *Bradyrhizobium japonicum* to paraquat-mediated oxidative stress. Appl. Environ. Microbiol. **77**(11): 3633–3643. doi:10.1128/AEM.00047-11.

Dong, R., Zhang, J., Huan, H., Bai, C., Chen, Z., and Liu, G. 2017. High salt tolerance of a *Bradyrhizobium* strain and its promotion of the growth of *Stylosanthes guianensis*. Int. J. Mol. Sci. **18**(8): 1625. doi:10.3390/ijms18081625.

Downie, J.A. 2010. The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. FEMS Microbiol. Rev. **34**(2): 150–170. doi:10.1111/j.1574-6976.2009.00205.x.

Draghi, W.O., Del Papa, M.F., Hellweg, C., Watt, S.A., Watt, T.F., Barsch, A., Lozano, M.J., Salas, M.E., López, J.L., Albicoro, F.J., Nilsson, J.F., Torres Tejerizo, G.A., Luna, M.F., Pistorio, M., Boiardi, J.L., Puhler, A., Weidner, S., Niehaus, K., and Lagares, A. 2016. A consolidated analysis of the physiologic and molecular responses induced under acid stress in the legume-symbiont model-soil bacterium *Sinorhizobium meliloti*. Sci. Rep. **6**(1): 29278. doi:10.1038/srep29278.

Dwivedi, S.L., Sahrawat, K.L., Upadhyaya, H.D., Mengoni, A., Galardini, M., Bazzicalupo, M., Biondi, E.G., Hungria, M., Kaschuk, G., Blair, M.W., and Ortiz, R. 2015. Advances in host

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

913 plant and rhizobium genomics to enhance symbiotic nitrogen fixation in grain legumes. Adv.
914 Agron. 129:1–116. doi:10.1016/bs.agron.2014.09.001.
915 Encarnación, S., del Carmen Vargas, M., Dunn, M.F., Dávalos, A., Mendoza, G., Mora, Y., and
916 Mora, J. 2002. AniA regulates reserve polymer accumulation and global protein expression in
917 *Rhizobium etli*. J. Bacteriol. **184**(8): 2287–2295. doi:10.1128/JB.184.8.2287-2295.2002.
918 Encarnación, S., Guzmán, Y., Dunn, M.F., Hernández, M., del Carmen Vargas, M., and Mora, J.
919 2003. Proteome analysis of aerobic and fermentative metabolism in *Rhizobium etli* CE3.
920 Proteomics **3**(6): 1077–1085. doi:10.1002/pmic.200300427.
921 Erisman, J.W., Sutton, M.A., Galloway, J., Klimont, Z., and Winiwarter, W. 2008. How a century
922 of ammonia synthesis changed the world. Nat. Geosci. **1**(10): 636–639. doi:10.1038/ngeo325.
923 Evans, J., Barnet, Y.M., and Vincent, J.M. 1979. Effect of a bacteriophage on colonisation and
924 nodulation of clover roots by paired strains of *Rhizobium trifolii*. Can. J. Microbiol. **25**(9):
925 974–978.
926 Fan, Y., Liu, J., Lyu, S., Wang, Q., Yang, S., and Zhu, H. 2017. The soybean *Rfg1* gene restricts
927 nodulation by *Sinorhizobium fredii* USDA193. Front. Plant Sci. **8**: 1548.
928 doi:10.3389/fpls.2017.01548.
929 Fei, F., diCenzo, G.C., Bowdish, D.M.E., McCarry, B.E., and Finan, T.M. 2016. Effects of
930 synthetic large-scale genome reduction on metabolism and metabolic preferences in a
931 nutritionally complex environment. Metabolomics **12**(2): 23. doi:10.1007/s11306-015-0928-
932 y.
933 Finan, T.M., Kunkel, B., De Vos, G.F., and Signer, E.R. 1986. Second symbiotic megaplasmid in
934 *Rhizobium meliloti* carrying exopolysaccharide and thiamine synthesis genes. J. Bacteriol.
935 **167**(1): 66–72. doi:10.1128/jb.167.1.66-72.1986.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

936 Finkel, O.M., Castrillo, G., Herrera Paredes, S., Salas González, I., and Dangl, J.L. 2017.
937 Understanding and exploiting plant beneficial microbes. Curr. Opin. Plant Biol. **38**: 155–163.
938 doi:10.1016/j.pbi.2017.04.018.
939 Fondi, M., and Liò, P. 2015. Multi -omics and metabolic modelling pipelines: challenges and tools
940 for systems microbiology. Microbiol Res **171**: 52–64. doi:10.1016/j.micres.2015.01.003.
941 Frederix, M., Edwards, A., Swiderska, A., Stanger, A., Karunakaran, R., Williams, A., Abbruscato,
942 P., Sanchez-Contreras, M., Poole, P.S., and Downie, J.A. 2014. Mutation of *praR* in
943 *Rhizobium leguminosarum* enhances root biofilms, improving nodulation competitiveness by
944 increased expression of attachment proteins. Mol. Microbiol. **93**(3): 464–478.
945 doi:10.1111/mmi.12670.
946 Fuhrer, T., Fischer, E., and Sauer, U. 2005. Experimental identification and quantification of
947 glucose metabolism in seven bacterial species. J. Bacteriol. **187**(5): 1581–1590.
948 doi:10.1128/JB.187.5.1581-1590.2005.
949 Gage, D.J. 2004. Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during
950 nodulation of temperate legumes. Microbiol. Mol. Biol. Rev. **68**(2): 280–300.
951 doi:10.1128/MMBR.68.2.280-300.2004.
952 Galardini, M., Mengoni, A., Brilli, M., Pini, F., Fioravanti, A., Lucas, S., Lapidus, A., Cheng, J.-
953 F., Goodwin, L., Pitluck, S., Land, M., Hauser, L., Woyke, T., Mikhailova, N., Ivanova, N.,
954 Daligault, H., Bruce, D., Detter, C., Tapia, R., Han, C., Teshima, H., Mocali, S., Bazzicalupo,
955 M., and Biondi, E.G. 2011. Exploring the symbiotic pangenome of the nitrogen-fixing
956 bacterium *Sinorhizobium meliloti*. BMC Genomics **12**(1): 235. doi:10.1186/1471-2164-12-
957 235.

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958 Galardini, M., Pini, F., Bazzicalupo, M., Biondi, E.G., and Mengoni, A. 2013. Replicon-dependent
959 bacterial genome evolution: the case of *Sinorhizobium meliloti*. Genome Biol. Evol. **5**(3): 542–
960 558. doi:10.1093/gbe/evt027.
961 Gao, J.-L., Weissenmayer, B., Taylor, A.M., Thomas-Oates, J., López-Lara, I.M., and Geiger, O.
962 2004. Identification of a gene required for the formation of lyso-ornithine lipid, an
963 intermediate in the biosynthesis of ornithine-containing lipids. Mol. Microbiol. **53**(6): 1757–
964 1770. doi:10.1111/j.1365-2958.2004.04240.x.
965 Gao, M., Barnett, M.J., Long, S.R., and Teplitski, M. 2010. Role of the *Sinorhizobium meliloti*
966 global regulator Hfq in gene regulation and symbiosis. Mol. Plant Microbe Interact. **23**(4):
967 355–365. doi:10.1094/MPMI-23-4-0355.
968 Gao, M., Chen, H., Eberhard, A., Gronquist, M.R., Robinson, J.B., Connolly, M., Teplitski, M.,
969 Rolfe, B.G., and Bauer, W.D. 2007. Effects of AiiA-mediated quorum quenching in
970 *Sinorhizobium meliloti* on quorum-sensing signals, proteome patterns, and symbiotic
971 interactions. Mol. Plant Microbe Interact. **20**(7): 843–856. doi:10.1094/MPMI-20-7-0843.
972 Gao, M., Chen, H., Eberhard, A., Gronquist, M.R., Robinson, J.B., Rolfe, B.G., and Bauer, W.D.
973 2005. *sinI*- and *expR*-dependent quorum sensing in *Sinorhizobium meliloti*. J. Bacteriol.
974 **187**(23): 7931–7944. doi:10.1128/JB.187.23.7931-7944.2005.
975 Gao, T.G., Xu, Y.Y., Jiang, F., Li, B.Z., Yang, J.S., Wang, E.T., and Yuan, H.L. 2015. Nodulation
976 characterization and proteomic profiling of *Bradyrhizobium liaoningense* CCBAU05525 in
977 response to water-soluble humic materials. Sci. Rep. **5**(1): 10836. doi:10.1038/srep10836.
978 Garcia-Fraile, P., Seaman, J.C., Karunakaran, R., Edwards, A., Poole, P.S., and Downie, J.A. 2015.
979 Arabinose and protocatechuate catabolism genes are important for growth of *Rhizobium*

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

leguminosarum biovar *viciae* in the pea rhizosphere. Plant Soil **390**(1-2): 251–264. doi:10.1007/s11104-015-2389-5.

García-de-los-Santos, A., López, E., Cubillas, C.A., Noel, K.D., Brom, S., and Romero, D. 2008. Requirement of a plasmid-encoded catalase for survival of *Rhizobium etli* CFN42 in a polyphenol-rich environment. Appl. Environ. Microbiol. **74**(8): 2398–2403. doi:10.1128/AEM.02457-07.

Geddes, B.A., Ryu, M.-H., Mus, F., Garcia Costas, A., Peters, J.W., Voigt, C.A., and Poole, P. 2015. Use of plant colonizing bacteria as chassis for transfer of N_2-fixation to cereals. Curr. Opin. Biotech. **32**: 216–222. doi:10.1016/j.copbio.2015.01.004.

Gemperline, E., Jayaraman, D., Maeda, J., Ané, J.-M., and Li, L. 2015. Multifaceted investigation of metabolites during nitrogen fixation in *Medicago* via high resolution MALDI-MS imaging and ESI-MS. J. Am. Soc. Mass Spectrom. **26**(1): 149–158. doi:10.1007/s13361-014-1010-0.

Ghobakhlou, A., Laberge, S., Antoun, H., Wishart, D.S., Xia, J., Krishnamurthy, R., and Mandal, R. 2013. Metabolomic analysis of cold acclimation of Arctic *Mesorhizobium* sp. strain N33. PLOS One **8**(12): e84801. doi:10.1371/journal.pone.0084801.

Ghobakhlou, A.-F., Johnston, A., Harris, L., Antoun, H., and Laberge, S. 2015. Microarray transcriptional profiling of Arctic *Mesorhizobium* strain N33 at low temperature provides insights into cold adaption strategies. BMC Genomics **16**(1): 383. doi:10.1186/s12864-015-1611-4.

Gibson, K.E., Barnett, M.J., Toman, C.J., Long, S.R., and Walker, G.C. 2007. The symbiosis regulator CbrA modulates a complex regulatory network affecting the flagellar apparatus and cell envelope proteins. J. Bacteriol. **189**(9): 3591–3602. doi:10.1128/JB.01834-06.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1002 Gibson, K.E., Kobayashi, H., and Walker, G.C. 2008. Molecular determinants of a symbiotic
1003 chronic infection. Annu. Rev. Genet. **42**(1): 413–441.
1004 doi:10.1146/annurev.genet.42.110807.091427.
1005 Giraud, E., Moulin, L., Vallenet, D., Barbe, V., Cytryn, E., Avarre, J.-C., Jaubert, M., Simon, D.,
1006 Cartieaux, F., Prin, Y., Bena, G., Hannibal, L., Fardoux, J., Kojadinovic, M., Vuillet, L., Lajus,
1007 A., Cruveiller, S., Rouy, Z., Mangenot, S., Segurens, B., Dossat, C., Franck, W.L., Chang, W.-
1008 S., Saunders, E., Bruce, D., Richardson, P., Normand, P., Dreyfus, B., Pignol, D., Stacey, G.,
1009 Emerich, D., Verméglio, A., Médigue, C., and Sadowsky, M. 2007. Legumes symbioses:
1010 absence of *nod* genes in photosynthetic bradyrhizobia. Science **316**(5829): 1307–1312.
1011 doi:10.1126/science.1139548.
1012 Gomes, D.F., da Silva Batista, J.S., Schiavon, A.L., Andrade, D.S., and Hungria, M. 2012a.
1013 Proteomic profiling of *Rhizobium tropici* PRF 81: identification of conserved and specific
1014 responses to heat stress. BMC Microbiol. **12**(1): 84. doi:10.1186/1471-2180-12-84.
1015 Gomes, D.F., da Silva Batista, J.S., Torres, A.R., de Souza Andrade, D., Galli-Terasawa, L.V., and
1016 Hungria, M. 2012b. Two-dimensional proteome reference map of *Rhizobium tropici* PRF 81
1017 reveals several symbiotic determinants and strong resemblance with agrobacteria. Proteomics
1018 **12**(6): 859–863. doi:10.1002/pmic.201100406.
1019 Gomes, D.F., da Silva Batista, J.S., Rolla, A.A.P., da Silva, L.P., Bloch, C., Galli-Terasawa, L.V.,
1020 and Hungria, M. 2014. Proteomic analysis of free-living *Bradyrhizobium diazoefficiens*:
1021 highlighting potential determinants of a successful symbiosis. BMC Genomics **15**(1): 643.
1022 doi:10.1186/1471-2164-15-643.
1023 González, V., Acosta, J.L., Santamaría, R.I., Bustos, P., Fernández, J.L., Hernández González,
1024 I.L., Díaz, R., Flores, M., Palacios, R., Mora, J., and Dávila, G. 2010. Conserved symbiotic

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

plasmid DNA sequences in the multireplicon pangenomic structure of *Rhizobium etli*. Appl. Environ. Microbiol. **76**(5): 1604–1614. doi:10.1128/AEM.02039-09.

González, V., Bustos, P., Ramírez-Romero, M.A., Medrano-Soto, A., Salgado, H., Hernández-González, I., Hernández-Celis, J.C., Quintero, V., Moreno-Hagelsieb, G., Girard, L., Rodríguez, O., Flores, M., Cevallos, M.A., Collado-Vides, J., Romero, D., and Dávila, G. 2003. The mosaic structure of the symbiotic plasmid of *Rhizobium etli* CFN42 and its relation to other symbiotic genome compartments. Genome Biol. **4**(6): R36. doi:10.1186/gb-2003-4-6-r36.

Gourion, B., Sulser, S., Frunzke, J., Francez-Charlot, A., Stiefel, P., Pessi, G., Vorholt, J.A., Fischer, H.M. 2009. The PhyR-sigma(EcfG) signalling cascade is involved in stress response and symbiotic efficiency in *Bradyrhizobium japonicum*. Mol. Microbiol. **73**(2): 291-305. doi:10.1111/j.1365-2958.2009.06769.x.

Griffitts, J.S., and Long, S.R. 2008. A symbiotic mutant of *Sinorhizobium meliloti* reveals a novel genetic pathway involving succinoglycan biosynthetic functions. Mol. Microbiol. **67**(6): 1292–1306. doi:10.1111/j.1365-2958.2008.06123.x.

Griffitts, J.S., Carlyon, R.E., Erickson, J.H., Moulton, J.L., Barnett, M.J., Toman, C.J., and Long, S.R. 2008. A *Sinorhizobium meliloti* osmosensory two-component system required for cyclic glucan export and symbiosis. Mol. Microbiol. **69**(2): 479–490. doi:10.1111/j.1365-2958.2008.06304.x.

