Curr Microbiol (2016) 72:804–816
DOI 10.1007/s00284-016-1005-0

REVIEW ARTICLE

Antibiotics Resistance in *Rhizobium:* Type, Process, Mechanism and Benefit for Agriculture

Judith Naamala[1] · Sanjay K. Jaiswal[2] · Felix D. Dakora[2]

Received: 30 October 2015 / Accepted: 7 January 2016 / Published online: 20 February 2016

Abstract The use of high-quality rhizobial inoculants on agricultural legumes has contributed substantially to the N economy of farming systems through inputs from biological nitrogen fixation (BNF). Large populations of symbiotically effective rhizobia should be available in the rhizosphere for symbiotic BNF with host plants. The rhizobial populations should also be able to compete and infect host plants. However, the rhizosphere comprises large populations of different microorganisms. Some of these microorganisms naturally produce antibiotics which are lethal to susceptible rhizobial populations in the soil. Therefore, intrinsic resistance to antibiotics is a desirable trait for the rhizobial population. It increases the rhizobia's chances of growth, multiplication and persistence in the soil. With a large population of rhizobia in the soil, infectivity of host plants and the subsequent BNF efficiency can be guaranteed. This review, therefore, puts together findings by various researchers on antibiotic resistance in bacteria with the main emphasis on rhizobia. It describes the different modes of action of different antibiotics, the types of antibiotic resistance exhibited by rhizobia, the mechanisms of acquisition of antibiotic resistance in rhizobia and the levels of tolerance of different rhizobial species to different antibiotics.

✉ Sanjay K. Jaiswal
sanjaysiswa@gmail.com

[1] Department of Crop Sciences, Tshwane University of Technology, Arcadia Campus, Pretoria, South Africa

[2] Department of Chemistry, Tshwane University of Technology, Arcadia Campus, Pretoria, South Africa

Introduction

Biological nitrogen fixation (BNF) is an environmentally friendly and easily accessible source of N for agricultural crops' N nutrition [111]. BNF occurs when a group of prokaryotes known as diazotrophs reduce atmospheric N_2 to ammonia via a nitrogenase enzyme complex [111, 174]. The legume-rhizobia symbiosis is the most important BNF process in agriculture in terms of quantities of N fixed [174]. It takes place in root nodules harbouring the rhizobia. In cases where the populations of indigenous soil rhizobia are insufficient, commercial rhizobial strains (inoculants) are introduced into the soil [19]. However, the establishment of these introduced rhizobial strains in the soil, their infectivity and symbiotic effectiveness is affected by a number of biotic and abiotic factors [2, 4, 20, 33, 41, 90, 174]. Such factors include, but are not limited to, antibiotics which are introduced into the soil by antibiotic-producing organisms and by faeces and urine [50, 101] because under concentrated animal feeding operations worldwide, antibiotics are commonly used to treat animal disease and promote animal growth. Ben et al. [14] reported that in China over 8000 t of antibiotics are used as feed additives each year. In Turkey, the usage of veterinary antibiotics in feeding operations has been reported to be 33 % of total pharmaceutical consumption [76]. About 16 million kg of antibiotics are used annually even in the USA, and 70 % are estimated to be used for non-therapeutic purposes [134]. So, the majority of used antibiotics are excreted in faeces and urine which persist and accumulate in soils after repeated manure application [74]. The

performance of *Rhizobium* inoculation in agricultural fields and its efficiency are critical due to competition occurring between the introduced rhizobia and the indigenous rhizobia present at high densities. Maximum nodule occupancy by the inoculant strain is achieved using *Rhizobium* strains having more host cultivar specificity, inoculum size and better competitiveness. Competition for nodulation is usually measured by comparing the ability of introduced *Rhizobium* strains to form nodules on the chosen host. Antibiotic resistance has been frequently used in distinguishing the introduced inoculant strain from indigenous rhizobia and monitoring their survival and occupancy of legume nodules [22, 143]. Antibiotic resistance is an ancient and naturally occurring phenomenon widespread in the environment [168]. The rhizosphere contains a mixture of metabolically active microbial populations that compete in this environment in relation to size, diversity and biochemical activity [90]. Production of antibiotics by some soil harbouring microorganisms mainly bacteria and fungi has been largely documented [10, 81, 92, 117]. Such organisms include Streptomyces like *Streptomyces coelicolor, Micromonospora purpurea* and *Streptomyces griseus* which produce actinorhodin, gentamycin and streptomycin, respectively [87, 156]. Spore-producing, filamentous, gram-positive actinomycetes, specifically Streptomyces species, produce the majority of known antibiotics [81]. The reasons for production of antibiotics by some soil harbouring microbes are not yet clear. Various reports have suggested a number of hypotheses for the production of antibiotics.