Guan, S.H., Gris, C., Cruveiller, S., Pouzet, C., Tasse, L., Leru, A., Maillard, A., Médigue, C., Batut, J., Masson-Boivin, C., and Capela, D. 2013. Experimental evolution of nodule intracellular infection in legume symbionts. ISME J. **7**(7): 1367–1377. doi:10.1038/ismej.2013.24.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1048 Guerreiro, N., Djordjevic, M.A., and Rolfe, B.G. 1999. Proteome analysis of the model
1049 microsymbiont *Sinorhizobium meliloti*: isolation and characterisation of novel proteins.
1050 Electrophoresis **20**(4-5): 818–825. doi:10.1002/(SICI)1522-
1051 2683(19990101)20:4/5<818::AID-ELPS818>3.0.CO;2-6.
1052 Guerreiro, N., Redmond, J.W., Rolfe, B.G., and Djordjevic, M.A. 1997. New *Rhizobium*
1053 *leguminosarum* flavonoid-induced proteins revealed by proteome analysis of differentially
1054 displayed proteins. Mol. Plant Microbe Interact. **10**(4): 506–516.
1055 doi:10.1094/MPMI.1997.10.4.506.
1056 Guerreiro, N., Stepkowski, T., Rolfe, B.G., and Djordjevic, M.A. 1998. Determination of plasmid-
1057 encoded functions in *Rhizobium leguminosarum* biovar *trifolii* using proteome analysis of
1058 plasmid-cured derivatives. Electrophoresis **19**(11): 1972–1979.
1059 doi:10.1002/elps.1150191115.
1060 Haag, A.F., Arnold, M.F.F., Myka, K.K., Kerscher, B., Dall'Angelo, S., Zanda, M., Mergaert, P.,
1061 and Ferguson, G.P. 2013. Molecular insights into bacteroid development during *Rhizobium*-
1062 legume symbiosis. FEMS Microbiol. Rev. **37**(3): 364–383. doi:10.1111/1574-6976.12003.
1063 Hagen, M.J., and Hamrick, J.L. 1996. Population level processes in *Rhizobium leguminosarum* bv.
1064 *trifolii*: the role of founder effects. Mol. Ecol. **5**(6): 707–714. doi:10.1111/j.1365-
1065 294X.1996.tb00367.x.
1066 Harrison, C.L., Crook, M.B., Peco, G., Long, S.R., and Griffitts, J.S. 2011. Employing site-specific
1067 recombination for conditional genetic analysis in *Sinorhizobium meliloti*. Appl. Environ.
1068 Microbiol. **77**(12): 3916–3922. doi:10.1128/AEM.00544-11.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1069 Harrison, S.P., Jones, D.G., and Young, J.P.W. 1989. *Rhizobium* population genetics: genetic
1070 variation within and between populations from diverse locations. Microbiology **135**(5): 1061–
1071 1069. doi:10.1099/00221287-135-5-1061.
1072 Hashem, F.M., and Angle, J.S. 1990. Rhizobiophage effects on nodulation, nitrogen fixation, and
1073 yield of field-grown soybeans (*Glycine max* L. Merr.). Biol. Fert. Soils **9**(4): 330–334.
1074 doi:10.1007/BF00634110.
1075 Haskett, T.L., Terpolilli, J.J., Bekuma, A., O'Hara, G.W., Sullivan, J.T., Wang, P., Ronson, C.W.,
1076 and Ramsay, J.P. 2016. Assembly and transfer of tripartite integrative and conjugative genetic
1077 elements. Proc. Natl. Acad. Sci. U.S.A. **113**(43): 12268–12273.
1078 doi:10.1073/pnas.1613358113.
1079 Haskett, T.L., Terpolilli, J.J., Ramachandran, V.K., Verdonk, C.J., Poole, P.S., O'Hara, G.W., and
1080 Ramsay, J.P. 2018. Sequential induction of three recombination directionality factors directs
1081 assembly of tripartite integrative and conjugative elements. PLOS Genet. **14**(3): e1007292.
1082 doi:10.1371/journal.pgen.1007292.
1083 Hawkins, J.P., Geddes, B.A., and Oresnik, I.J. 2017. Succinoglycan production contributes to
1084 acidic pH tolerance in *Sinorhizobium meliloti* Rm1021. Mol. Plant Microbe Interact. **30**(12):
1085 1009–1019. doi:10.1094/MPMI-07-17-0176-R.
1086 Hawkins, J.P., Ordonez, P.A., and Oresnik, I.J. 2018. Characterization of mutants that affect the
1087 non-oxidative pentose phosphate pathway in *Sinorhizobium meliloti*. J. Bacteriol. **200**(2):
1088 e00436–17. doi:10.1128/JB.00436-17.
1089 Heath, K.D., and Tiffin, P. 2009. Stabilizing mechanisms in a legume-rhizobium mutualism.
1090 Evolution **63**(3): 652–662. doi:10.1111/j.1558-5646.2008.00582.x.

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1091 Heath, K.D., Burke, P.V., and Stinchcombe, J.R. 2012. Coevolutionary genetic variation in the
1092 legume-rhizobium transcriptome. Mol. Ecol. **21**(19): 4735–4747. doi:10.1111/j.1365-
1093 294X.2012.05629.x.
1094 Heinz, E.B., and Streit, W.R. 2003. Biotin limitation in *Sinorhizobium meliloti* strain 1021 alters
1095 transcription and translation. Appl. Environ. Microbiol. **69**(2): 1206–1213.
1096 doi:10.1128/AEM.69.2.1206-1213.2003.
1097 Hellweg, C., Pühler, A., and Weidner, S. 2009. The time course of the transcriptomic response of
1098 *Sinorhizobium meliloti* 1021 following a shift to acidic pH. BMC Microbiol. **9**(1): 37.
1099 doi:10.1186/1471-2180-9-37.
1100 Hempel, J., Zehner, S., Göttfert, M., and Patschkowski, T. 2009. Analysis of the secretome of the
1101 soybean symbiont *Bradyrhizobium japonicum*. J. Biotechnol. **140**(1-2): 51–58.
1102 doi:10.1016/j.jbiotec.2008.11.002.
1103 Hinsinger, P., Bengough, A.G., Vetterlein, D., and Young, I.M. 2009. Rhizosphere: biophysics,
1104 biogeochemistry and ecological relevance. Plant Soil **321**(1-2): 117–152. doi:10.1007/s11104-
1105 008-9885-9.
1106 Hirsch, A.M., Wilson, K.J., Jones, J.D., Bang, M., Walker, V.V., and Ausubel, F.M. 1984.
1107 *Rhizobium meliloti* nodulation genes allow *Agrobacterium tumefaciens* and *Escherichia coli*
1108 to form pseudonodules on alfalfa. J. Bacteriol. **158**(3): 1133–1143.
1109 Hirsch, P.R. 1979. Plasmid-determined bacteriocin production by *Rhizobium leguminosarum*.
1110 Microbiology **113**(2): 219–228. doi:10.1099/00221287-113-2-219.
1111 Hooykaas, P.J., Snijdewint, F.G., and Schilperoort, R.A. 1982. Identification of the Sym plasmid
1112 of *Rhizobium leguminosarum* strain 1001 and its transfer to and expression in other rhizobia
1113 and *Agrobacterium tumefaciens*. Plasmid **8**(1): 73–82. doi:10.1016/0147-619X(82)90042-7.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1114 Hu, G., Li, Y., and Zhou, J. 2007. Biological characteristics of plasmids of *Mesorhizobium huakuii*
1115 HN3015 from *Astragalus sinicus*. World J. Microbiol. Biotechnol. **23**(6): 845–851.
1116 doi:10.1007/s11274-006-9308-0.
1117 Hynes, M.F., and McGregor, N.F. 1990. Two plasmids other than the nodulation plasmid are
1118 necessary for formation of nitrogen-fixing nodules by *Rhizobium leguminosarum*. Mol.
1119 Microbiol. **4**(4): 567–574. doi:10.1111/j.1365-2958.1990.tb00625.x.
1120 Hynes, M.F., Quandt, J., O'Connell, M.P., and Puhler, A. 1989. Direct selection for curing and
1121 deletion of *Rhizobium* plasmids using transposons carrying the *Bacillus subtilis sacB* gene.
1122 Gene **78**(1): 111–120. doi:10.1016/0378-1119(89)90319-3.
1123 Hynes, M.F., Simon, R., Müller, P., Niehaus, K., Labes, M., and Pühler, A. 1986. The two
1124 megaplasmids of *Rhizobium meliloti* are involved in the effective nodulation of alfalfa. Mol.
1125 Gen. Genet. **202**(3): 356–362. doi:10.1007/BF00333262.
1126 Irar, S., González, E.M., Arrese-Igor, C., and Marino, D. 2014. A proteomic approach reveals new
1127 actors of nodule response to drought in split-root grown pea plants. Physiol. Plant. **152**(4):
1128 634–645. doi:10.1111/ppl.12214.
1129 Ivleva, N.B., Groat, J., Staub, J.M., and Stephens, M. 2016. Expression of active subunit of
1130 nitrogenase via integration into plant organelle genome. PLOS One **11**(8): e0160951.
1131 doi:10.1371/journal.pone.0160951.
1132 Jebbar, M., Sohn-Bosser, L., Bremer, E., Bernard, T., and Blanco, C. 2005. Ectoine-induced
1133 proteins in *Sinorhizobium meliloti* include an ectoine ABC-type transporter involved in
1134 osmoprotection and ectoine catabolism. J. Bacteriol. **187**(4): 1293–1304.
1135 doi:10.1128/JB.187.4.1293-1304.2005.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1136 Jeon, J.-M., Lee, H.-I., Donati, A.J., So, J.-S., Emerich, D.W., and Chang, W.-S. 2011. Whole-
1137 genome expression profiling of *Bradyrhizobium japonicum* in response to hydrogen peroxide.
1138 Mol. Plant Microbe Interact. **24**(12): 1472–1481. doi:10.1094/MPMI-03-11-0072.
1139 Ji, Z.J., Yan, H., Cui, Q.G., Wang, E.T., Chen, W.F., and Chen, W.X. 2017. Competition between
1140 rhizobia under different environmental conditions affects the nodulation of a legume. Syst.
1141 Appl. Microbiol. **40**(2): 114–119. doi:10.1016/j.syapm.2016.12.003.
1142 Jiménez-Guerrero, I., Acosta Jurado, S., Del Cerro, P., Navarro-Gómez, P., López-Baena, F.J.,
1143 Ollero, F.J., Vinardell, J.M., and Pérez-Montaño, F. 2017. Transcriptomic studies of the effect
1144 of *nod* gene-inducing molecules in rhizobia: different weapons, one purpose. Genes **9**(1): 1.
1145 doi:10.3390/genes9010001.
1146 Jones, K.M., Kobayashi, H., Davies, B.W., Taga, M.E., and Walker, G.C. 2007. How rhizobial
1147 symbionts invade plants: the *Sinorhizobium–Medicago* model. Nat. Rev. Microbiol. **5**(8):
1148 619–633. doi:10.1038/nrmicro1705.
1149 Jozefkowicz, C., Brambilla, S., Frare, R., Stritzler, M., Puente, M., Piccinetti, C., Soto, G., and
1150 Ayub, N. 2017. Microevolution rather than large genome divergence determines the
1151 effectiveness of legume-rhizobia symbiotic interaction under field conditions. J. Mol. Evol.
1152 **85**(3-4): 79–83. doi:10.1007/s00239-017-9808-6.
1153 Kajiwara, H., Kaneko, T., Ishizaka, M., Tajima, S., and Kouchi, H. 2003. Protein profile of
1154 symbiotic bacteria *Mesorhizobium loti* MAFF303099 in mid-growth phase. Biosci.
1155 Biotechnol. Biochem. **67**(12): 2668–2673. doi:10.1271/bbb.67.2668.
1156 Karunakaran, R., Haag, A.F., East, A.K., Ramachandran, V.K., Prell, J., James, E.K., Scocchi, M.,
1157 Ferguson, G.P., and Poole, P.S. 2010. BacA is essential for bacteroid development in nodules

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1158 of galegoid, but not phaseoloid, legumes. J. Bacteriol. **192**(11): 2920–2928.
1159 doi:10.1128/JB.00020-10.

1160 Karunakaran, R., Ramachandran, V.K., Seaman, J.C., East, A.K., Mouhsine, B., Mauchline, T.H.,
1161 Prell, J., Skeffington, A., and Poole, P.S. 2009. Transcriptomic analysis of *Rhizobium*
1162 *leguminosarum* biovar *viciae* in symbiosis with host plants *Pisum sativum* and *Vicia cracca*.
1163 J. Bacteriol. **191**(12): 4002–4014. doi:10.1128/JB.00165-09.

1164 Keum, Y.S., Seo, J.S., Li, Q.X., and Kim, J.H. 2008. Comparative metabolomic analysis of
1165 *Sinorhizobium* sp. C4 during the degradation of phenanthrene. Appl. Microbiol. Biotechnol.
1166 **80**(5): 863–872. doi:10.1007/s00253-008-1581-4.

1167 Kiers, E.T., and Denison, R.F. 2008. Sanctions, cooperation, and the stability of plant-rhizosphere
1168 mutualisms. Annu. Rev. Ecol. Evol. Syst. **39**(1): 215–236.
1169 doi:10.1146/annurev.ecolsys.39.110707.173423.

1170 Kiers, E.T., Rousseau, R.A., West, S.A., and Denison, R.F. 2003. Host sanctions and the legume-
1171 rhizobium mutualism. Nature **425**(6953): 78–81. doi:10.1038/nature01931.

1172 Klonowska, A., Melkonian, R., Miché, L., Tisseyre, P., and Moulin, L. 2018. Transcriptomic
1173 profiling of *Burkholderia phymatum* STM815, *Cupriavidus taiwanensis* LMG19424 and
1174 *Rhizobium mesoamericanum* STM3625 in response to *Mimosa pudica* root exudates
1175 illuminates the molecular basis of their nodulation competitiveness and symbiotic
1176 evolutionary history. BMC Genomics **19**(1): 105. doi:10.1186/s12864-018-4487-2.

1177 Kondorosi, E., Mergaert, P., and Kereszt, A. 2013. A paradigm for endosymbiotic life: cell
1178 differentiation of *Rhizobium* bacteria provoked by host plant factors. Annu. Rev. Microbiol.
1179 **67**(1): 611–628. doi:10.1146/annurev-micro-092412-155630.

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1180 Krol, E., and Becker, A. 2004. Global transcriptional analysis of the phosphate starvation response
1181 in *Sinorhizobium meliloti* strains 1021 and 2011. Mol. Genet. Genomics **272**(1).
1182 doi:10.1007/s00438-004-1030-8.
1183 Krol, E., and Becker, A. 2011. ppGpp in *Sinorhizobium meliloti*: biosynthesis in response to
1184 sudden nutritional downshifts and modulation of the transcriptome. Mol. Microbiol. **81**(5):
1185 1233–1254. doi:10.1111/j.1365-2958.2011.07752.x.
1186 Krol, E., Klaner, C., Gnau, P., Kaever, V., Essen, L.-O., and Becker, A. 2016. Cyclic
1187 mononucleotide- and Clr-dependent gene regulation in *Sinorhizobium meliloti*. Microbiology
1188 **162**(10): 1840–1856. doi:10.1099/mic.0.000356.
1189 Krysciak, D., Grote, J., Rodriguez Orbegoso, M., Utpatel, C., Förstner, K.U., Li, L., Schmeisser,
1190 C., Krishnan, H.B., and Streit, W.R. 2014. RNA sequencing analysis of the broad-host-range
1191 strain *Sinorhizobium fredii* NGR234 identifies a large set of genes linked to quorum sensing-
1192 dependent regulation in the background of a *traI* and *ngrI* deletion mutant. Appl. Environ.
1193 Microbiol. **80**(18): 5655–5671. doi:10.1128/AEM.01835-14.
1194 Kumar, D., Yadav, A.K., Kadimi, P.K., Nagaraj, S.H., Grimmond, S.M., and Dash, D. 2013.
1195 Proteogenomic analysis of *Bradyrhizobium japonicum* USDA110 using GenoSuite, an
1196 automated multi-algorithmic pipeline. Mol. Cell Proteomics **12**(11): 3388–3397.
1197 doi:10.1074/mcp.M112.027169.
1198 Lagares, A., Borella, G.C., Linne, U., Becker, A., and Valverde, C. 2017. Regulation of
1199 polyhydroxybutyrate accumulation in *Sinorhizobium meliloti* by the trans-encoded small RNA
1200 MmgR. J. Bacteriol. **199**(8): e00776–16. doi:10.1128/JB.00776-16.
1201 Laguerre, G. 1992. Plasmid profiles and restriction fragment length polymorphism of *Rhizobium*
1202 *leguminosarum* bv. *viciae* in field populations. FEMS Microbio. Lett. **101**(1): 17–26.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1203 Lamouche, F., Gully, D., Chaumeret, A., Nouwen, N., Verly, C., Pierre, O., Sciallano, C., Fardoux,
1204 J., Jeudy, C., Szűcs, A., Mondy, S., Salon, C., Nagy, I., Kereszt, A., Dessaux, Y., Giraud, E.,
1205 Mergaert, P., and Alunni, B. 2018. Transcriptomic dissection of *Bradyrhizobium* sp. strain
1206 ORS285 in symbiosis with *Aeschynomene* spp. inducing different bacteroid morphotypes with
1207 contrasted symbiotic efficiency. Environ. Microbiol. doi:10.1111/1462-2920.14292.
1208 Landeta, C., Dávalos, A., Cevallos, M.Á., Geiger, O., Brom, S., and Romero, D. 2011. Plasmids
1209 with a chromosome-like role in rhizobia. J. Bacteriol. **193**(6): 1317–1326.
1210 doi:10.1128/JB.01184-10.
1211 Lang, C., Smith, L.S., and Long, S.R. 2018. Characterization of novel plant symbiosis mutants
1212 using a new multiple gene-expression reporter *Sinorhizobium meliloti* strain. Front. Plant Sci.
1213 **9**: 76. doi:10.3389/fpls.2018.00076.
1214 Laranjo, M., Alexandre, A., and Oliveira, S. 2014. Genes commonly involved in acid tolerance
1215 are not overexpressed in the plant microsymbiont *Mesorhizobium loti* MAFF303099 upon
1216 acidic shock. Appl. Microbiol. Biotechnol. **98**(16): 7137–7147. doi:10.1007/s00253-014-
1217 5875-4.
1218 Laranjo, M., Alexandre, A., and Oliveira, S. 2017. Global transcriptional response to salt shock of
1219 the plant microsymbiont *Mesorhizobium loti* MAFF303099. Res. Microbiol. **168**(1): 55–63.
1220 doi:10.1016/j.resmic.2016.07.006.
1221 Lardi, M., Murset, V., Fischer, H.-M., Mesa, S., Ahrens, C.H., Zamboni, N., and Pessi, G. 2016.
1222 Metabolomic profiling of *Bradyrhizobium diazoefficiens*-induced root nodules reveals both
1223 host plant-specific and developmental signatures. Int. J. Mol. Sci. **17**(6): 815.
1224 doi:10.3390/ijms17060815.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1225 Larrainzar, E., and Wienkoop, S. 2017. A proteomic view on the role of legume symbiotic
1226 interactions. Front. Plant Sci. **8**: 1267. doi:10.3389/fpls.2017.01267.
1227 Lehman, A.P., and Long, S.R. 2018. OxyR-dependent transcription response of *Sinorhizobium*
1228 *meliloti* to oxidative stress. J. Bacteriol. **200**(7): e00622–17. doi:10.1128/JB.00622-17.
1229 Li, Y., Tian, C.F., Chen, W.F., Wang, L., Sui, X.H., and Chen, W.X. 2013. High-resolution
1230 transcriptomic analyses of *Sinorhizobium* sp. NGR234 bacteroids in determinate nodules of
1231 *Vigna unguiculata* and indeterminate nodules of *Leucaena leucocephala*. PLOS One **8**(8):
1232 e70531. doi:10.1371/journal.pone.0070531.
1233 Li, Z., Zu, C., Wang, C., Yang, J., Yu, H., and Wu, H. 2016. Different responses of rhizosphere
1234 and non-rhizosphere soil microbial communities to consecutive *Piper nigrum* L. monoculture.
1235 Sci. Rep. **6**(1): 35825. doi:10.1038/srep35825.
1236 Liu, L.X., Li, Q.Q., Zhang, Y.Z., Hu, Y., Jiao, J., Guo, H.J., Zhang, X.X., Zhang, B., Chen, W.X.,
1237 and Tian, C.F. 2017a. The nitrate-reduction gene cluster components exert lineage-dependent
1238 contributions to optimization of *Sinorhizobium* symbiosis with soybeans. Environ. Microbiol.
1239 **19**(12): 4926–4938. doi:10.1111/1462-2920.13948.
1240 Liu, T., Tian, C.F., and Chen, W.X. 2015. Site-specific Ser/Thr/Tyr phosphoproteome of
1241 *Sinorhizobium meliloti* at stationary phase. PLOS One **10**(9): e0139143.
1242 doi:10.1371/journal.pone.0139143.
1243 Liu, X., Luo, Y., Mohamed, O.A., Liu, D., and Wei, G. 2014. Global transcriptome analysis of
1244 *Mesorhizobium alhagi* CCNWXJ12-2 under salt stress. BMC Microbiol. **14**(1): 1.
1245 doi:10.1186/s12866-014-0319-y.
1246 Liu, Y., Jiang, X., Guan, D., Zhou, W., Ma, M., Zhao, B., Cao, F., Li, L., and Li, J. 2017b.
1247 Transcriptional analysis of genes involved in competitive nodulation in *Bradyrhizobium*