Hypotheses for Antibiotic Production by Some Soil Microbes

Competition

This hypothesis suggests that microbial organisms produce antibiotics for competition purposes [10, 92]. The hypothesis is supported by three lines of evidence [117]. First, various studies have indicated that production of antibiotics from bacteria inhibits the growth of pathogenic fungi of plants [71, 151]. Secondly, a survey on tropical rhizobia reported a prevalence of isolates which were highly tolerant to naturally produced antibiotics [126, 130]. This implies that rhizobial strains susceptible to the naturally produced antibiotics succumbed to the antibiotics. Lastly, when populations of bacteria and antibiotic-producing microbes were mixed, the densities of bacterial populations sensitive to antibiotics were significantly reduced [154]. It is assumed that production of antibiotics gives the producing organism an advantage over non-producing microbial populations [10, 92, 167].

Coordination of Developmental Process in the Organism

Dworkin et al. [48] suggested that some organisms produce antibiotics as a measure of coordinating their developmental stages. For instance, the antibiotics may maintain the dormancy of the spores produced by the antibiotic-producing organisms. This hypothesis is supported by the observation of antibiotic production and spore formation in the same organism that occurred at almost the same time [48].

Symbiotic Associations

This hypothesis was suggested by Kumbhar and Watve [87]. An example of the symbiosis between Streptomyces and beewolf digger wasp was given in support of the hypothesis [85]. The Streptomyces produces antibiotics which protect the beewolf digger wasp's cocoons from fungal infection hence increasing the larvae's chances of survival in the soil [75]. In return, the Streptomyces acquires nutrients from the beewolf digger wasp.

Homeostasis

It is hypothesised that the antibiotics act as signalling molecules for homeostasis regulation in the organism [92]. It is believed that at low concentrations, antibiotics may positively impact the susceptible bacterial species [87]. For instance, Bader et al. [11] showed that sub-lethal concentrations of antibiotics boost the growth of bacteria on solid media. Enhancement of motility and/or mutation of the bacterial cell by sub-lethal doses of the antibiotics was also reported [92]. Sub-lethal doses of antibiotics have also been reported to aid in exopolysaccharide synthesis. High concentrations of antibiotics, on the other hand, inhibit the growth of susceptible bacterial species [38].

Predation is the most recent suggested hypothesis. Since most antibiotic-producing organisms (e.g. Streptomyces) are predators, it is hypothesised that they produce antibiotics to aid the predation process. The hypothesis is supported by the fact that antibiotics are produced at the end of the growth period (when nutrients have been depleted) or under nutrient stress. The antibiotics lyse live cells of susceptible bacterial species, thereby providing nutrients for the organism [87].

Despite the conflicting hypotheses as to why some soil organisms produce antibiotics [87], the fact still remains that some microorganisms in the soil produce antibiotics. The produced antibiotics then inhibit growth and/or kill susceptible populations of the soil bacteria [10, 92]. Different classes of antibiotics exhibit different modes of action on the bacteria.

Mode of Action of Antibiotics

Interference with Cell Wall Synthesis

This can be through interfering with enzymes required for the production of the peptidoglycan layer [101]. The antibiotic agent acts by forming covalent complexes with enzymes that generate the mature peptidoglycan molecule [99]. This mode of action is common in β-lactam antibiotics like penicillin and vancomycin. As a result, the bacterial cell wall is weakened resulting into bacterial death due to osmotic pressure [6, 83].

Inhibition of Protein Synthesis

The antibiotic agent binds to the 16S and 23S rRNA hence inhibiting protein translation [2]. Tetracycline, actinomycin, aminoglycoside, oxazolidinone and macrolide-lincosamide-streptogramin (MLS) like kanamycin and streptomycin use this mode of action [50]. Streptomycin, for example, interferes with the 30S subunit of the bacteria's ribosomal RNA thereby affecting translation [164]. Chloramphenicol also inhibits protein synthesis in both gram-positive and gram-negative bacteria. This is due to its affinity for the peptidyl transferase of the 50S ribosomal subunit of 70S ribosomes [136]. The binding of chloramphenicol to peptidyl transferase enzyme prevents elongation of the peptide chain [138]. The target sites and function of some commonly used antibiotics with rhizobial cells are indicated in Table 1.

Inhibition of Nucleic Acid Synthesis

This results from inhibition of either DNA or RNA synthesis. For example, fluoroquinolones inhibit DNA synthesis and also cause lethal double-strand DNA breaks during replication [46]. Bleomycin also uses this mode of action.

Inhibition of Metabolic Pathway

The antibiotic may block the pathway for folic acid synthesis in the bacteria [119]. For example, trimethoprim antibiotic acts by inhibiting folic acid metabolism in the bacteria [36].

Disruption of Bacterial Membrane Structure

The antibiotic may increase the rhizobial membrane permeability causing leakage of bacterial cell content [24, 148]. Lipopeptides use this mode of action [2]. For instance, daptomycin is reported to insert its lipid tail into the bacterial cell membrane leading to depolarisation of the bacterial membrane and eventual death of the bacterium [24].