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

diazoefficiens at the presence of soybean root exudates. Sci. Rep. **7**(1): 10946. doi:10.1038/s41598-017-11372-0.

Long, S.R. 1996. Rhizobium symbiosis: nod factors in perspective. Plant Cell **8**(10): 1885–1898. doi:10.1105/tpc.8.10.1885.

López-Lara, I.M., Gao, J.-L., Soto, M.J., Solares-Pérez, A., Weissenmayer, B., Sohlenkamp, C., Verroios, G.P., Thomas-Oates, J., and Geiger, O. 2005. Phosphorus-free membrane lipids of *Sinorhizobium meliloti* are not required for the symbiosis with alfalfa but contribute to increased cell yields under phosphorus-limiting conditions of growth. Mol. Plant Microbe Interact. **18**(9): 973–982.. doi:10.1094/MPMI-18-0973.

López-Torrejón, G., Jiménez-Vicente, E., Buesa, J.M., Hernandez, J.A., Verma, H.K., and Rubio, L.M. 2016. Expression of a functional oxygen-labile nitrogenase component in the mitochondrial matrix of aerobically grown yeast. Nat. Commun. **7**: 11426. doi:10.1038/ncomms11426.

Lu, M., Jiao, S., Gao, E., Song, X., Li, Z., Hao, X., Rensing, C., and Wei, G. 2017. Transcriptome response to heavy metals in *Sinorhizobium meliloti* CCNWSX0020 reveals new metal resistance determinants that also promote bioremediation by *Medicago lupulina* in metal contaminated soil. Appl. Environ. Microbiol. **83**(20): e01244–17. doi:10.1128/AEM.01244-17.

Lupwayi, N.Z., Clayton, G.W., and Rice, W.A. 2006. Rhizobial inoculants for legume crops. J. Crop Im. **15**(2): 289–321. doi:10.1300/J411v15n02_09.

MacLean, A.M., Finan, T.M., and Sadowsky, M.J. 2007. Genomes of the symbiotic nitrogen-fixing bacteria of legumes. Plant Physiol. **144**(2): 615–622. doi:10.1104/pp.107.101634.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1270 Mandal, S., Mandal, M., Pati, B., Das, A., and Ghosh, A. 2009. Proteomics view of a *Rhizobium*
1271 isolate response to arsenite [As(III)] stress. Acta Microbiol. Immunol. Hung. **56**(2): 157–167.
1272 doi:10.1556/AMicr.56.2009.2.4.
1273 Marchetti, M., Capela, D., Glew, M., Cruveiller, S., Chane-Woon-Ming, B., Gris, C., Timmers,
1274 T., Poinsot, V., Gilbert, L.B., Heeb, P., Médigue, C., Batut, J., and Masson-Boivin, C. 2010.
1275 Experimental evolution of a plant pathogen into a legume symbiont. PLOS Biol. **8**(1):
1276 e1000280–10. doi:10.1371/journal.pbio.1000280.
1277 Marchetti, M., Clerissi, C., Yousfi, Y., Gris, C., Bouchez, O., Rocha, E., Cruveiller, S., Jauneau,
1278 A., Capela, D., and Masson-Boivin, C. 2017. Experimental evolution of rhizobia may lead to
1279 either extra- or intracellular symbiotic adaptation depending on the selection regime. Mol.
1280 Ecol. **26**(7): 1818–1831. doi:10.1111/mec.13895.
1281 Marchetti, M., Jauneau, A., Capela, D., Remigi, P., Gris, C., Batut, J., and Masson-Boivin, C.
1282 2014. Shaping bacterial symbiosis with legumes by experimental evolution. Mol. Plant
1283 Microbe Interact. **27**(9): 956–964. doi:10.1094/MPMI-03-14-0083-R.
1284 Martínez, E., Palacios, R., and Sánchez, F. 1987. Nitrogen-fixing nodules induced by
1285 *Agrobacterium tumefaciens* harboring *Rhizobium phaseoli* plasmids. J. Bacteriol. **169**(6):
1286 2828–2834. doi:10.1128/jb.169.6.2828-2834.1987.
1287 Martínez-Salazar, J.M., Salazar, E., Encarnación, S., Ramírez-Romero, M.A., and Rivera, J. 2009.
1288 Role of the extracytoplasmic function sigma factor RpoE4 in oxidative and osmotic stress
1289 responses in *Rhizobium etli*. J. Bacteriol. **191**(13): 4122–4132. doi:10.1128/JB.01626-08.
1290 Marx, H., Minogue, C.E., Jayaraman, D., Richards, A.L., Kwiecien, N.W., Siahpirani, A.F.,
1291 Rajasekar, S., Maeda, J., Garcia, K., Del Valle-Echevarria, A.R., Volkening, J.D., Westphall,
1292 M.S., Roy, S., Sussman, M.R., Ané, J.-M., and Coon, J.J. 2016. A proteomic atlas of the

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1293 legume *Medicago truncatula* and its nitrogen-fixing endosymbiont *Sinorhizobium meliloti*.
1294 Nat. Biotechnol. **34**(11): 1198–1205. doi:10.1038/nbt.3681.
1295 Masson-Boivin, C., Giraud, E., Perret, X., and Batut, J. 2009. Establishing nitrogen-fixing
1296 symbiosis with legumes: how many rhizobium recipes? Trends Microbiol. **17**(10): 458–466.
1297 doi:10.1016/j.tim.2009.07.004.
1298 Maunoury, N., Redondo-Nieto, M., Bourcy, M., Van de Velde, W., Alunni, B., Laporte, P.,
1299 Durand, P., Agier, N., Marisa, L., Vaubert, D., Delacroix, H., Duc, G., Ratet, P., Aggerbeck,
1300 L., Kondorosi, E., and Mergaert, P. 2010. Differentiation of symbiotic cells and
1301 endosymbionts in *Medicago truncatula* nodulation are coupled to two transcriptome-switches.
1302 PLOS One **5**(3): e9519. doi:10.1371/journal.pone.0009519.
1303 Maynaud, G., Brunel, B., Mornico, D., Durot, M., Severac, D., Dubois, E., Navarro, E., Cleyet-
1304 Marel, J.-C., and Le Quéré, A. 2013. Genome-wide transcriptional responses of two metal-
1305 tolerant symbiotic *Mesorhizobium* isolates to zinc and cadmium exposure. BMC Genomics
1306 **14**(1): 292. doi:10.1186/1471-2164-14-292.
1307 Mendum, T.A., Clark, I.M., and Hirsch, P.R. 2001. Characterization of two novel *Rhizobium*
1308 *leguminosarum* bacteriophages from a field release site of genetically-modified rhizobia.
1309 Antonie Van Leeuwenhoek **79**(2): 189–197. doi:10.1023/A:1010238412538.
1310 Meneses, N., Mendoza-Hernández, G., and Encarnación, S. 2010. The extracellular proteome of
1311 *Rhizobium etli* CE3 in exponential and stationary growth phase. Proteome Sci. **8**(1): 51.
1312 doi:10.1186/1477-5956-8-51.
1313 Meneses, N., Taboada, H., Dunn, M.F., Vargas, M.D.C., Buchs, N., Heller, M., and Encarnación,
1314 S. 2017. The naringenin-induced exoproteome of *Rhizobium etli* CE3. Arch. Microbiol.
1315 **199**(5): 737–755. doi:10.1007/s00203-017-1351-8.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1316 Mergaert, P., Nikovics, K., Kelemen, Z., Maunoury, N., Vaubert, D., Kondorosi, A., and
1317 Kondorosi, E. 2003. A novel family in *Medicago truncatula* consisting of more than 300
1318 nodule-specific genes coding for small, secreted polypeptides with conserved cysteine motifs.
1319 Plant Physiol. **132**(1): 161–173. doi:10.1104/pp.102.018192.
1320 Mesa, S., Hauser, F., Friberg, M., Malaguti, E., Fischer, H.-M., and Hennecke, H. 2008.
1321 Comprehensive assessment of the regulons controlled by the FixLJ-FixK2-FixK1 cascade in
1322 *Bradyrhizobium japonicum*. J. Bacteriol. **190**(20): 6568–6579. doi:10.1128/JB.00748-08.
1323 Miao, J., Zhang, N., Liu, H., Wang, H., Zhong, Z., and Zhu, J. 2018. Soil commensal rhizobia
1324 promote *Rhizobium etli* nodulation efficiency through CinR-mediated quorum sensing. Arch.
1325 Microbiol. **27**: 462. doi:10.1007/s00203-018-1478-2.
1326 Milunovic, B., diCenzo, G.C., Morton, R.A., and Finan, T.M. 2014. Cell growth inhibition upon
1327 deletion of four toxin-antitoxin loci from the megaplasmids of *Sinorhizobium meliloti*. J.
1328 Bacteriol. **196**(4): 811–824. doi:10.1128/JB.01104-13.
1329 Mitsch, M.J., diCenzo, G.C., Cowie, A., and Finan, T.M. 2018. Succinate transport is not essential
1330 for symbiotic nitrogen fixation by *Sinorhizobium meliloti* nor *Rhizobium leguminosarum*.
1331 Appl. Environ. Microbiol **84**(1): e01561–17. doi:10.1128/AEM.01561-17.
1332 Moënne Loccoz, Y. 1995. Plasmids influence growth of rhizobia in the rhizosphere of clover. Soil
1333 Biol. Biochem. **27**(8): 1001–1004. doi:10.1016/0038-0717(95)00035-D.
1334 Mozo, T., Cabrera, E., and Ruiz-Argüeso, T. 1988. Diversity of plasmid profiles and conservation
1335 of symbiotic nitrogen fixation genes in newly isolated *Rhizobium* strains nodulating sulla
1336 (*Hedysarum coronarium* L.). Appl. Environ. Microbiol. **54**(5): 1262–1267.
1337 Mus, F., Crook, M.B., Garcia, K., Garcia Costas, A., Geddes, B.A., Kouri, E.D., Paramasivan, P.,
1338 Ryu, M.-H., Oldroyd, G.E.D., Poole, P.S., Udvardi, M.K., Voigt, C.A., Ané, J.-M., and Peters,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

J.W. 2016. Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Appl. Environ. Microbiol. **82**(13): 3698–3710. doi:10.1128/AEM.01055-16.

Mutuma, S.P., Okello, J.J., Karanja, N.K., and Woomer, P.I. 2014. Smallholder farmers' use and profitability of legume inoculants in western Kenya. Afr. Crop Sci. J. **22**(3): 205–214. doi:10.4314/acsj.v22i3.

Münchbach, M., Dainese, P., Staudenmann, W., Narberhaus, F., and James, P. 1999. Proteome analysis of heat shock protein expression in *Bradyrhizobium japonicum*. Eur. J. Biochem. **264**(1): 39–48. doi:10.1046/j.1432-1327.1999.00567.x.

Nakatsukasa, H., Uchiumi, T., Kucho, K.-I., Suzuki, A., Higashi, S., and Abe, M. 2008. Transposon mediation allows a symbiotic plasmid of *Rhizobium leguminosarum* bv. *trifolii* to become a symbiosis island in *Agrobacterium* and *Rhizobium*. J. Gen. Appl. Microbiol. **54**(2): 107–118. doi:10.2323/jgam.54.107.

Nambu, M., Tatsukami, Y., Morisaka, H., Kuroda, K., and Ueda, M. 2015. Quantitative time-course proteome analysis of *Mesorhizobium loti* during nodule maturation. J. Proteom. **125**: 112–120. doi:10.1016/j.jprot.2015.04.034.

Natera, S.H., Guerreiro, N., and Djordjevic, M.A. 2000. Proteome analysis of differentially displayed proteins as a tool for the investigation of symbiosis. Mol. Plant Microbe Interact. **13**(9): 995–1009. doi:10.1094/MPMI.2000.13.9.995.

Nguyen, H.P., Miwa, H., Kaneko, T., Sato, S., and Okazaki, S. 2017. Identification of *Bradyrhizobium elkanii* genes involved in incompatibility with *Vigna radiata*. Genes **8**(12): 374. doi:10.3390/genes8120374.

Nogales, J., Domínguez-Ferreras, A., Amaya-Gomez, C.V., van Dillewijn, P., Cuellar, V., Sanjuán, J., Olivares, J., and Soto, M.J. 2010. Transcriptome profiling of a *Sinorhizobium*

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meliloti fadD mutant reveals the role of rhizobactin 1021 biosynthesis and regulation genes in the control of swarming. BMC Genomics **11**(1): 157. doi:10.1186/1471-2164-11-157.

Novikova, N., and Safronova, V. 1992. Transconjugants of *Agrobacterium radiobacter* harbouring sym genes of *Rhizobium galegae* can form an effective symbiosis with *Medicago sativa*. FEMS Microbio. Lett. **93**(3): 261–268. doi:10.1111/j.1574-6968.1992.tb05107.x.

O'Callaghan, M. 2016. Microbial inoculation of seed for improved crop performance: issues and opportunities. Appl. Microbiol. Biotechnol. **100**(13): 5729–5746. doi:10.1007/s00253-016-7590-9.

Ogden, A.J., Gargouri, M., Park, J., Gang, D.R., and Kahn, M.L. 2017. Integrated analysis of zone-specific protein and metabolite profiles within nitrogen-fixing *Medicago truncatula-Sinorhizobium medicae* nodules. PLOS One **12**(7): e0180894. doi:10.1371/journal.pone.0180894.

Oger, E., Marino, D., Guigonis, J.-M., Pauly, N., and Puppo, A. 2012. Sulfenylated proteins in the *Medicago truncatula-Sinorhizobium meliloti* symbiosis. J. Proteom. **75**(13): 4102–4113. doi:10.1016/j.jprot.2012.05.024.

Okazaki, S., Zehner, S., Hempel, J., Lang, K., and Göttfert, M. 2009. Genetic organization and functional analysis of the type III secretion system of *Bradyrhizobium elkanii*. FEMS Microbio. Lett. **295**(1): 88–95. doi:10.1111/j.1574-6968.2009.01593.x.

Oldroyd, G.E., and Dixon, R. 2014. Biotechnological solutions to the nitrogen problem. Curr. Opin. Biotech. **26**: 19–24. doi:10.1016/j.copbio.2013.08.006.

Oldroyd, G.E.D. 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat. Rev. Microbiol. **11**(4): 252–263. doi:10.1038/nrmicro2990.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1385 Oldroyd, G.E.D., and Downie, J.A. 2008. Coordinating nodule morphogenesis with rhizobial
1386 infection in legumes. Annu. Rev. Plant Biol. **59**(1): 519–546.
1387 doi:10.1146/annurev.arplant.59.032607.092839.
1388 Oldroyd, G.E.D., Murray, J.D., Poole, P.S., and Downie, J.A. 2011. The rules of engagement in
1389 the legume-rhizobial symbiosis. Annu. Rev. Genet. **45**(1): 119–144. doi:10.1146/annurev-
1390 genet-110410-132549.
1391 Oresnik, I.J., Twelker, S., and Hynes, M.F. 1999. Cloning and characterization of a *Rhizobium*
1392 *leguminosarum* gene encoding a bacteriocin with similarities to RTX toxins. Appl. Environ.
1393 Microbiol. **65**(7): 2833–2840.
1394 Orikasa, Y., Nodasaka, Y., Ohyama, T., Okuyama, H., Ichise, N., Yumoto, I., Morita, N., Wei,
1395 M., and Ohwada, T. 2010. Enhancement of the nitrogen fixation efficiency of genetically-
1396 engineered *Rhizobium* with high catalase activity. J. Biosci. Bioeng. **110**(4): 397–402.
1397 doi:10.1016/j.jbiosc.2010.04.007.
1398 Paau, A.S. 1991. Improvement of *Rhizobium* inoculants by mutation, genetic engineering and
1399 formulation. Biotechnol. Adv. **9**(2): 173–184.
1400 Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T.G., Fookes, M.,
1401 Falush, D., Keane, J.A., and Parkhill, J. 2015. Roary: rapid large-scale prokaryote pan genome
1402 analysis. Bioinformatics **31**(22): 3691–3693. doi:10.1093/bioinformatics/btv421.
1403 Pahua, V.J., Stokes, P.J.N., Hollowell, A.C., Regus, J.U., Gano-Cohen, K.A., Wendlandt, C.E.,
1404 Quides, K.W., Lyu, J.Y., and Sachs, J.L. 2018. Fitness variation among host species and the
1405 paradox of ineffective rhizobia. J. Evol. Biol. **31**(4): 599–610. doi:10.1111/jeb.13249.
1406 Pan, H., and Wang, D. 2017. Nodule cysteine-rich peptides maintain a working balance during
1407 nitrogen-fixing symbiosis. Nat. Plants **3**(5): 17048. doi:10.1038/nplants.2017.48.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1408 Pankhurst, C.E., Macdonald, P.E., and Reeves, J.M. 1986. Enhanced nitrogen fixation and
1409 competitiveness for nodulation of *Lotus pedunculatus* by a plasmid-cured derivative of
1410 *Rhizobium loti*. Microbiology **132**(8): 2321–2328. doi:10.1099/00221287-132-8-2321.
1411 Park, S., Croteau, P., and Boering, K.A. 2012. Trends and seasonal cycles in the isotopic
1412 composition of nitrous oxide since 1940. Nat. Geosci. **5**(4): 261–265.
1413 Pech-Canul, Á., Nogales, J., Miranda-Molina, A., Álvarez, L., Geiger, O., Soto, M.J., and López-
1414 Lara, I.M. 2011. FadD is required for utilization of endogenous fatty acids released from
1415 membrane lipids. J. Bacteriol. **193**(22): 6295–6304. doi:10.1128/JB.05450-11.
1416 Penterman, J., Abo, R.P., De Nisco, N.J., Arnold, M.F.F., Longhi, R., Zanda, M., and Walker,
1417 G.C. 2014. Host plant peptides elicit a transcriptional response to control the *Sinorhizobium*
1418 *meliloti* cell cycle during symbiosis. Proc. Natl. Acad. Sci. U.S.A. **111**(9): 3561–3566.
1419 doi:10.1073/pnas.1400450111.
1420 Penttinen, P., Räsänen, L.A., Lortet, G., and Lindström, K. 2013. Stable isotope labelling reveals
1421 that NaCl stress decreases the production of *Ensifer* (*Sinorhizobium*) *arboris*
1422 lipochitooligosaccharide signalling molecules. FEMS Microbio. Lett. **349**(2): 117–126.
1423 doi:10.1111/1574-6968.12303.
1424 Peralta, H., Aguilar, A., Díaz, R., Mora, Y., Martínez-Batallar, G., Salazar, E., Vargas-Lagunas,
1425 C., Martínez, E., Encarnación, S., Girard, L., and Mora, J. 2016. Genomic studies of nitrogen-
1426 fixing rhizobial strains from *Phaseolus vulgaris* seeds and nodules. BMC Genomics **17**(1):
1427 711. doi:10.1186/s12864-016-3053-z.
1428 Perry, B.J., Akter, M.S., and Yost, C.K. 2016. The use of transposon insertion sequencing to
1429 interrogate the core functional genome of the legume symbiont *Rhizobium leguminosarum*.
1430 Front. Microbiol. **7**: 1873. doi:10.3389/fmicb.2016.01873.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1431 Perry, B.J., and Yost, C.K. 2014. Construction of a *mariner*-based transposon vector for use in
1432 insertion sequence mutagenesis in selected members of the *Rhizobiaceae*. BMC Microbiol.
1433 **14**(1): 298. doi:10.1186/s12866-014-0298-z.
1434 Pessi, G., Ahrens, C.H., Rehrauer, H., Lindemann, A., Hauser, F., Fischer, H.-M., and Hennecke,
1435 H. 2007. Genome-wide transcript analysis of *Bradyrhizobium japonicum* bacteroids in
1436 soybean root nodules. Mol. Plant Microbe Interact. **20**(11): 1353–1363. doi:10.1094/MPMI-
1437 20-11-1353.
1438 Pérez Carrascal, O.M., VanInsberghe, D., Juárez, S., Polz, M.F., Vinuesa, P., and González, V.
1439 2016. Population genomics of the symbiotic plasmids of sympatric nitrogen-fixing *Rhizobium*
1440 species associated with *Phaseolus vulgaris*. Environ. Microbiol. **18**(8): 2660–2676.
1441 doi:10.1111/1462-2920.13415.
1442 Pérez-Giménez, J., Covelli, J.M., López, M.F., Althabegoiti, M.J., Ferrer-Navarro, M.,
1443 Mongiardini, E.J., and Lodeiro, A.R. 2012. Soybean seed lectin prevents the accumulation of
1444 S-adenosyl methionine synthetase and the S1 30S ribosomal protein in *Bradyrhizobium*
1445 *japonicum* under C and N starvation. Curr. Microbiol. **65**(4): 465–474. doi:10.1007/s00284-
1446 012-0180-x.
1447 Pérez-Montaño, F., Del Cerro, P., Jiménez-Guerrero, I., López-Baena, F.J., Cubo, M.T., Hungria,
1448 M., Megías, M., and Ollero, F.J. 2016a. RNA-seq analysis of the *Rhizobium tropici* CIAT 899
1449 transcriptome shows similarities in the activation patterns of symbiotic genes in the presence
1450 of apigenin and salt. BMC Genomics **17**(1): 198. doi:10.1186/s12864-016-2543-3.
1451 Pérez-Montaño, F., Jiménez-Guerrero, I., Acosta-Jurado, S., Navarro-Gómez, P., Ollero, F.J.,
1452 Ruiz-Sainz, J.E., López-Baena, F.J., and Vinardell, J.M. 2016b. A transcriptomic analysis of

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1453 the effect of genistein on *Sinorhizobium fredii* HH103 reveals novel rhizobial genes putatively
1454 involved in symbiosis. Sci. Rep. **6**(1): 31592. doi:10.1038/srep31592.
1455 Pfau, T., Christian, N., Masakapalli, S.K., Sweetlove, L.J., Poolman, M.G., and Ebenhoeh, O.
1456 2016. The intertwined metabolism of *Medicago truncatula* and its nitrogen fixing symbiont
1457 *Sinorhizobium meliloti* elucidated by genome-scale metabolic models. bioRxiv: **067348**.
1458 doi:10.1101/067348.
1459 Pini, F., De Nisco, N.J., Ferri, L., Penterman, J., Fioravanti, A., Brilli, M., Mengoni, A.,
1460 Bazzicalupo, M., Viollier, P.H., Walker, G.C., and Biondi, E.G. 2015. Cell cycle control by
1461 the master regulator CtrA in *Sinorhizobium meliloti*. PLOS Genet. **11**(5): e1005232.
1462 doi:10.1371/journal.pgen.1005232.
1463 Pini, F., East, A.K., Appia-Ayme, C., Tomek, J., Karunakaran, R., Mendoza-Suárez, M., Edwards,
1464 A., Terpolilli, J.J., Roworth, J., Downie, J.A., and Poole, P.S. 2017. Bacterial biosensors for
1465 *in vivo* spatiotemporal mapping of root secretion. Plant Physiol. **174**(3): 1289–1306.
1466 doi:10.1104/pp.16.01302.
1467 Pini, F., Galardini, M., Bazzicalupo, M., and Mengoni, A. 2011. Plant-bacteria association and
1468 symbiosis: are there common genomic traits in *Alphaproteobacteria*? Genes **2**(4): 1017–1032.
1469 doi:10.3390/genes2041017.
1470 Pobigaylo, N., Szymczak, S., Nattkemper, T.W., and Becker, A. 2008. Identification of genes
1471 relevant to symbiosis and competitiveness in *Sinorhizobium meliloti* using signature-tagged
1472 mutants. Mol. Plant Microbe Interact. **21**(2): 219–231. doi:10.1094/MPMI-21-2-0219.
1473 Pobigaylo, N., Wetter, D., Szymczak, S., Schiller, U., Kurtz, S., Meyer, F., Nattkemper, T.W., and
1474 Becker, A. 2006. Construction of a large signature-tagged mini-Tn*5* transposon library and its

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

application to mutagenesis of *Sinorhizobium meliloti*. Appl. Environ. Microbiol. **72**(6): 4329–4337. doi:10.1128/AEM.03072-05.

Poole, P., Ramachandran, V., and Terpolilli, J. 2018. Rhizobia: from saprophytes to endosymbionts. Nat. Rev. Microbiol. **16**(5): 291-303. doi:10.1038/nrmicro.2017.171.

Porter, S.S., Chang, P.L., Conow, C.A., Dunham, J.P., and Friesen, M.L. 2017. Association mapping reveals novel serpentine adaptation gene clusters in a population of symbiotic *Mesorhizobium*. ISME J. **11**(1): 248–262. doi:10.1038/ismej.2016.88.

Price, P.A., Tanner, H.R., Dillon, B.A., Shabab, M., Walker, G.C., and Griffitts, J.S. 2015. Rhizobial peptidase HrrP cleaves host-encoded signaling peptides and mediates symbiotic compatibility. Proc. Natl. Acad. Sci. U.S.A. **112**(49): 15244–15249. doi:10.1073/pnas.1417797112.

Queiroux, C., Washburn, B.K., Davis, O.M., Stewart, J., Brewer, T.E., Lyons, M.R., and Jones, K.M. 2012. A comparative genomics screen identifies a *Sinorhizobium meliloti* 1021 *sodM*-like gene strongly expressed within host plant nodules. BMC Microbiol. **12**(1): 74. doi:10.1186/1471-2180-12-74.

Quides, K.W., Stomackin, G.M., Lee, H.-H., Chang, J.H., and Sachs, J.L. 2017. *Lotus japonicus* alters *in planta* fitness of *Mesorhizobium loti* dependent on symbiotic nitrogen fixation. PLOS One **12**(9): e0185568. doi:10.1371/journal.pone.0185568.

Rachwał, K., Matczyńska, E., and Janczarek, M. 2015. Transcriptome profiling of a *Rhizobium leguminosarum* bv. *trifolii rosR* mutant reveals the role of the transcriptional regulator RosR in motility, synthesis of cell-surface components, and other cellular processes. BMC Genomics **16**(1): 1111. doi:10.1186/s12864-015-2332-4.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1497 Ramachandran, V.K., East, A.K., Karunakaran, R., Downie, J.A., and Poole, P.S. 2011. Adaptation
1498 of *Rhizobium leguminosarum* to pea, alfalfa and sugar beet rhizospheres investigated by
1499 comparative transcriptomics. Genome Biol. **12**(10): R106. doi:10.1186/gb-2011-12-10-r106.
1500 Ramírez, M., Valderrama, B., Arredondo-Peter, R., Soberón, M., Mora, J., and Hernández, G.
1501 1999. *Rhizobium etli* genetically engineered for the heterologous expression of *Vitreoscilla* sp.
1502 hemoglobin: effects on free-living and symbiosis. Mol. Plant Microbe Interact. **12**(11): 1008–
1503 1015. doi:10.1094/MPMI.1999.12.11.1008.
1504 Reeve, W.G., Tiwari, R.P., Guerreiro, N., Stubbs, J., Dilworth, M.J., Glenn, A.R., Rolfe, B.G.,
1505 Djordjevic, M.A., and Howieson, J.G. 2004. Probing for pH-regulated proteins in
1506 *Sinorhizobium medicae* using proteomic analysis. J. Mol. Microbiol. Biotechnol. **7**(3): 140–
1507 147. doi:10.1159/000078657.
1508 Remigi, P., Capela, D., Clerissi, C., Tasse, L., Torchet, R., Bouchez, O., Batut, J., Cruveiller, S.,
1509 Rocha, E.P.C., and Masson-Boivin, C. 2014. Transient hypermutagenesis accelerates the
1510 evolution of legume endosymbionts following horizontal gene transfer. PLOS Biol. **12**(9):
1511 e1001942. doi:10.1371/journal.pbio.1001942.
1512 Remigi, P., Zhu, J., Young, J.P.W., and Masson-Boivin, C. 2016. Symbiosis within symbiosis:
1513 Evolving nitrogen-fixing legume symbionts. Trends Microbiol. **24**(1): 63–75.
1514 doi:10.1016/j.tim.2015.10.007.
1515 Resendis-Antonio, O., Hernández, M., Mora, Y., and Encarnación, S. 2012. Functional modules,
1516 structural topology, and optimal activity in metabolic networks. PLOS Comput. Biol. **8**(10):
1517 e1002720. doi:10.1371/journal.pcbi.1002720.
1518 Resendis-Antonio, O., Hernández, M., Salazar, E., Contreras, S., Batallar, G.M., Mora, Y., and
1519 Encarnación, S. 2011. Systems biology of bacterial nitrogen fixation: high-throughput

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

technology and its integrative description with constraint-based modeling. BMC Syst. Biol. **5**(1): 120. doi:10.1186/1752-0509-5-120.

Resendis-Antonio, O., Reed, J.L., Encarnación, S., Collado-Vides, J., and Palsson, B.Ø. 2007. Metabolic reconstruction and modeling of nitrogen fixation in *Rhizobium etli*. PLOS Comput. Biol. **3**(10): 1887–1895. doi:10.1371/journal.pcbi.0030192.

Reyes-Pérez, A., Vargas, M.D.C., Hernández, M., Aguirre-von-Wobeser, E., Pérez-Rueda, E., and Encarnación, S. 2016. Transcriptomic analysis of the process of biofilm formation in *Rhizobium etli* CFN42. Arch. Microbiol. **198**(9): 847–860. doi:10.1007/s00203-016-1241-5.

Rodrigues, J.A., López-Baena, F.J., Ollero, F.J., Vinardell, J.M., Espuny, M.D.R., Bellogín, R.A., Ruiz-Sainz, J.E., Thomas, J.R., Sumpton, D., Ault, J., and Thomas-Oates, J. 2007. NopM and NopD are rhizobial nodulation outer proteins: identification using LC-MALDI and LC-ESI with a monolithic capillary column. J. Proteome Res. **6**(3): 1029–1037. doi:10.1021/pr060519f.

Rogel, M.A., Hernández-Lucas, I., Kuykendall, L.D., Balkwill, D.L., and Martinez-Romero, E. 2001. Nitrogen-fixing nodules with *Ensifer adhaerens* harboring *Rhizobium tropici* symbiotic plasmids. Appl. Environ. Microbiol. **67**(7): 3264–3268. doi:10.1128/AEM.67.7.3264-3268.2001.

Rogers, C., and Oldroyd, G.E.D. 2014. Synthetic biology approaches to engineering the nitrogen symbiosis in cereals. J. Exp. Bot. **65**(8): 1939–1946. doi:10.1093/jxb/eru098.

Roux, B., Rodde, N., Jardinaud, M.-F., Timmers, T., Sauviac, L., Cottret, L., Carrère, S., Sallet, E., Courcelle, E., Moreau, S., Debellé, F., Capela, D., de Carvalho-Niebel, F., Gouzy, J., Bruand, C., and Gamas, P. 2014. An integrated analysis of plant and bacterial gene expression

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

in symbiotic root nodules using laser-capture microdissection coupled to RNA sequencing. Plant J. **77**(6): 817–837. doi:10.1111/tpj.12442.