Although antibiotic resistance in bacteria is a threat in the health sector [2, 150, 166], it is a desirable trait in both indigenous and introduced (commercial inoculants) rhizobial populations [5, 9, 174]. Resistance to antibiotics increases the rhizobium's chances of survival in the rhizosphere. Rhizobial strains should be resistant to concentrations of antibiotics that inhibit the growth of other soil

Table 1 Some commonly used antibiotics with legume symbionts, their target sites and function

Serial no.	Antibiotic	Target sites	Function	Reference
1.	Streptomycin	16S rDNA	Inhibit protein synthesis	[70]
2.	Neomycin	16S rDNA	Inhibit protein synthesis	[102]
3.	Kanamycin	16S rDNA	Misreading and inhibit the translocation of the tRNA-mRNA complex and prevent protein synthesis	[133]
4.	Erythromycin	23S rDNA	Inhibit protein synthesis	[43]
5.	Ampicillin	Trans-peptidase	Irreversible inhibition of enzyme trans-peptidase which is needed by bacteria to make their cell wall	[158]
6.	Penicillin	Trans-peptidase	Inhibition of trans-peptidase	[158]
7.	Chloramphenicol	23S rRNA (peptidyltransferase)	Prevent protein chain elongation	[135]
8.	Tetracycline	Aminoacyl-tRNA	Inhibit protein synthesis	[28]
9.	Nalidixic acid	DNA gyrase	Block DNA replication by formation cleavage complexes	[120]
10.	Rifampicin	RNA polymerase	Inhibit DNA-dependent RNA polymerase synthesis	[23]
11.	Gentamicin	16S rRNA	Inhibit protein synthesis	[104]
12	Spectinomycin	16S rRNA	Inhibit protein synthesis	[70]

bacteria and they should be able to retain their infectivity and symbiotic effectiveness. While the majority of rhizobial strains in the soil are susceptible to antibiotics, others have developed resistance in response to naturally produced antibiotics [16, 164, 167]. The resistance may be developed towards one or multiple antibiotic classes [5]. Unlike resistance to some soil stress, which may be dependent on the origin of the rhizobial strains, antibiotic resistance is not [167].

Rhizobial genome organisation has been widely studied. Usually, these bacteria have one chromosome and several plasmids and/or megaplasmids that may represent 50 % of the genome. Symbiotic genes are typically located in megaplasmids, known as pSyms. Non-symbiotic plasmids may encode locally adaptive traits that confer phenotypic advantages, such as heavy metal or antibiotic resistance genes. In evolutionary terms, plasmid-encoded genes have the advantage of being more easily exchangeable within a certain population than genes located in the chromosome [3].

Therefore, increase in resistance of rhizobia to antibiotics is mainly due to mobile genes on plasmids that can readily spread through rhizobial populations [86]. It is also important to note that the more rhizobial populations are exposed to antibiotic agents, the more they develop resistance due to selective pressure.

Types/Forms of Rhizobial Resistance to Antibiotics

There are two forms of antibiotic resistance in bacteria, namely intrinsic/innate and acquired resistance [2, 150].

Intrinsic/innate resistance occurs naturally in a bacterial species [99]. It results from genes which naturally occur in the bacterium's chromosome [2]. Such genes include *ampC* β-lactamase found in gram-negative bacteria. Cole and Elkan [30] reported that *R. japonicum* (now *Bradyrhizobium japonicum*) carries extra chromosomal antibiotic resistance genes. Intrinsic resistance may also result from the multiple drug resistance efflux systems within the bacterial cell. Gram-negative bacteria (including rhizobia) are intrinsically resistant to the activity of macrolides because macrolides are slow to traverse the bacterial cell wall [99]. It is also because the macrolides are simultaneously extruded by the activity of efflux pumps [82]. Intrinsic resistance could also result from a lack of the antibiotic target site [99]. Bacterial species with intrinsic resistance could be resistant to a single or multiple classes of antibiotics [150].