Saad, M.M., Kobayashi, H., Marie, C., Brown, I.R., Mansfield, J.W., Broughton, W.J., and Deakin, W.J. 2005. NopB, a type III secreted protein of *Rhizobium* sp. strain NGR234, is associated with pilus-like surface appendages. J. Bacteriol. **187**(3): 1173–1181. doi:10.1128/JB.187.3.1173-1181.2005.

Saalbach, G., Erik, P., and Wienkoop, S. 2002. Characterisation by proteomics of peribacteroid space and peribacteroid membrane preparations from pea (*Pisum sativum*) symbiosomes. Proteomics **2**(3): 325–337.

Sachs, J.L., Ehinger, M.O., and Simms, E.L. 2010. Origins of cheating and loss of symbiosis in wild *Bradyrhizobium*. J. Evol. Biol. **23**(5): 1075–1089. doi:10.1111/j.1420-9101.2010.01980.x.

Sachs, J.L., Quides, K.W., and Wendlandt, C.E. 2018. Legumes versus rhizobia: a model for ongoing conflict in symbiosis. New Phytol. **29**: 925. doi:10.1111/nph.15222.

Salas, M.E., Lozano, M.J., López, J.L., Draghi, W.O., Serrania, J., Torres Tejerizo, G.A., Albicoro, F.J., Nilsson, J.F., Pistorio, M., Del Papa, M.F., Parisi, G., Becker, A., and Lagares, A. 2017. Specificity traits consistent with legume-rhizobia coevolution displayed by *Ensifer meliloti* rhizosphere colonization. Environ. Microbiol. **282**: 219. doi:10.1111/1462-2920.13820.

Salazar, E., Díaz-Mejía, J.J., Moreno-Hagelsieb, G., Martínez-Batallar, G., Mora, Y., Mora, J., and Encarnación, S. 2010. Characterization of the NifA-RpoN regulon in *Rhizobium etli* in free life and in symbiosis with *Phaseolus vulgaris*. Appl. Environ. Microbiol. **76**(13): 4510–4520. doi:10.1128/AEM.02007-09.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1564 Saramago, M., Peregrina, A., Robledo, M., Matos, R.G., Hilker, R., Serrania, J., Becker, A.,
1565 Arraiano, C.M., and Jiménez-Zurdo, J.I. 2017. *Sinorhizobium meliloti* YbeY is an
1566 endoribonuclease with unprecedented catalytic features, acting as silencing enzyme in
1567 riboregulation. Nucleic Acids Res. **45**(3): 1371–1391. doi:10.1093/nar/gkw1234.
1568 Sarma, A.D., and Emerich, D.W. 2005. Global protein expression pattern of *Bradyrhizobium*
1569 *japonicum* bacteroids: a prelude to functional proteomics. Proteomics **5**(16): 4170–4184.
1570 doi:10.1002/pmic.200401296.
1571 Sarma, A.D., and Emerich, D.W. 2006. A comparative proteomic evaluation of culture grown vs
1572 nodule isolated *Bradyrhizobium japonicum*. Proteomics **6**(10): 3008–3028.
1573 doi:10.1002/pmic.200500783.
1574 Schellenberger, J., Que, R., Fleming, R.M.T., Thiele, I., Orth, J.D., Feist, A.M., Zielinski, D.C.,
1575 Bordbar, A., Lewis, N.E., Rahmanian, S., Kang, J., Hyduke, D.R., and Palsson, B.Ø. 2011.
1576 Quantitative prediction of cellular metabolism with constraint-based models: the COBRA
1577 Toolbox v2.0. Nat. Protoc. **6**(9): 1290–1307. doi:10.1038/nprot.2011.308.
1578 Schlüter, J.-P., Reinkensmeier, J., Daschkey, S., Evguenieva-Hackenberg, E., Janssen, S., Jänicke,
1579 S., Becker, J.D., Giegerich, R., and Becker, A. 2010. A genome-wide survey of sRNAs in the
1580 symbiotic nitrogen-fixing alpha-proteobacterium *Sinorhizobium meliloti*. BMC Genomics
1581 **11**(1): 245. doi:10.1186/1471-2164-11-245.
1582 Schroeder, B.K., House, B.L., Mortimer, M.W., Yurgel, S.N., Maloney, S.C., Ward, K.L., and
1583 Kahn, M.L. 2005. Development of a functional genomics platform for *Sinorhizobium meliloti*:
1584 construction of an ORFeome. Appl. Environ. Microbiol. **71**(10): 5858–5864.
1585 doi:10.1128/AEM.71.10.5858-5864.2005.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1586 Serrania, J., Johner, T., Rupp, O., Goesmann, A., and Becker, A. 2017. Massive parallel insertion
1587 site sequencing of an arrayed *Sinorhizobium meliloti* signature-tagged mini-Tn*5* transposon
1588 mutant library. J. Biotechnol. doi:10.1016/j.jbiotec.2017.02.019.
1589 Seshadri, R., Reeve, W.G., Ardley, J.K., Tennessen, K., Woyke, T., Kyrpides, N.C., and Ivanova,
1590 N.N. 2015. Discovery of novel plant interaction determinants from the genomes of 163 root
1591 nodule bacteria. Sci. Rep. **5**(1): 16825. doi:10.1038/srep16825.
1592 Shamseldin, A., Nyalwidhe, J., and Werner, D. 2006. A proteomic approach towards the analysis
1593 of salt tolerance in *Rhizobium etli* and *Sinorhizobium meliloti* strains. Curr. Microbiol. **52**(5):
1594 333–339. doi:10.1007/s00284-005-6472-7.
1595 Silva, C., Kan, F.L., and Martínez-Romero, E. 2007. Population genetic structure of *Sinorhizobium*
1596 *meliloti* and S. *medicae* isolated from nodules of *Medicago* spp. in Mexico. FEMS Microbiol.
1597 Ecol. **60**(3): 477–489. doi:10.1111/j.1574-6941.2007.00301.x.
1598 Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H., and
1599 Berg, G. 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient
1600 gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl. Environ.
1601 Microbiol. **67**(10): 4742–4751. doi:10.1128/AEM.67.10.4742-4751.2001.
1602 Sobrero, P., Schlüter, J.-P., Lanner, U., Schlosser, A., Becker, A., and Valverde, C. 2012.
1603 Quantitative proteomic analysis of the Hfq-regulon in *Sinorhizobium meliloti* 2011. PLOS One
1604 **7**(10): e48494. doi:10.1371/journal.pone.0048494.
1605 Sohlenkamp, C., de Rudder, K.E., Rohrs, V., Lopéz-Lara, I.M., and Geiger, O. 2000. Cloning and
1606 characterization of the gene for phosphatidylcholine synthase. J. Biol. Chem. **275**(25): 18919–
1607 18925. doi:10.1074/jbc.M000844200.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1608 Sohlenkamp, C., de Rudder, K.E.E., and Geiger, O. 2004. Phosphatidylethanolamine is not
1609 essential for growth of *Sinorhizobium meliloti* on complex culture media. J. Bacteriol. **186**(6):
1610 1667–1677. doi:10.1128/JB.186.6.1667-1677.2004.
1611 Spini, G., Decorosi, F., Cerboneschi, M., Tegli, S., Mengoni, A., Viti, C., and Giovannetti, L.
1612 2015. Effect of the plant flavonoid luteolin on *Ensifer meliloti* 3001 phenotypic responses.
1613 Plant Soil **399**(1): 159–178. doi:10.1007/s11104-015-2659-2.
1614 Starker, C.G., Parra-Colmenares, A.L., Smith, L., Mitra, R.M., and Long, S.R. 2006. Nitrogen
1615 fixation mutants of *Medicago truncatula* fail to support plant and bacterial symbiotic gene
1616 expression. Plant Physiol. **140**(2): 671–680. doi:10.1104/pp.105.072132.
1617 Stasiak, G., Mazur, A., Wielbo, J., Marczak, M., Żebracki, K., Koper, P., and Skorupska, A. 2014.
1618 Functional relationships between plasmids and their significance for metabolism and
1619 symbiotic performance of *Rhizobium leguminosarum* bv. *trifolii*. J. Appl. Genet. **55**(4): 515–
1620 527. doi:10.1007/s13353-014-0220-2.
1621 Stockwell, S.B., Reutimann, L., and Guerinot, M.L. 2012. A role for *Bradyrhizobium japonicum*
1622 ECF16 sigma factor EcfS in the formation of a functional symbiosis with soybean. Mol. Plant
1623 Microbe Interact. **25**(1): 119–128. doi:10.1094/MPMI-07-11-0188.
1624 Strodtman, K.N., Stevenson, S.E., Waters, J.K., Mawhinney, T.P., Thelen, J.J., Polacco, J.C., and
1625 Emerich, D.W. 2017. The bacteroid periplasm in soybean nodules is an interkingdom
1626 symbiotic space. Mol. Plant Microbe Interact. **30**(12): 997–1008. doi:10.1094/MPMI-12-16-
1627 0264-R.
1628 Sugawara, M., Epstein, B., Badgley, B.D., Unno, T., Xu, L., Reese, J., Gyaneshwar, P., Denny,
1629 R., Mudge, J., Bharti, A.K., Farmer, A.D., May, G.D., Woodward, J.E., Médigue, C., Vallenet,
1630 D., Lajus, A., Rouy, Z., Martinez-Vaz, B., Tiffin, P., Young, N.D., and Sadowsky, M.J. 2013.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1631 Comparative genomics of the core and accessory genomes of 48 *Sinorhizobium* strains
1632 comprising five genospecies. Genome Biol. **14**(2): R17. doi:10.1186/gb-2013-14-2-r17.
1633 Sullivan, J.T., and Ronson, C.W. 1998. Evolution of rhizobia by acquisition of a 500-kb symbiosis
1634 island that integrates into a phe-tRNA gene. Proc. Natl. Acad. Sci. U.S.A. **95**(9): 5145–5149.
1635 doi:10.1073/pnas.95.9.5145.
1636 Sullivan, J.T., Patrick, H.N., Lowther, W.L., Scott, D.B., and Ronson, C.W. 1995. Nodulating
1637 strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the
1638 environment. Proc. Natl. Acad. Sci. U.S.A. **92**(19): 8985–8989. doi:10.1073/pnas.92.19.8985.
1639 Talebi, M.B., Bahar, M., Saeidi, G., Mengoni, A., and Bazzicalupo, M. 2008. Diversity of
1640 *Sinorhizobium* strains nodulating *Medicago sativa* from different Iranian regions. FEMS
1641 Microbio. Lett. **288**(1): 40–46. doi:10.1111/j.1574-6968.2008.01329.x.
1642 Tanthanuch, W., Tittabutr, P., Mohammed, S., Matthiesen, R., Yamabhai, M., Manassila, M.,
1643 Jensen, O.N., Boonkerd, N., and Teaumroong, N. 2010. Identification of salt-tolerant
1644 *Sinorhizobium* sp. strain BL3 membrane proteins based on proteomics. Microbes Environ.
1645 **25**(4): 275–280. doi:10.1264/jsme2.ME09185.
1646 Tatsukami, Y., Nambu, M., Morisaka, H., Kuroda, K., and Ueda, M. 2013. Disclosure of the
1647 differences of *Mesorhizobium loti* under the free-living and symbiotic conditions by
1648 comparative proteome analysis without bacteroid isolation. BMC Microbiol. **13**(1): 180.
1649 BioMed Central. doi:10.1186/1471-2180-13-180.
1650 Terpolilli, J.J., Masakapalli, S.K., Karunakaran, R., Webb, I.U.C., Green, R., Watmough, N.J.,
1651 Kruger, N.J., Ratcliffe, R.G., and Poole, P.S. 2016. Lipogenesis and redox balance in nitrogen-
1652 fixing pea bacteroids. J. Bacteriol. **198**(20): 2864–2875. doi:10.1128/JB.00451-16.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1653 Thiele, I., and Palsson, B.Ø. 2010. A protocol for generating a high-quality genome-scale
1654 metabolic reconstruction. Nat. Protoc. **5**(1): 93–121. doi:10.1038/nprot.2009.203.
1655 Tian, C.F., Zhou, Y.J., Zhang, Y.M., Li, Q.Q., Zhang, Y.Z., Li, D.F., Wang, S., Wang, J., Gilbert,
1656 L.B., Li, Y.R., and Chen, W.X. 2012. Comparative genomics of rhizobia nodulating soybean
1657 suggests extensive recruitment of lineage-specific genes in adaptations. Proc. Natl. Acad. Sci.
1658 U.S.A. **109**(22): 8629–8634. doi:10.1073/pnas.1120436109.
1659 Tian, Z., Zou, H., Li, J., Zhang, Y., Liu, Y., Yu, G., Zhu, J., Rüberg, S., Becker, A., and Wang, Y.
1660 2006. Transcriptome analysis of *Sinorhizobium meliloti* nodule bacteria in *nifA* mutant
1661 background. Chinese Sci. Bull. **51**(17): 2079–2086. doi:10.1007/s11434-006-2092-2.
1662 Tiricz, H., Szűcs, A., Farkas, A., Pap, B., Lima, R.M., Maróti, G., Kondorosi, E., and Kereszt, A.
1663 2013. Antimicrobial nodule-specific cysteine-rich peptides induce membrane depolarization-
1664 associated changes in the transcriptome of *Sinorhizobium meliloti*. Appl. Environ. Microbiol.
1665 **79**(21): 6737–6746. doi:10.1128/AEM.01791-13.
1666 Todd, J.D., Sawers, G., and Johnston, A.W.B. 2005. Proteomic analysis reveals the wide-ranging
1667 effects of the novel, iron-responsive regulator RirA in *Rhizobium leguminosarum* bv. *viciae*.
1668 Mol. Genet. Genomics **273**(2): 197–206. doi:10.1007/s00438-005-1127-8.
1669 Tolin, S., Arrigoni, G., Moscatiello, R., Masi, A., Navazio, L., Sablok, G., and Squartini, A. 2013.
1670 Quantitative analysis of the naringenin-inducible proteome in *Rhizobium leguminosarum* by
1671 isobaric tagging and mass spectrometry. Proteomics **13**(12-13): 1961–1972.
1672 doi:10.1002/pmic.201200472.
1673 Toro, N., Martínez-Abarca, F., Molina-Sánchez, M.D., García-Rodríguez, F.M., and Nisa-
1674 Martínez, R. 2018. Contribution of mobile group II introns to *Sinorhizobium meliloti* genome
1675 evolution. Front. Microbiol. **9**: 627. doi:10.3389/fmicb.2018.00627.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1676 Torres-Quesada, O., Millán, V., Nisa-Martínez, R., Bardou, F., Crespi, M., Toro, N., and Jiménez-
1677 Zurdo, J.I. 2013. Independent activity of the homologous small regulatory RNAs AbcR1 and
1678 AbcR2 in the legume symbiont *Sinorhizobium meliloti*. PLOS One **8**(7): e68147.
1679 doi:10.1371/journal.pone.0068147.
1680 Torres-Quesada, O., Oruezabal, R.I., Peregrina, A., Jofré, E., Lloret, J., Rivilla, R., Toro, N., and
1681 Jiménez-Zurdo, J.I. 2010. The *Sinorhizobium meliloti* RNA chaperone Hfq influences central
1682 carbon metabolism and the symbiotic interaction with alfalfa. BMC Microbiol. **10**(1): 71.
1683 doi:10.1186/1471-2180-10-71.
1684 Torres-Quesada, O., Reinkensmeier, J., Schlüter, J.-P., Robledo, M., Peregrina, A., Giegerich, R.,
1685 Toro, N., Becker, A., and Jiménez-Zurdo, J.I. 2014. Genome-wide profiling of Hfq-binding
1686 RNAs uncovers extensive post-transcriptional rewiring of major stress response and symbiotic
1687 regulons in *Sinorhizobium meliloti*. RNA Biol. **11**(5): 563–579. doi:10.4161/rna.28239.
1688 Truchet, G., Rosenberg, C., Vasse, J., Julliot, J.S., Camut, S., and Dénarié, J. 1984. Transfer of
1689 *Rhizobium meliloti* pSym genes into *Agrobacterium tumefaciens*: host-specific nodulation by
1690 atypical infection. J. Bacteriol. **157**(1): 134–142.
1691 Twelker, S., Oresnik, I.J., and Hynes, M.F. 1999. Bacteriocins of *Rhizobium Leguminosarum*. *In*
1692 Highlights of Nitrogen Fixation Research. Springer US, Boston, MA. pp. 105–108.
1693 doi:10.1007/978-1-4615-4795-2_20.
1694 Udvardi, M., and Poole, P.S. 2013. Transport and metabolism in legume-rhizobia symbioses.
1695 Annu. Rev. Plant Biol. **64**(1): 781–805. doi:10.1146/annurev-arplant-050312-120235.
1696 Ulvé, V.M., Sevin, E.W., Cheron, A., and Barloy-Hubler, F. 2007. Identification of chromosomal
1697 alpha-proteobacterial small RNAs by comparative genome analysis and detection in
1698 *Sinorhizobium meliloti* strain 1021. BMC Genomics **8**(1): 467. doi:10.1186/1471-2164-8-467.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1699 Van de Velde, W., Zehirov, G., Szatmari, A., Debreczeny, M., Ishihara, H., Kevei, Z., Farkas, A.,
1700 Mikulass, K., Nagy, A., Tiricz, H., Satiat-Jeunemaître, B., Alunni, B., Bourge, M., Kucho, K.-
1701 I., Abe, M., Kereszt, A., Maróti, G., Uchiumi, T., Kondorosi, E., and Mergaert, P. 2010. Plant
1702 peptides govern terminal differentiation of bacteria in symbiosis. Science **327**(5969): 1122–
1703 1126. doi:10.1126/science.1184057.
1704 van Dillewijn, P., Soto, M.J., Villadas, P.J., and Toro, N. 2001. Construction and environmental
1705 release of a *Sinorhizobium meliloti* strain genetically modified to be more competitive for
1706 alfalfa nodulation. Appl. Environ. Microbiol. **67**(9): 3860–3865.
1707 doi:10.1128/AEM.67.9.3860-3865.2001.
1708 VanYperen, R.D., Orton, T.S., and Griffitts, J.S. 2015. Genetic analysis of signal integration by
1709 the *Sinorhizobium meliloti* sensor kinase FeuQ. Microbiology **161**(Pt 2): 244–253.
1710 doi:10.1099/mic.0.000002.
1711 Vauclare, P., Bligny, R., Gout, E., and Widmer, F. 2013. An overview of the metabolic differences
1712 between *Bradyrhizobium japonicum* 110 bacteria and differentiated bacteroids from soybean
1713 (*Glycine max*) root nodules: an *in vitro* 13C- and 31P-nuclear magnetic resonance
1714 spectroscopy study. FEMS Microbio. Lett. **343**(1): 49–56. doi:10.1111/1574-6968.12124.
1715 Vences-Guzmán, M.A., Geiger, O., and Sohlenkamp, C. 2008. *Sinorhizobium meliloti* mutants
1716 deficient in phosphatidylserine decarboxylase accumulate phosphatidylserine and are strongly
1717 affected during symbiosis with alfalfa. J. Bacteriol. **190**(20): 6846–6856.
1718 doi:10.1128/JB.00610-08.
1719 Vercruysse, M., Fauvart, M., Jans, A., Beullens, S., Braeken, K., Cloots, L., Engelen, K., Marchal,
1720 K., and Michiels, J. 2011. Stress response regulators identified through genome-wide