Acquired resistance occurs when susceptible rhizobial strains become resistant to the antibiotic agent they were susceptible to [99, 150]. The new resistant strains proliferate and spread under selective pressure of the antibiotic agent [150]. Unlike intrinsic resistance, acquired resistance only occurs to some strains of a species [99]. Acquired resistance is brought in bacteria through mutation in genes targeted by antibiotics and the transfer of resistance determinants born on plasmids, bacteriophages, transposons and other mobile genetic material [2, 66]. The acquired genes may provide resistance to a whole class of antimicrobials [99]. These genes are frequently associated with large transferable extra chromosomal DNA elements called plasmids, on which may be other mobile DNA elements such as transposons and integrons [99]. Gene transfer may be intra- or inter-genera [31]. This implies that a rhizobial strain may acquire or transfer genes for antibiotic resistance from/to members of the same or different genera [30]. This is referred to as horizontal gene transfer [156]. Kuykendall [88], reported the transfer of plasmid genes (R factor) to and between genetically marked strains of *R. japonicum*. The plasmid gene transfer was from *P. aeruginosa* and *E. coli* to *R. japonicum*. However, the *B. japonicum* requires more time for mating and for phenotypic expression compared to other bacteria. In cases of mutation, the antibiotic target gene within the bacterium may be altered or expelled leaving no site to which the antibiotic agent can bind [150]. Antibiotic-resistant (AR) phenotypes come about due to chromosomal DNA mutations [147]. The mutations alter existing bacterial proteins, through processes like transformation which can create mosaic proteins [97]. AR phenotypes can also be brought about by transfer and acquisition of new genetic material between bacteria of the same or different species or genera [97, 110, 147]. Alternatively, mutations may cause the bacterium to produce enzymes which inactivate the antibiotic agent before it reaches the target site [150]. Mutations may also lead to the altering of the bacterium's outer membrane protein channel which the antimicrobial agents require entering the bacterium [150]. This makes it impossible for the antimicrobial agent to access the target sites within the bacterial cell. Lastly, the bacterium can up-regulate efflux pumps which expel the antibiotic agent from the bacterial cell [66, 101]. It should be noted that some rhizobial species/strains lose their symbiotic effectiveness and infectivity once they mutate to AR forms [175, 176]. Other rhizobial species/strains, however, maintain their infectivity and symbiotic effectiveness [63]. Acquired resistance resulting from chromosomal mutations and selection is termed vertical evolution [150]. Resistance resulting from the acquisition of genetic material from other organisms is referred to as horizontal evolution [150].

Processes Through Which Rhizobia Acquire Antibiotic Resistance Genes

Since soil is a reservoir of antibiotic-resistant genes (ARGs) [51], transfer of antibiotic resistance determinant genes among rhizobia is effected by three main parasexual

processes, namely conjugation [68, 69], transformation [122] and transduction [84]. Conjugation and transduction often transfer particular parts of the chromosomes that are associated with the integration sites of episomal elements. Transformation is a generalised mechanism that can in principle mediate the transfer of any part of the chromosome [97].

Conjugation involves the transfer of DNA from one rhizobial cell to another rhizobial cell through direct contact [8]. During conjugation, the antibiotic resistance determinant genes are carried on conjugative plasmids and conjugative transposons from the donor bacteria to the recipient bacteria [2, 66, 150]. More than a single plasmid can occur in a host *Rhizobium* and their multiplication does not depend on the host. Transposons, on the other hand, are a mobile genetic material which may exist on plasmids, integrate into other transposons or in the host's chromosome. They can also aid in the transfer of endogenous plasmids from one bacterium to another [2]. Beringer [16] showed the ability of *R. leguminosarum* to donate and receive R factors belonging to the compatibility class P (which are associated with plasmids carrying antibiotic resistance genes) in mixed culture with other bacteria. The same author reported the transfer of R factors RP4, RK2 and R6886 carrying resistance to kanamycin/neomycin, tetracycline and carbenicillin from *E. coli* to *R. leguminosarum*. Datta et al. [37] also reported the transfer of R factors (Rp4) carrying high levels of resistance to carbenicillin from *E. coli* to *R. trifolii* and *R. meliloti* by conjugation. Transfer of antibiotic resistance genes from *R. japonicum* to *Agrobacterium tumefaciens* through conjugation was also reported [29].

Transformation is a process through which a rhizobium acquires free/naked DNA from the environment and incorporates it into its genome [89, 150] by homologous recombination or transposition [156]. A wide range of DNA fragments can be transferred between bacteria by transformation [97]. The free DNA is normally released to the environment as a result of cell lysis of the donor bacterium. Balassa [12] reported acquisition of resistance to streptomycin by three rhizobial species (*R. japonicum*, *R. meliloti* and *R. lupini*) through transformation. However, the frequency of transformation varied between the three rhizobial species. Acquisition of penicillin resistance genes by *R. phaseolus* and *R. leguminosarum* strains through transformation was also reported [53]. There are three requirements for bacterial transformation, namely opportunity (presence of exogenous DNA in the immediate environment of the host *Rhizobium*), DNA uptake (presence of DNA uptake mechanisms in the host rhizobium) and incorporation of the acquired DNA into the host rhizobium's chromosome [94, 97]. Since transformation can allow transfer of any part of the chromosome, it can be equated to recombination in sexual organisms with the one exception that a single part of the chromosome is transferred per a single process of transformation [77].

Transduction is a rare process of gene transfer. It occurs when the antibiotic resistance determinant gene is transferred from the donor bacterium to the recipient *Rhizobium* by a bacterial phage [2, 150]. Instead of the phage DNA, bacterial DNA is packaged into the phage head and injected into the recipient *Rhizobium* [156]. There are two types of transduction, namely generalised and specialised transduction. In generalised transduction, any element of DNA is packaged into the phage head [156]. In specialised transduction, only DNA adjacent to the phage insertion site is packaged. Kowalski [84], Sik and Orosz [141] reported gene transfer in rhizobia by transduction. *Rhizobium* phage 16-3 transferred genetic material to *R. meliloti* strain 41 [141].