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

transcriptome analysis of the (p)ppGpp-dependent response in *Rhizobium etli*. Genome Biol. **12**(2): R17. doi:10.1186/gb-2011-12-2-r17.

Wang, C., Sheng, X., Equi, R.C., Trainer, M.A., Charles, T.C., and Sobral, B.W.S. 2007. Influence of the poly-3-hydroxybutyrate (PHB) granule-associated proteins (PhaP1 and PhaP2) on PHB accumulation and symbiotic nitrogen fixation in *Sinorhizobium meliloti* Rm1021. J. Bacteriol. **189**(24): 9050–9056. doi:10.1128/JB.01190-07.

Wang, D., Wang, Y.C., Wu, L.J., Liu, J.X., Zhang, P., Jiao, J., Yan, H., Liu, T., Tian, C.F., and Chen, W.X. 2016. Construction and pilot screening of a signature-tagged mutant library of *Sinorhizobium fredii*. Arch. Microbiol. **198**(2): 91–99. doi:10.1007/s00203-015-1161-9.

Wang, Q., Yang, S., Liu, J., Terecskei, K., Ábrahám, E., Gombár, A., Domonkos, Á., Szűcs, A., Körmöczi, P., Wang, T., Fodor, L., Mao, L., Fei, Z., Kondorosi, E., Kaló, P., Kereszt, A., and Zhu, H. 2017. Host-secreted antimicrobial peptide enforces symbiotic selectivity in *Medicago truncatula*. Proc. Natl. Acad. Sci. U.S.A. **114**(26): 6854–6859. doi:10.1073/pnas.1700715114.

Wang, X., Yang, J.-G., Chen, L., Wang, J.-L., Cheng, Q., Dixon, R., and Wang, Y.-P. 2013. Using synthetic biology to distinguish and overcome regulatory and functional barriers related to nitrogen fixation. PLOS One **8**(7): e68677. doi:10.1371/journal.pone.0068677.

Weissenmayer, B., Gao, J.-L., López-Lara, I.M., and Geiger, O. 2002. Identification of a gene required for the biosynthesis of ornithine-derived lipids. Mol. Microbiol. **45**(3): 721–733. doi:10.1046/j.1365-2958.2002.03043.x.

Weissenmayer, B., Geiger, O., and Benning, C. 2000. Disruption of a gene essential for sulfoquinovosyldiacylglycerol biosynthesis in *Sinorhizobium meliloti* has no detectable effect on root nodule symbiosis. Mol. Plant Microbe Interact. **13**(6): 666–672. doi:10.1094/MPMI.2000.13.6.666.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1744 Westhoek, A., Field, E., Rehling, F., Mulley, G., Webb, I., Poole, P.S., and Turnbull, L.A. 2017.
1745 Policing the legume-Rhizobium symbiosis: a critical test of partner choice. Sci. Rep. **7**(1):
1746 1419. doi:10.1038/s41598-017-01634-2.
1747 Wheatley, R.M., Ramachandran, V.K., Geddes, B.A., Perry, B.J., Yost, C.K., and Poole, P.S. 2017.
1748 The role of O_2 in the growth of *Rhizobium leguminosarum* bv. *viciae* 3841 on glucose and
1749 succinate. J. Bacteriol. **199**(1): e00572–16–16. doi:10.1128/JB.00572-16.
1750 Wilson, R.A., Handley, B.A., and Beringer, J.E. 1998. Bacteriocin production and resistance in a
1751 field population of *Rhizobium leguminosarum* biovar *viciae*. Soil Biol. Biochem. **30**(3): 413–
1752 417. doi:10.1016/S0038-0717(97)00123-5.
1753 Wong, C.H., Pankhurst, C.E., Kondorosi, A., and Broughton, W.J. 1983. Morphology of root
1754 nodules and nodule-like structures formed by *Rhizobium* and *Agrobacterium* strains
1755 containing a *Rhizobium meliloti* megaplasmid. J. Cell Biol. **97**(3): 787–794.
1756 doi:10.1083/jcb.97.3.787.
1757 Yamaya-Ito, H., Shimoda, Y., Hakoyama, T., Sato, S., Kaneko, T., Hossain, M.S., Shibata, S.,
1758 Kawaguchi, M., Hayashi, M., KOUCHI, H., and Umehara, Y. 2018. Loss-of-function of
1759 ASPARTIC PEPTIDASE NODULE-INDUCED 1 (APN1) in *Lotus japonicus* restricts
1760 efficient nitrogen-fixing symbiosis with specific *Mesorhizobium loti* strains. Plant J. **93**(1): 5–
1761 16. doi:10.1111/tpj.13759.
1762 Yang, J., Xie, X., Wang, X., Dixon, R., and Wang, Y.-P. 2014. Reconstruction and minimal gene
1763 requirements for the alternative iron-only nitrogenase in *Escherichia coli*. Proc. Natl. Acad.
1764 Sci. U.S.A. **111**(35): E3718–25. doi:10.1073/pnas.1411185111.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1765 Yang, J., Xie, X., Yang, M., Dixon, R., and Wang, Y.-P. 2017a. Modular electron-transport chains
1766 from eukaryotic organelles function to support nitrogenase activity. Proc. Natl. Acad. Sci.
1767 U.S.A. **114**(12): E2460–E2465. doi:10.1073/pnas.1620058114.
1768 Yang, S., Wang, Q., Fedorova, E., Liu, J., Qin, Q., Zheng, Q., Price, P.A., Pan, H., Wang, D.,
1769 Griffitts, J.S., Bisseling, T., and Zhu, H. 2017b. Microsymbiont discrimination mediated by a
1770 host-secreted peptide in *Medicago truncatula*. Proc. Natl. Acad. Sci. U.S.A. **114**(26): 6848–
1771 6853. doi:10.1073/pnas.1700460114.
1772 Yang, Y., Hu, X.-P., and Ma, B.-G. 2017c. Construction and simulation of the *Bradyrhizobium*
1773 *diazoefficiens* USDA110 metabolic network: a comparison between free-living and symbiotic
1774 states. Mol. Biosyst. **13**(3): 607–620. doi:10.1039/c6mb00553e.
1775 Ye, H., Gemperline, E., Venkateshwaran, M., Chen, R., Delaux, P.-M., Howes-Podoll, M., Ané,
1776 J.-M., and Li, L. 2013. MALDI mass spectrometry-assisted molecular imaging of metabolites
1777 during nitrogen fixation in the *Medicago truncatula-Sinorhizobium meliloti* symbiosis. Plant
1778 J. **75**(1): 130–145. doi:10.1111/tpj.12191.
1779 Young, J.P.W., Crossman, L.C., Johnston, A.W., Thomson, N.R., Ghazoui, Z.F., Hull, K.H.,
1780 Wexler, M., Curson, A.R., Todd, J.D., Poole, P.S., Mauchline, T.H., East, A.K., Quail, M.A.,
1781 Churcher, C., Arrowsmith, C., Cherevach, I., Chillingworth, T., Clarke, K., Cronin, A., Davis,
1782 P., Fraser, A., Hance, Z., Hauser, H., Jagels, K., Moule, S., Mungall, K., Norbertczak, H.,
1783 Rabbinowitsch, E., Sanders, M., Simmonds, M., Whitehead, S., and Parkhill, J. 2006. The
1784 genome of *Rhizobium leguminosarum* has recognizable core and accessory components.
1785 Genome Biol. **7**(4): R34. doi:10.1186/gb-2006-7-4-r34.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1786 Yuan, Z.-C., Zaheer, R., Morton, R., and Finan, T.M. 2006. Genome prediction of PhoB regulated
1787 promoters in *Sinorhizobium meliloti* and twelve proteobacteria. Nucleic Acids Res. **34**(9):
1788 2686–2697. doi:10.1093/nar/gkl365.
1789 Yurgel, S.N., Mortimer, M.W., Rice, J.T., Humann, J.L., and Kahn, M.L. 2013a. Directed
1790 construction and analysis of a *Sinorhizobium meliloti* pSymA deletion mutant library. Appl.
1791 Environ. Microbiol. **79**(6): 2081–2087. doi:10.1128/AEM.02974-12.
1792 Yurgel, S.N., Rice, J., and Kahn, M.L. 2013b. Transcriptome analysis of the role of GlnD/GlnBK
1793 in nitrogen stress adaptation by *Sinorhizobium meliloti* Rm1021. PLOS One **8**(3): e58028.
1794 doi:10.1371/journal.pone.0058028.
1795 Zamani, M., diCenzo, G.C., Milunovic, B., and Finan, T.M. 2017. A putative 3-hydroxyisobutyryl-
1796 CoA hydrolase is required for efficient symbiotic nitrogen fixation in *Sinorhizobium meliloti*
1797 and *Sinorhizobium fredii* NGR234. Environ. Microbiol. **19**(1): 218–236. doi:10.1111/1462-
1798 2920.13570.
1799 Zavaleta-Pastor, M., Sohlenkamp, C., Gao, J.L., Guan, Z., Zaheer, R., Finan, T.M., Raetz, C.R.H.,
1800 Lopéz-Lara, I.M., and Geiger, O. 2010. *Sinorhizobium meliloti* phospholipase C required for
1801 lipid remodeling during phosphorus limitation. Proc. Natl. Acad. Sci. U.S.A. **107**(1): 302–307.
1802 doi:10.1073/pnas.0912930107.
1803 Zhang, X.-S., and Cheng, H.-P. 2006. Identification of *Sinorhizobium meliloti* early symbiotic
1804 genes by use of a positive functional screen. Appl. Environ. Microbiol. **72**(4): 2738–2748.
1805 doi:10.1128/AEM.72.4.2738-2748.2006.
1806 Zhang, Y., Smallbone, L.A., diCenzo, G.C., Morton, R., and Finan, T.M. 2016. Loss of malic
1807 enzymes leads to metabolic imbalance and altered levels of trehalose and putrescine in the
1808 bacterium *Sinorhizobium meliloti*. **16**(1): 491. doi:10.1186/s12866-016-0780-x.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27033v2 | CC BY 4.0 Open Access | rec: 18 Jul 2018, publ: 18 Jul 2018

1809 Zhao, H., Li, M., Fang, K., Chen, W., and Wang, J. 2012. *In silico* insights into the symbiotic
1810 nitrogen fixation in *Sinorhizobium meliloti* via metabolic reconstruction. PLOS One **7**(2):
1811 e31287. doi:10.1371/journal.pone.0031287.
1812 Zou, L., Gastebois, A., Mathieu-Demazière, C., Sorroche, F., Masson-Boivin, C., Batut, J., and
1813 Garnerone, A.-M. 2017. Transcriptomic insight in the control of legume root secondary
1814 infection by the *Sinorhizobium meliloti* transcriptional regulator Clr. Front. Microbiol. **8**:
1815 1236. doi:10.3389/fmicb.2017.01236.

SUPPLEMENTARY MATERIAL LEGENDS

File S1. Contains the supplementary methods and associated references, and Figure S1.

Figure S1. *In silico* predicted flux distributions and reaction essentialities. Example data obtained through metabolic modelling. Results from *in silico* metabolic modelling of *S. meliloti* metabolism using the iGD726 metabolic reconstruction are shown (diCenzo et al. 2018). Growth was simulated using (**A**) glucose or (**B**) succinate as the sole carbon source. The pathways represent central carbon metabolism, with the exception of the pentose phosphate pathway (for simplicity). The width of the line represents the amount of flux through the reaction; a doubling in the width corresponds to a ten-fold flux increase. Arrows indicate the direction of flux. Colours indicate if the reaction is essential (red) or non-essential (blue). Genes associated with reactions are based on the information present in iGD726, but they are not necessarily comprehensive. See the Supplementary Materials for details on the modelling procedures used. A version without gene names is provided as Figure 7 in manuscript.

File S2. Contains an enlarged version of the phylogeny of Figure 1, the Newick formatted phylogeny and associated annotation file of Figure 1, and a README file to describe the contents of the archive.

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TABLES AND FIGURES

Table 1. Studies of rhizobial species employing -omics technologies.

Topic of the study	Organisms	Approaches	References
Characterization of proteins			
SyrM (Nod factor synthesis)	*S. meliloti*	Transcriptomics	(Barnett and Long 2015)
NodD (Nod factor synthesis)	*S. meliloti*	Transcriptomics	(Capela et al. 2005)
NolR (Nod factor synthesis)	*S. meliloti*	Proteomics	(Chen et al. 2000a, 2005)
FixL/FixJ/FixK (nitrogen fixation)	*S. meliloti*; *B. diazoefficiens*	Transcriptomics	(Bobik et al. 2006, Mesa et al. 2008)
CbrA (cell cycle and symbiosis)	*S. meliloti*	Transcriptomics	(Gibson et al. 2007)
CtrA (cell cycle)	*S. meliloti*	Transcriptomics	(Pini et al. 2015)
RosR (exopolysaccharide synthesis)	*R. leguminosarum* bv. *trifolii*	Transcriptomics	(Rachwał et al. 2015)
FeuP/FeuQ (osmoadaptation and symbiosis)	*S. meliloti*	Transcriptomics	(Griffitts et al. 2008)
ECF sigma factors (stress response and symbiosis)	*R. etli*; *B. diazoefficiens*	Transcriptomics	(Gourion et al. 2009, Martínez-Salazar et al. 2009, Stockwell et al. 2012)
Clr (cAMP dependent regulator, nodulation)	*S. meliloti*	Transcriptomics	(Krol et al. 2016, Zou et al. 2017)
PraR (quorum sensing and biofilms)	*R. leguminosarum* bv. *viciae*	Transcriptomics	(Frederix et al. 2014)
PckR (central carbon metabolism)	*S. meliloti*	Transcriptomics	(diCenzo et al. 2017b)
PhoB (phosphate limitation response)	*S. meliloti*	Transcriptomics	(Krol and Becker 2004, Yuan et al. 2006)
GlnD/GlnBK (nitrogen limitation response)	*S. meliloti*	Transcriptomics	(Yurgel et al. 2013b)
NtrY/NtrX (nitrogen metabolism)	*S. meliloti*	Transcriptomics	(Calatrava-Morales et al. 2017)
Tkt2/Tal (central carbon metabolism)	*S. meliloti*	Proteomics; metabolomics	(Hawkins et al. 2018)
OxyR (oxidative stress)	*S. meliloti*	Transcriptomics	(Lehman and Long 2018)
RelA/Rsh (stringent response)	*S. meliloti*; *R. etli*	Transcriptomics	(Vercruysse et al. 2011, Krol and Becker 2011)
RirA (iron limitation)	*S. meliloti*; *R. leguminosarum* bv. *viciae*	Transcriptomics; proteomics	(Todd et al. 2005, Chao et al. 2005)
LeuB (leucine biosynthesis)	*S. meliloti*	Metabolomics	(Barsch et al. 2004)
AniA (PHB accumulation)	*R. etli*	Proteomics	(Encarnación et al. 2002)
FadD (Fatty acid metabolism)	*S. meliloti*	Lipid analysis	(Nogales et al. 2010)
PHB cycle mutations	*S. meliloti*	Transcriptomics; proteomics	(Wang et al. 2007, D'Alessio et al. 2017)
Dme/Tme (Malic enzymes)	*S. meliloti*	Transcriptomics; metabolomics	(Zhang et al. 2016)
Membrane lipid biosynthetic proteins (Cfa, PlsC, PlcP, Pcs, Pmt, OlsA, OlsB, PssA, Psd, SqdB, BtaA)	*S. meliloti*	Lipidomics and lipid analysis	(de Rudder et al. 1999, Weissenmayer et al. 2000, Sohlenkamp et al. 2000, de Rudder et al. 2000, Weissenmayer et al. 2002, Sohlenkamp et al. 2004, Gao et al. 2004, López-Lara et al. 2005, Vences-Guzmán et al. 2008, Basconcillo et al. 2009a, 2009c, Zavaleta-Pastor et al. 2010, Pech-Canul et al. 2011)
Riboregulation			
Small RNAs (sRNA)	*S. meliloti*	Transcriptomics	(Ulvé et al. 2007, Schlüter et al. 2010)
Hfq (RNA binding protein)	*S. meliloti*	Transcriptomics; proteomics	(Barra-Bily et al. 2010, Torres-Quesada et al. 2010, Gao et al. 2010, Sobrero et al. 2012)
Hfq binding RNAs	*S. meliloti*	Transcriptomics	(Torres-Quesada et al. 2014)
YbeY (endoribonuclease)	*S. meliloti*	Transcriptomics	(Saramago et al. 2017)
AbrC1/AbrC2 (sRNAs, nutrient uptake)	*S. meliloti*	Proteomics	(Torres-Quesada et al. 2013)
MmgR (sRNA, PHB accumulation)	*S. meliloti*	Proteomics	(Lagares et al. 2017)
Environmental adaptation and stresses			
Growth in root hairs	*B. diazoefficiens*	Metabolomics	(Brechenmacher et al. 2010)

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Oxic versus microoxic growth	*S. meliloti; B. diazoefficiens*	Transcriptomics	(Ampe et al. 2003, Becker et al. 2004, Bobik et al. 2006, Pessi et al. 2007)
Aerobic, micro-aerobic, anaerobic growth	*B. diazoefficiens*	Proteomics	(Dainese-Hatt et al. 1999)
Rhizosphere adaptation	*R. leguminosarum bv. viciae*	Transcriptomics	(Ramachandran et al. 2011)
Growth in presence of root exudates	*B. diazoefficiens; B. phymatum; C. taiwanensis; R. mesoamericanum*	Transcriptomics	(Liu et al. 2017b, Klonowska et al. 2018)
Seed endophytic growth	*R. phaseoli*; *S. americanum*	Proteomics	(Peralta et al. 2016)
Stationary phase adaptation	*S. meliloti; R. etli*	Proteomics	(Guerreiro et al. 1999, Meneses et al. 2010)
Phosphate limitation	*S. meliloti*	Lipidomics and lipid analysis	(Basconcillo et al. 2009a, 2009b, 2009c, Zavaleta-Pastor et al. 2010)
Biotin limitation	*S. meliloti*	Proteomics	(Heinz and Streit 2003)
Carbon limitation	*S. meliloti*	Proteomics	(Djordjevic et al. 2007)
Nitrogen limitation	*S. meliloti*	Proteomics	(Djordjevic et al. 2007)
Osmotic/salt stress	*S. meliloti*; *M. loti*; *M. alhagi*; *R. etli*; *S.* sp. BL3; *B. spp*.	Transcriptomics; proteomics	(Jebbar et al. 2005, Shamseldin et al. 2006, Dominguez-Ferreras et al. 2006, Tanthanuch et al. 2010, Liu et al. 2014, Laranjo et al. 2017, Dong et al. 2017)
Oxidative stress	*B. diazoefficiens*	Transcriptomics	(Donati et al. 2011, Jeon et al. 2011)
Acid stress	*S. meliloti*; *M. loti*; *S. medicae*	Transcriptomics; proteomics; metabolomics; lipidomics	(Reeve et al. 2004, Basconcillo et al. 2009a, Hellweg et al. 2009, Laranjo et al. 2014, Draghi et al. 2016)
Heat stress	*M. loti*; *R. tropici*; *B. diazoefficiens*	Transcriptomics; proteomics	(Münchbach et al. 1999, Gomes et al. 2012a, Alexandre et al. 2014)
Cold stress	*M.* sp. N33	Transcriptomics; metabolomics	(Ghobakhlou et al. 2013, 2015)
Heavy metal stress	*S. meliloti*; *M. metallidurans*; *M.* sp. STM 4661; *R.* sp. VMA301; *R.* sp. E20-8	Transcriptomics; proteomics; metabolomics	(Mandal et al. 2009, Maynaud et al. 2013, Lu et al. 2017, Cardoso et al. 2017b)
Dessication stress	*B. diazoefficiens*	Transcriptomics	(Cytryn et al. 2007)
Herbicide exposure	*R. leguminosarum bv. viciae*	Metabolomics	(Bhat et al. 2014)
Cellular processes			
Flavanoid perception	*S. meliloti*; *S. fredii*; *R. tropici*; *R. leguminosarum* bv. *trifolii*; *R. leguminosarum* bv. *viciae*; *R. etli*; *B. liaoningense*; *B. japonicum*; *B. diazoefficiens*	Transcriptomics; proteomics	(Guerreiro et al. 1997, Chen et al. 2000b, Ampe et al. 2003, Barnett et al. 2004, Capela et al. 2005, Hempel et al. 2009, da Silva Batista and Hungria 2012, Tolin et al. 2013, Arrigoni et al. 2013, Gao et al. 2015, Pérez-Montaño et al. 2016a, 2016b, Meneses et al. 2017)
Nod factor production	*S. arboris*	Metabolite analysis	(Penttinen et al. 2013)
Cell cycle	*S. meliloti*	Transcriptomics	(De Nisco et al. 2014, Pini et al. 2015)
Response to NCR peptides	*S. meliloti*	Transcriptomics	(Tiricz et al. 2013, Penterman et al. 2014)
Secretome / Type III secretion	*B. diazoefficiens*; *S. fredii*; *B. elkanii*	Proteomics	(Saad et al. 2005, Rodrigues et al. 2007, Hempel et al. 2009, Okazaki et al. 2009)
Biofilm formation	*R. etli*	Transcriptomics	(Reyes-Pérez et al. 2016)
Quorum sensing	*S. fredii*; *S. meliloti*; *R. leguminosarum* bv. *viciae*	Transcriptomics; proteomics	(Chen et al. 2003, Gao et al. 2005, Cantero et al. 2006, Gao et al. 2007, Krysciak et al. 2014)
Motility	*S. meliloti*	Transcriptomics	(Nogales et al. 2010)
Aerobic vs fermentative growth	*R. etli*	Proteomics	(Encarnación et al. 2003)
Lectin perception	*B. diazoefficiens*	Proteomics	(Pérez-Giménez et al. 2012)
Catabolism of phenanthrene	*S.* sp. C4	Metabolomics	(Keum et al. 2008)
Symbiotic island transfer	*M. ciceri*	Transcriptomics	(Haskett et al. 2018)
Nodule analyses			
Integrated plant + bacterium analyses	*S. meliloti* - *M. truncatula*; *S. meliloti* - *M. alba*; *S. medicae* - *M. truncatula*; *R. leguminosarum* bv. *viciae* - *P. sativum*	Transcriptomics; proteomics	(Natera et al. 2000, Barnett et al. 2004, Heath et al. 2012, Roux et al. 2014, Marx et al. 2016, Ogden et al. 2017, Mitsch et al. 2018)
Bacteroids vs free-living	*S. meliloti* - *M. truncatula*; *S. meliloti* - *M. sativa*; *S. meliloti* - *M. alba*; *R.*	Transcriptomics; proteomics; metabolomics	(Ampe et al. 2003, Becker et al. 2004, Djordjevic 2004, Barnett et al. 2004, Sarma and Emerich 2006, Djordjevic et al. 2007, Pessi et al.

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	leguminosarum bv. *viciae* - *P. sativum*; *R. leguminosarum* bv. *viciae* - *V. cracca; B. diazoefficiens* - *G. max*; *M. loti* - *L. japonicus*; *R. etli* - *P. vulgaris*		2007, Karunakaran et al. 2009, Resendis-Antonio et al. 2012, Vauclare et al. 2013, Tatsukami et al. 2013)
Nodules of varying maturity	*S. meliloti* - *M. truncatula*; *S. meliloti* - *M. sativa; B.* sp. ORS 278 - *A. indica*; *M. loti* - *L. japonicus*	Transcriptomics; proteomics	(Ampe et al. 2003, Capela et al. 2006, Delmotte et al. 2014, Nambu et al. 2015, Lardi et al. 2016, Marx et al. 2016)
Plant/bacterium mutants	*S. meliloti* - *M. truncatula*; *S. meliloti* - *M. sativa*; *B. diazoefficiens* - *G. max*	Transcriptomics; metabolomics	(Tian et al. 2006, Starker et al. 2006, Capela et al. 2006, Maunoury et al. 2010, Ye et al. 2013, Gemperline et al. 2015, Lardi et al. 2016)
Nodule zones	*S. meliloti* - *M. truncatula; S. medicae* - *M. truncatula*	Transcriptomics; proteomics; metabolomics	(Roux et al. 2014, Ogden et al. 2017)
Reference nodule proteome/transcriptome	*B. diazoefficiens* - *G. max; R. etli* - *P. vulgaris*	Transcriptomics; proteomics	(Sarma and Emerich 2005, Delmotte et al. 2010, Resendis-Antonio et al. 2011)
Sulfenylated nodule proteome	*S. meliloti* - *M. truncatula*	Proteomics	(Oger et al. 2012)
Peribacteroid space/membrane proteome	*R. leguminosarum bv. viciae* - *P. sativum*	Proteomics	(Saalbach et al. 2002)
Bacteroid periplasm	*B. diazoefficiens – G. max*	Proteomics	(Strodtman et al. 2017)
FixJ in nodules	*S. meliloti* - *M. truncatula*	Transcriptomics	(Barnett et al. 2004)
BacA in nodules	*R. leguminosarum bv. viciae*	Transcriptomics	(Karunakaran et al. 2010)
NifA in nodules	*R. etli* - *P. vulgaris*	Proteomics	(Salazar et al. 2010)
Control of plant immunity (plant *symCRK* mutant)	*M. truncatula* - *S.medicae*	Proteomics	(Berrabah et al. 2018)
C4-dicarboxylate transport	*R. leguminosarum bv. viciae* - *P. sativum*	Transcriptomics	(Mitsch et al. 2018)
Lipogenesis	*R. leguminosarum bv. viciae* - *P. sativum*	Metabolomics	(Terpolilli et al. 2016)
Effect of genetic variation	*S. meliloti* - *M. truncatula*; *S. medicae* - *M. truncatula*	Transcriptomics	(Heath et al. 2012, Burghardt et al. 2017)
Effect of plant host	*S. meliloti* - *M. truncatula*; *S. meliloti* - *M. sativa*; *R. leguminosarum* bv. *viciae* - *P. sativum*; *R. leguminosarum* bv. *viciae* - *V. cracca*; *S. fredii* - *V. unguiculata*; *S. fredii* - *L. leucocephala*; *B. diazoefficiens* - *G. max*; *B. diazoefficiens* - *V. radiata*; *B. diazoefficiens* - *V. unguiculata*; *B. diazoefficiens* - *M. atropurpureum*	Transcriptomics; metabolomics	(Ampe et al. 2003, Karunakaran et al. 2009, Li et al. 2013, Lardi et al. 2016)
Bacteroid morphology	*B.* sp. ORS285 - *Aeschynomene* spp.	Transcriptomics	(Lamouche et al. 2018)
Effect of field growth	*B. diazoefficiens* - *G. max*	Proteomics	(Delmotte et al. 2010)
Root versus stem nodules	*B.* sp. ORS278 - *A. indica*	Proteomics	(Delmotte et al. 2014)
Drought stress	*R. leguminosarum* - *P. sativum*	Proteomics	(Irar et al. 2014)
Other			
Reference free-living proteome	*S. meliloti; M. loti*; *B. diazoefficiens*; *B. japonicum*; *R. tropici*	Proteomics	(Kajiwara et al. 2003, Djordjevic et al. 2007, da Silva Batista et al. 2010, Gomes et al. 2012b, Kumar et al. 2013, Gomes et al. 2014)
Phosphorylated free-living proteome	*S. meliloti*	Proteomics	(Liu et al. 2015)
Effects of genome reduction	*S. meliloti*; *R. leguminosarum* bv. *trifolii*	Transcriptomics; proteomics; metabolomics	(Guerreiro et al. 1998, Chen et al. 2000b, Fei et al. 2016)
Growth with different carbon sources	*S. meliloti*; *R. leguminosarum* bv. *viciae*	Metabolomics; fluxomics	(Barsch et al. 2004, Fuhrer et al. 2005, Terpolilli et al. 2016)
Membrane lipid composition	*S. meliloti*	Lipidomics	(Basconcillo et al. 2009b)

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Table 2. Summary of the fields covered in this review.

Field	Goal	Key lessons
Socio-microbiology	Study the interactions between rhizobia and other organisms that influence environmental growth and the symbiotic process.	Rhizobia interact with many organisms (eukaryotes, prokaryotes, viruses), influencing symbiotic efficiency and competition for nodule occupancy.
Genetics and molecular biology	Characterize the precise function of individual genes and their role in complex biological processes including symbiosis.	Laid the groundwork for modern studies with rhizobia, establishing the fundamentals of the symbiotic interactions.
High-throughput functional genomics	Perform genome-scale analyses to identify genomic elements that contribute to a particular process, growth in a specific environment, or symbiosis.	Elucidated countless loci of relevance to symbiosis or other phenotypes (e.g., rhizosphere growth), highlighting the complexity of these processes.
Large-scale genome manipulation	Generate massive addition or deletions in the genomic content of the cell, and characterize the phenotypic effects and symbiotic effects.	Genes related to symbiosis are spread throughout the genome, and gain of classical symbiotic genes is not necessary sufficient for symbiosis.
Genomics	Compare genome content between strains or species to identify loci likely contributing to a particular phenotype, such as symbiosis.	Rhizobia show massive genome variations that could be related to symbiotic efficiency, and it appears that no gene is common and specific to rhizobia.
Transcriptomics	Identify changes in gene expression across environments or during symbiosis to gain insights into important functions and regulatory mechanisms.	Major transcriptional changes occur during symbiosis, and there is a constant transcriptional rewiring throughout the developmental process.
Proteomics	Identify changes in protein levels across environments or during symbiosis to gain insights into important functions and regulatory mechanisms.	Have provided information into the biochemical pathways that are active during symbiosis, and the roles of proteins and ncRNAs in free-living cells.
Metabolite analysis	Identify changes in metabolite concentrations, or reaction fluxes, across environments or during symbiosis to gain insights into metabolism.	Has been used to identify metabolic pathways active in symbiosis, and has been instrumental in studying the genetics of membrane lipid biosynthesis.
Metabolic modelling	Use computer simulations to predict effect of genomic or environmental alterations on metabolism, and to integrate multi-level omics data.	Used to predict changes in metabolism during shifts in environments and during symbiosis, but application to rhizobia has so far been limited.
Synthetic biology	Engineer cells for the purpose of studying a biological process, or for engineering improved abilities, such as greater symbiotic efficiency.	Has demonstrated the feasibility of at least the initial steps of engineering improved rhizobial inoculants and of transferring BNF directly to plants.