Mechanisms of Rhizobial Resistance to Antibiotic

Mechanisms of resistance to antibiotics in rhizobia can be described as active or passive based on how the mechanisms are put in place. Passive resistance is not directly put in place to resist a particular antibiotic or class of antibiotics. Instead, it results from general adaptive processes, for example, the presence of specific barriers on the outer membrane of gram-negative bacteria. Active resistance involves three main mechanisms, namely modification of antibiotic target site, active efflux of the antimicrobial agent from the bacterial cell and enzymatic modification/degradation of the antimicrobial agent. All active mechanisms require changes in their genetic programming in response to the presence of antibiotic agents [165]. Other resistance mechanisms include the acquisition of alternative metabolic pathways, over production of the target enzyme and permeability changes in the bacterial cell wall.

Modification of the Antibiotics Target Site

The commonest targets of antibiotic agents include enzymes involved in major life processes like cell wall synthesis, protein synthesis and nucleic acid synthesis [101, 150]. Such sites include DNA gyrase, topoisomerase IV, dihydrofolate reductase and penicillin-binding protein (PBP) sites [45, 99, 146]. Rhizobia may modify antibiotic target sites through mutation, for example, mutation of the ribosomal RNA [101, 165]. The mutation may be a point mutation or mutation of the entire chromosome [2]. Gram-negative bacteria (including rhizobia) develop resistance to fluoroquinolones and coumarin by a mutation in the DNA gyrase and topoisomerase IV. Some antibiotics like rifamycins arrest transcription by interacting with RpoB, MLS

classes of antibiotic target ribosome to inhibit protein translation. Some bacteria acquire resistance to penicillin through mutation in the PBP.

Some bacteria modify the antibiotic target site by expression of a variant of the target site. The variant may have similar or close to similar functions as the antibiotic target site but with less or no affinity to the antibiotic agent [97, 156]. Some bacteria acquire penicillin resistance through the acquisition of entirely new PBP which have reduced affinity for penicillin or do not bind the penicillin at all [156].

The other modification could be reprogramming the biosynthetic pathway which is the case in the resistance to glycopeptide antibiotics [165].

Enzymatic Modification

In this case, the bacteria synthesise enzymes which selectively target the antibiotic agent [165]. The bacterium acquires genes which encode enzymes that destroy or break down the antibiotic agent to a new form before it reaches the target site. The new form is normally non-toxic to the bacterial cell [66, 150]. Such genes are normally introduced into the bacterial cell from the exterior. Rhizobia acquire resistance to β-lactams like penicillin by the production of one or more β-lactamases that inactivate the antibiotic agent [115]. The β-lactamases inactivate the antibiotics by cleaving their chemical bonds by hydrolysis [156, 166]. Five genes have been reported with regard to bacterial resistance to aminoglycosides (e.g. streptomycin and kanamycin), namely *armA* [54] *npmA*, *rmtA*, *rmtB*, *rmtC* and *rmtD* [31, 42]. Other enzymes modify the antibiotic to a form which impairs the target site binding [165]. The modification, however, requires co- substrates like ATP, acetyl-CoA, glutathione and NAD^+ for activity [165]. The best examples of such enzymes are the aminoglycoside acetyltransferases and chloramphenicol acetyltransferases (CATs) which confer resistance in some bacteria to aminoglycosides and chloramphenicol, respectively [109, 138, 165]. CATs inactivate most antibiotics in the chloramphenicol group except florfenicol which has structural modifications. CATs are coded by two genes—classical *catA* determinants and novel *catB* variants [156]. These two genes differ significantly in their structure. There are also redox enzymes which inactivate the antibiotic agent by the redox reaction [165]. For example, the redox enzyme Tet X oxidises tetracycline [114, 171]. Lyases follow non-hydrolytic or non-oxidative routes to cleavage off carbon–carbon, carbon–oxygen, carbon–nitrogen and carbon–sulphur bonds [108]. The commonest example is the Vgb lyase which is responsible for type B-streptogramin resistance.

Development of Efflux Pumps

Efflux pumps are membrane-associated proteins [165]. The efflux pumps actively extrude antibiotic agents from the bacterial cell before it reaches the target site [66, 150]. This lowers the concentration of the antibiotic agent in the bacterial cell [99]. Because, it is an active process, it implies that energy in the form of ATP is required. The antibiotic agent is excluded from the bacterial system without being altered or degraded. This mechanism may lead to multiple antibiotics resistance. This is because a single efflux pump may extrude multiple antibiotic agents. It should be noted that the efflux pumps not only extrude antibiotic agents from the bacteria's system but also other chemically and structurally unrelated compounds. There are five families of efflux pumps: the major facilitator super family (MFS), the adenosine triphosphate (ATP)-binding cassette (ABC) super family, the resistance-nodulation-cell division (RND) sub family, the small multi drug resistance (SMR) family and the multidrug and toxic compound extrusion (MATE) family [98, 113, 117, 132]. Some chloramphenicol-specific efflux proteins like CmlA, expression of the resistance determinant is mediated through an inducible mechanism of translational attenuation. In most of the gram-negative bacteria (rhizobia), intrinsic antibiotic resistance is attributed to the expression of the RND efflux system (s) that allows them to transport drugs from the cytoplasm and across the inner and outer membranes of the cell envelope [2]. The outer membrane of gram-negative cell envelope is a barrier to both hydrophobic and hydrophilic compounds. These organisms have evolved porin proteins (OmpF in *E. coli*) that function as non-specific entry and exit centres for antibiotics [2]. The tetracycline efflux proteins contain 12 (TetA-E) putative transmembrane-spanning segments in gram-negative bacteria. Expression of proteins is controlled by a transcription repressor such as TetR. The antibiotics inactivate the repressor and allow expression of the tetracycline efflux systems [2]. In *R. etli*, *Rhizobium* multiresistance genes (*rmrA* and *rmrB*) against phytoalexin and salicylic acids were identified [59]. The protein encoded by gene *rmrA* belongs to membrane fusion protein (MFP), is similar in sequence, length, hydropathy plots and proteins encoded by genes adjacent to multiresistance proteins EmrA and VceA of *Escherichia coli* and *Vibrio cholera*, respectively, while RmrB protein encoded by *rmrB* gene belongs to the drug resistance protein family. An *R. etli rmrA* mutant was complemented with the antibiotic resistance genes *emrAB* from *E. coli* [59].

Alteration/Changes in the Permeability of the Cell Wall

Bacterial strains acquire resistance to antibiotics by changing their cell wall structure. This may be through

Table 2 Antibiotic resistance and susceptibility of different *Rhizobium* and *Bradyrhizobium species*

Rhizobium/Bradyrhizobium sps.	Isolated from	chl	eryth	amp	kan	Neo	Str	Gent	Pen	Pefl	Reference
		μg/ml									
R. giardinii	*Phaseolus vulgaris*	nd	100	nd	S	S	S	5	–	–	[160]
R. huautlense	*Sesbania herbacea*	S	5	S	5	5		S	–	–	[159]
R. indigoferae	*Indigofera spp.*	5	50	5	S	5	5		–	–	[163]
R. calliandrae	*Calliandra grandiflora*	S	–	–	–	–	–	–	–	5	[128]
R. mayense		S	–	–	–	–	–	–	–	S	
R. jaguaris		S	–	–	–	–	–	–		S	
R. laguerreae	*Vicia faba*	–	S	R	–	S	–	S	R	–	[131]
R. lemnae	*Lemna aequinoctialis*	–	–	–	–	–	S	S	10	–	[79]
R. leucaenae	*Phaseolus vulgaris*	S	–	–	S	–	–	–	–	–	[127]
R. loessense	*Astragalus*	5	S	100	S	–	S	–	–	–	[162]
R. lusitanum	*Phaseolus vulgaris*	–	2	S	–	S	–	S	10	–	[155]
R. mesosinicum	*Albizia, Kummerowia and Dalbergia*	–	100	100	50	S	100	S	–	–	[91]
R. miluonense	*Lespedeza*	5	S	100	S	5	5	S	–	–	[61]
R. multihospitium	*Native legumes of China*	100	50	300	50	50	100	–	–	–	[65]
R. leguminosarum	*Phaseolus vulgaris*	–	–	50	–	–	S	–	–	–	[152]
R. fredii	*Glycine max*	–	–	–	20	S	S	S	50	–	[137]
R. halophytocola	*Coastal dune plant*	10	20	S	S	–	–	–	–	–	[18]
R. herbae	*Wild legumes of China*	–	S	–	50	S	S	5	–	–	[160]
R. oryzae	*Oryza alta*	S	S	100	S	–	S	–	–	–	[118]
R. sphaerophysae	*Sphaerophysa salsula*	100	50	100	S	–	–	–	–	–	[170]
R. taibaishanense	*Kummerowia striata*	–	–	50	S	–	50	–	–	–	[172]
R. tropici CIAT 899	*Phaseolus vulgaris*	30	15	10	–	S	10	–	10	–	[34]
R. tubonense	*Oxytropis glabra*	–	100	–	50	50	5	–	–	–	[177]
R. alamii	*Arabidopsis thaliana*	–	–	–	S	–	S	–	–	–	[15]
R. alkalisoli	*Caragana intermedia*	5	50	300	50	5	5	–	–	–	[96]
R. azibense	*Phaseolus vulgaris*	80	–	–	10	S	5	–	–	–	[105]
R. cauense	*Kummerowia stipulacea*	50	50	100	5	5	5	–	–	–	[93]
R. etli CFN42	*Phaseolus vulgaris*	–	–	50	–	–	300	–	–	–	[152]
R. fabae	*Vicia faba*	5	5	5	5	5	100	–	–	–	[152]
R. freirei	*Phaseolus vulgaris*	S	15	S	–	S	S	–	10	–	[34]
Bradyrhizobium arachidis	*Arachis hypogaea*	50	50	S	–	–	5	50	–	–	[161]
B. betae	*Beta vulgaris*	30	15	–	–	–	–	R	R	–	[40, 129]
B. canariense	*Genistoid legumes*	30	100	–	–	–	10	–	10	–	[40, 157]
B. cytisi	*Cytisus villosus*	–	R	R	–	S	–	S	R	–	[25]
B. daqingense	*Glycine max*	50	50	–	–	–	–	50	–	–	[161]
B. diazoefficiens	*Glycine max*	30	15	10	–	S	S	–	10	–	[40]
B. japonicum USDA 6	*Glycine max*	30	15	S	–	S	S	–	S	–	[40]
B. ganzhouense	*Acacia melanoxylon*	300	100	–	100	50	5	5	–	–	[95]
B. huanghuaihaiense	*Glycine max*	50	100	50	5	50	5	100	–	–	[178]
B. iriomotense	*Entada koshunensis*	S	100	–	S	–	–	–	–	–	[72]
B. lablabi	*Lablab purpureus*	50	5	50	–	–	5	5	–	–	[27]
B. liaoningense	*Glycine max*	50	S	–	–	30	–	25	S	–	[169]
B. manausense	*Vigna unguiculata*	50	30	25	S	10	S	10	10	–	[142]
B. oligotrophicum	*Rice*	–	–	–	–	–	–	–	–	–	[124]
B. ottawaense	*Glycine max*	100	50	–	100	–	–	–	10	–	[173]
B. pachyrhizi	*Pachyrhizus erosus*	–	50	1	S	S	–	S	2	–	[125]
B. jicamae		–	S	1	S	S	–	S	2	–	