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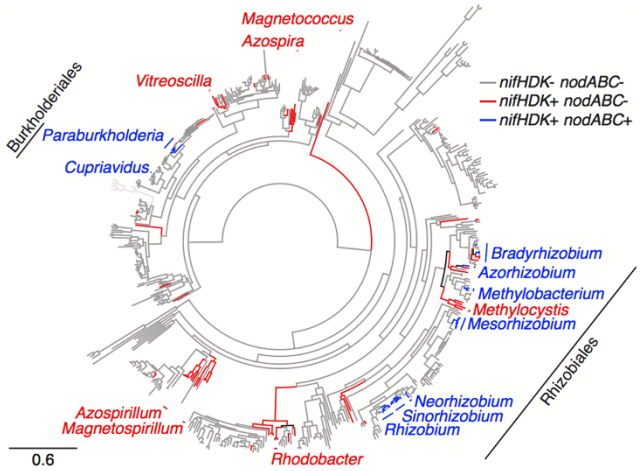

Figure 1. Phylogenetic distribution of biological nitrogen fixation. A maximum likelihood phylogeny of the α- and β-proteobacteria based on 23 highly conserved proteins (Frr, NusA, RplB, RplC, RplD, RplK, RplL, RplM, RplN, RplP, RplS, RplT, RpmA, RpoB, RpsB, RpsC, RpsE, RpsI, RpsJ, RpsK, RpsM, RpsS, Tsf). Species with at least one strain carrying the *nifHDK* nitrogen fixation genes are coloured red (sample genera are indicated), while species with at least one strain carrying the *nifHDK* nitrogen fixation genes and the *nodABC* nodulation genes are coloured blue (the genera and families are indicated). No strains have the nodulation genes but lack the nitrogen fixation genes. Branch lengths for the taxa that include the genera *Tremblaya*, *Hodgkinia*, and *Liberibacter* (light grey) were shortened for presentation. The complete phylogeny, including bootstrap values, is provided in File S2. See the Methods in File S1 for details on phylogeny construction and gene identification.

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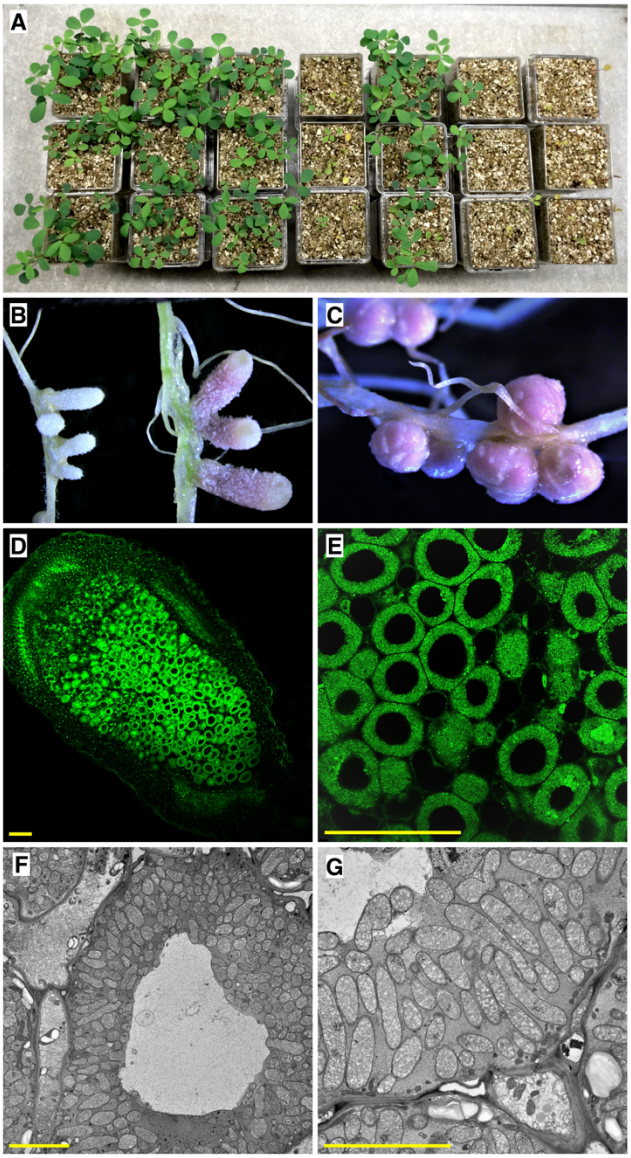

Figure 2. Visualizing the rhizobium – legume symbiosis. (**A**) A picture of *Medicago alba* (white sweet clover) plants grown in nitrogen free conditions. Plants in columns 1-3 and 5 are inoculated with nitrogen-fixing *S. meliloti*; plants in columns 4 and 6 are inoculated with a *S. meliloti* mutant unable to fix nitrogen; and plants in column 7 are not inoculated with *S. meliloti*. (**B**) *Medicago sativa* (alfalfa) root nodules, induced by *S. meliloti*. On the left are ineffective, white nodules containing a *S. meliloti* mutant unable to fix nitrogen; on the right are effective, pink nodules containing *S. meliloti* cells actively fixing nitrogen. (**C**) Effective, pink nodules of *Vigna unguiculata* (cowpea) containing *S. fredii* NGR234 cells actively fixing nitrogen. (**D**, **E**) Confocal micrographs of alfalfa root nodules filled with *S. meliloti* cells. The nodule sections were stained with a fluorescent nucleic acid binding dye (Syto9); the green colour is indicative of the presence of *S. meliloti*. Scale bar represents 250 µm. (**F**, **G**) Transmission electron micrographs of alfalfa root nodules, showing plant cells filled with nitrogen fixing *S. meliloti* bacteroids. Scale bar represents 25 µm.

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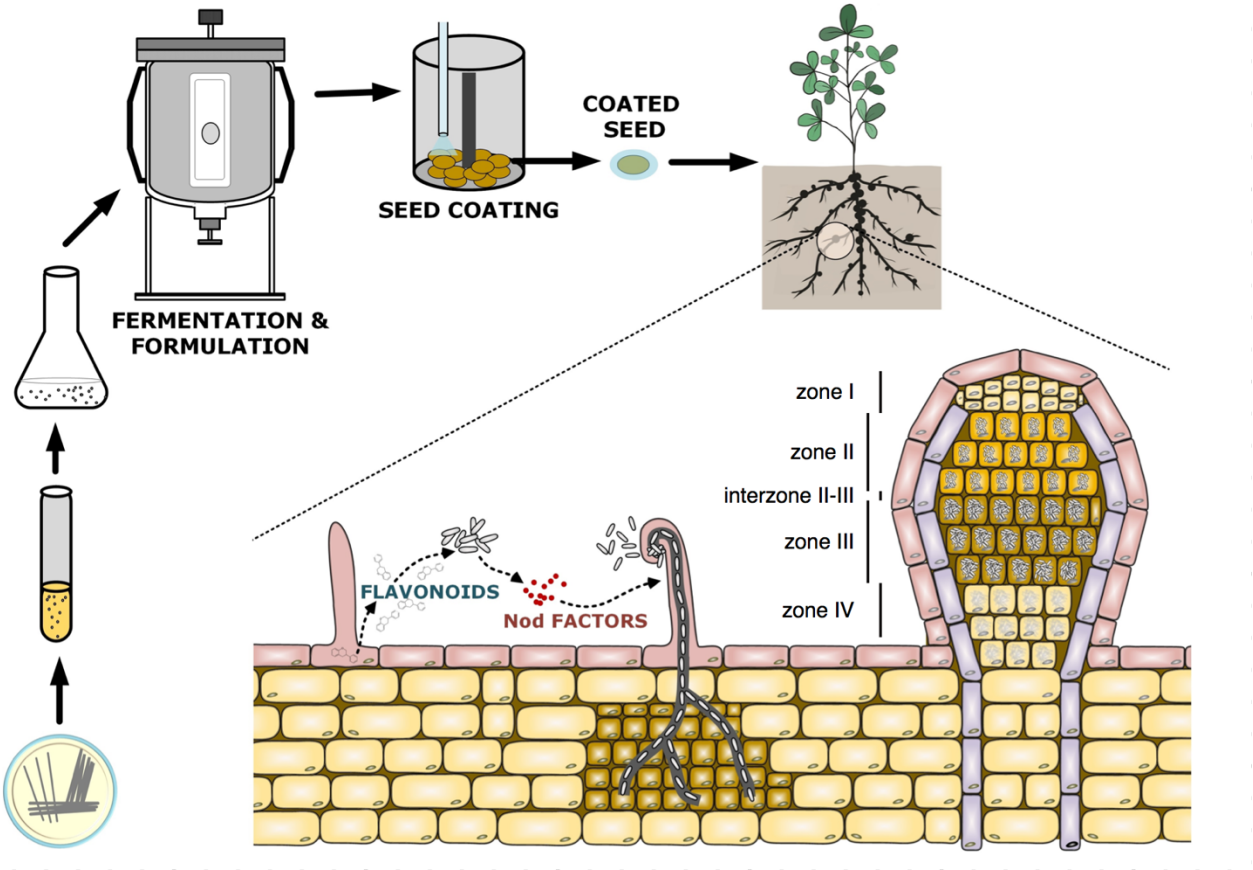

Figure 3. Schematic representation of the life-cycle of rhizobia. For inoculants, the life-cycle begins in an industrial environment, where the strain is grown in a fermenter. Inoculants are transported to the agricultural field, possibly as a seed coating. In the soil, the rhizobia must exploit nutrients, cope with the stresses, and compete with the indigenous microbiomes. The symbiotic interaction begins following an exchange of signals by the rhizobia and legume. Curling of a legume root hair traps a rhizobium cell, which then penetrates the cell wall of the root hair. The rhizobium proceeds to proliferates in a growing infection thread that progresses to the cortical cells. Here, the rhizobia are released into the cytoplasm of specialized cortical cells, resulting in them being enclosed in a plant derived membrane (peribacteroid membrane). The rhizobia and plant undergo a differentiation process that involves massive transcriptional and metabolic shifts, resulting in the formation of a nitrogen-fixing nodule. Five distinct developmental zones of the nodule are shown, but are not drawn to scale: zone I – apical meristem; zone II – infection and differentiation zone; interzone II-III – a small region between zone II and zone III; zone III – the nitrogen fixing zone; zone IV – the senescence zone found in mature nodules.

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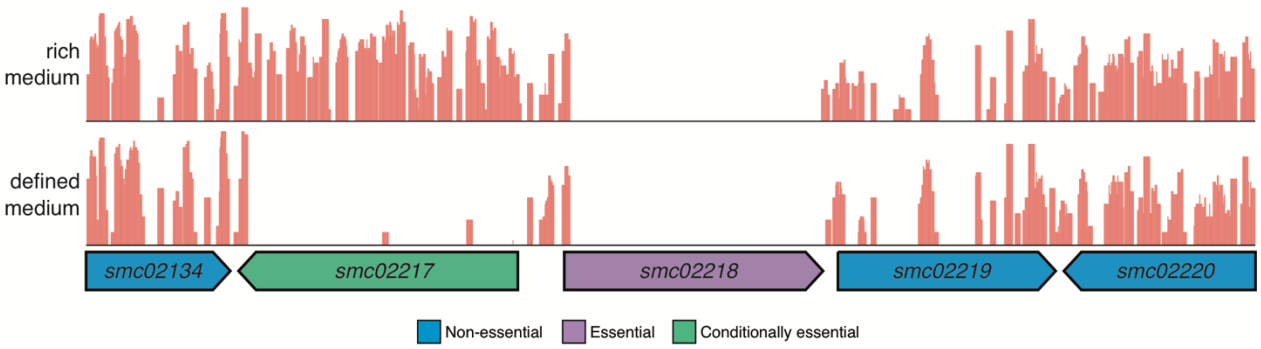

Figure 4. Example output of a Tn-seq study. A *S. meliloti* gene region illustrating the ability of Tn-seq to identify essential genes and environment-specific essential genes is shown. Published Tn-seq data (diCenzo et al. 2018) for *S. meliloti* grown in a nutritionally complex medium (rich medium) and a nutritionally defined medium (defined medium) were mapped to the *S. meliloti* chromosome. A five-gene region is shown. The location of the transposon insertions in the cell populations recovered from both conditions are indicated by the red bars, with the height of the bar representing the number of reads (log scale). Genes are colour coded based on their fitness classifications.

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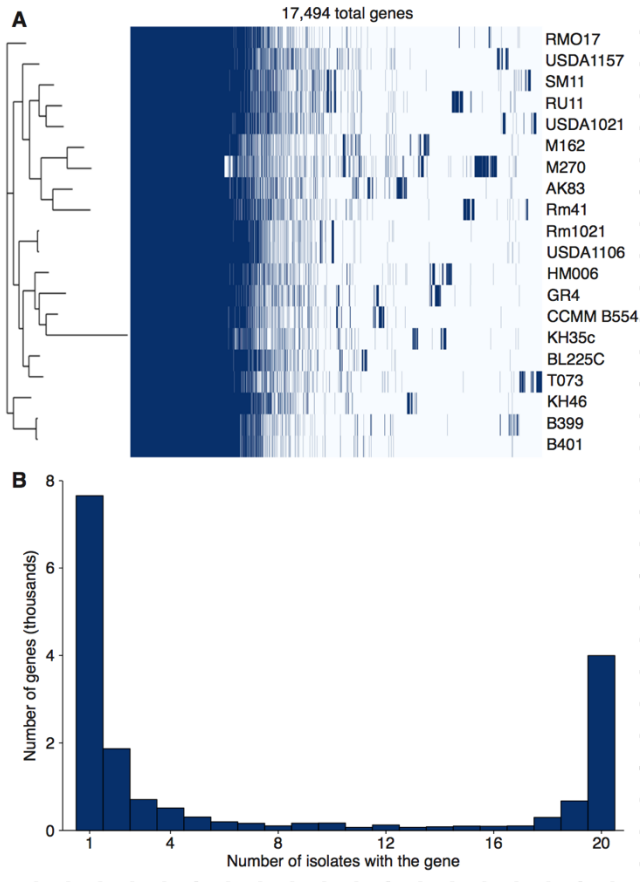

Figure 5. The *S. meliloti* pangenome. The pangenome of 20 *S. meliloti* strains with complete genomes was determined using Roary (Page et al. 2015). There was a median of 6,556 genes (standard deviation 362) per genome, and a total of 17,494 sets of orthologous proteins were detected of which 4,000 were present in all strains. (**A**) A heatmap showing gene presence (dark blue) or absence (light blue) in each of the 20 strains. A phylogeny built based on the 4,000 core genes is shown on the left, and the strain names are indicated on the right. (**B**) A histogram displaying the distribution of how many genomes each gene is found within. See the Methods in File S1 for details on pangenome construction.

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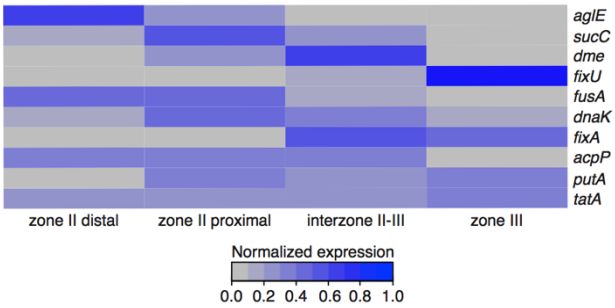

Figure 6. Expression patterns of select *S. meliloti* genes during symbiosis with *M. truncatula*.

The spatial expression patterns across a *M. truncatula* nodule of 10 *S. meliloti* genes, each displaying an unique pattern, is shown. Data was taken from the study of Roux *et al*. (Roux et al. 2014). The nodule zone is indicated along the bottom, and the gene names are given on the right. The plotted data is normalized across each zone; the darker the blue, the higher the expression of the gene in the indicated zone relative to other zones. For example, *aglE* is expressed strongly in the distal zone II but lowly in zone III, whereas *fixU* is primarily expressed in zone III. Colours provide no information on the expression of a gene compared to the other genes shown in the figure. Locations of each nodule zone are shown in Figure 3, with zone II distal and zone II proximal referring to the sections of zone II that are distal and proximal to the root, respectively.

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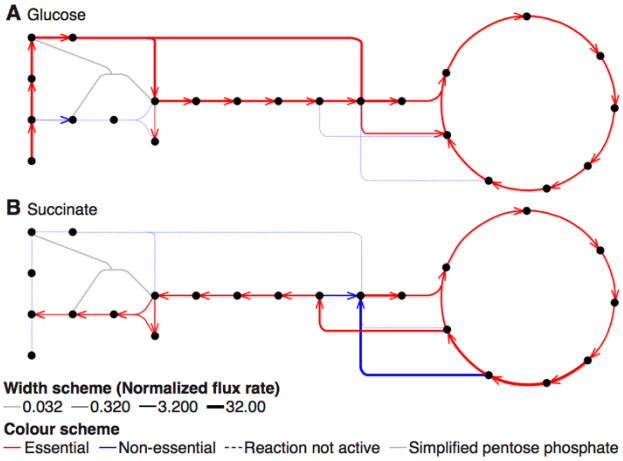

Figure 7. *In silico* predicted flux distributions and reaction essentialities. Example data obtained through metabolic modelling. Results from *in silico* metabolic modelling of *S. meliloti* metabolism using the iGD726 metabolic reconstruction are shown (diCenzo et al. 2018). Growth was simulated using (**A**) glucose or (**B**) succinate as the sole carbon source. The pathways represent central carbon metabolism, with the exception of the pentose phosphate pathway (for simplicity). The width of the line represents the amount of flux through the reaction; a doubling in the width corresponds to a ten-fold flux increase. Arrows indicate the direction of flux. Colours indicate if the reaction is essential (red) or non-essential (blue). See the Methods in File S1 for details on the modelling procedures used. A version with gene names is provided as Figure S1.

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