Table 2 continued

Rhizobium/Bradyrhizobium sps.	Isolated from	chl	eryth	amp	kan	Neo	Str	Gent	Pen	Pefl	Reference
		μg/ml									
B. paxllaeri	*Phaseolus lunatus*	–	50	–	S	–	S	5	–	–	[47]
B. icense		–	S	–	S	–	S	S	–	–	
B. retamae	*Retama sphaerocarpa and Retama monosperma*	–	50	1	–	5	–	S	2	–	[62]
B. rifense	*Cytisus villosus*	30	15	10	–	S	S	S	10	–	[26, 40]
B. yuanmingense	*Lespedeza*	30	15	S	S	S	S	–	S	–	[40]
B. neotropicale	*Centrolobium paraense*	–	30	–	–	–	S	10	S	–	[179]
B. elkani		50	50	50	–	5	–	5	–	–	[27]

Str streptomycin, *Neo* neomycin, *Kan* kanamycin, *Eryth* erythromycin, *Amp* ampicillin, *Pen* penicillin, *Chl* chloramphenicol, *Gen* gentamicin—not determined

changes in cell wall porins that limit movement of the antibiotic agent to the target site. The bacteria may also acquire genes for a metabolic pathway which produces altered bacterial cell walls. The altered cell walls may not contain binding sites for the antibiotic agent [150]. As a result, the antibiotic agent is excluded from the bacterial cell [97]. This mechanism of resistance may lead to multiple antibiotic resistance because more than one type of antibiotic agent may use the same cell wall porin [99].

Benefits of Antibiotic Use in Agriculture

Scientists have noted the potential benefits associated with using antibiotics to know the resistance or susceptibility level of rhizobium. The antibiotic-resistant *Rhizobium* makes itself competitive in soil environment to occupy high number of nodules in legumes [13, 56]. Large differences in degree of tolerance to antibiotics among fast- and slow-growing rhizobia have been reported [30, 52, 60, 112, 116]. Since the first exploitation of natural rhizobial strain-to-strain variations in intrinsic resistance to antibiotics (IAR) for identification and differentiation of nodule isolates from common bean [17] and chickpea [3], IAR has been used extensively in ecological studies to identify inoculant strains and to determine heterogeneity in natural populations [49]. Some of the intrinsic antibiotic resistance properties of *Rhizobium* and *Bradyrhizobium* sps. are shown in Table 2. Belachew [13] reported that field pea variety 'Wayitu' from Ethiopia showed the best combination with antibiotic resistant of strain EAL 302 for nodule occupancy. This method has proven practical, rapid and reliable with a discriminating ability dependent on the number of antibiotics and the concentrations used. IAR has been used either as a primary criterion or as a complement to other methods to describe diversity in nodule isolates from alfalfa [73, 140], pea [21, 153], clover [58, 64], *Phaseolus* sp. [7], chickpea [3, 55, 78], soybean [1, 44, 103, 106, 107, 123], cowpea [144, 167] and various tropical legumes [35, 100, 149]. High doses of antibiotics were used to get the mutant of bradyrhizobia to show resistant to soil rhizobiophage [5]. The authors had found that strain MPSR033 Sm^r V^r antibiotic and phage-resistant mutant of soybean nodulating *Bradyrhizobium* was highly competitive for nodule occupancy. Singh et al. [145] reported that soybean nodulating isolate ALSR12, a mutant of gentamicin and also phage-resistant, showed *ex*-planta nitrogenase activity comparable to the parent strain.

Numerous reports have indicated that agricultural practices, such as direct application of antibiotics or animal manure, provide positive selective pressure for antibiotic-resistant bacteria, often resulting in increased number and resistance level of these bacteria and ARGs in agricultural soils, and thereby expanding the level of native resistance in soil [80, 121]. In addition, a considerable amount of resistant bacteria added into the soil through manure amendment would lead to the spread of resistance to soil bacteria [57, 67]. The result of Anand et al. [5] showed that inoculation of soybean with an antibiotic and phage-resistant mutant of bradyrhizobia that showed the high ability for nitrogen fixation was effective in increasing soybean production in Indian soil.

Conclusion

Antibiotic-resistant genes (ARGs) found in bacterial chromosome and plasmid are increasing in number due to rapid accumulation of antibiotic in the environment [39, 166]. Antibiotic resistance has become one of the most important sustained driving forces for antibiotic discovery. The soil has been regarded as a rich source of ARGs, emanating from natural and anthropogenic processes. Although a high frequency of antibiotic resistance was

found in cultured and uncultured soil bacteria which represent a reservoir of new ARGs, use of metagenomic approach [51] can help in the preparation of single or multiple AR mutants of *Rhizobium* against these new antibiotic-producing microorganisms. Some rhizobial strains exhibit multiple antibiotic resistances [32, 167] but some possess no detectable antibiotic markers [32, 139]. In that situation, antibiotic markers could be introduced either by isolating spontaneous mutants or by transpositions.

Acknowledgments This work was supported with grants from the Bill and Melinda Gates Foundation Project on Capacity Building in Legume Sciences in Africa, the South African Department of Science and Technology, the Tshwane University of Technology, the National Research Foundation in Pretoria, and the South African Research Chair in Agrochemurgy and Plant Symbioses. JN is grateful for a competitive fellowship from the Bill and Melinda Gates Foundation Project on Capacity Building in Legume Sciences in Africa.

References

1. Abaidoo R, Keyser H, Singleton P, Borthakur D (2002) Comparison of molecular and antibiotic resistance profile methods for the population analysis of *Bradyrhizobium* spp. (TGx) isolates that nodulate the new TGx soybean cultivars in Africa. J Appl Microbiol 92:109–117
2. Alekshun MN, Levy SB (2007) Molecular mechanisms of antibacterial multidrug resistance. Cell 128:1037–1050
3. Alexandre A, Laranjo M, Oliveira S (2006) Natural populations of chickpea rhizobia evaluated by antibiotic resistance profiles and molecular methods. Microb Ecol 51:128–136
4. Alexandre A, Oliveira S (2011) Most heat-tolerant rhizobia show high induction of major chaperone genes upon stress. FEMS Microbiol Ecol 75:28–36
5. Anand A, Jaiswal SK, Dhar B, Vaishampayan A (2012) Surviving and thriving in terms of symbiotic performance of antibiotic and phage-resistant mutants of *Bradyrhizobium* of soybean [*Glycine max* (L.) Merrill]. Curr Microbiol 65:390–397
6. Andes D, Craig W (2005) Cephalosporins. In: Mandell GL, Bennett JE, Dolin R (eds) Principles and Practice of Infectious Diseases, 6. baskı''kitabında s. Elsevier Churchill Livingstone, Philadelphia, pp 294–311
7. Arredondo-Peter R, Escamilla E (1993) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein banding patterns among *Rhizobium leguminosarum* biovar phaseoli strains isolated from the Mexican bean *Phaseolus coccineus*. Appl Environ Microbiol 59:3960–3962
8. Aune TEV, Aachmann FL (2010) Methodologies to increase the transformation efficiencies and the range of bacteria that can be transformed. Appl Microbiol Biotechnol 85:1301–1313
9. Ausili P, Borisov A, Lindblad P, Mårtensson A (2002) Cadmium affects the interaction between peas and root nodule bacteria. Acta Agric Scand Sect B Plant Soil Sci 52:8–17
10. Baba T, Schneewind O (1998) Instruments of microbial warfare: bacteriocin synthesis, toxicity and immunity. Trends Microbiol 6:66–71
11. Bader MW, Navarre WW, Shiau W, Nikaido H, Frye JG, McClelland M, Fang FC, Miller SI (2003) Regulation of *Salmonela typhimurium* virulence gene expression by cationic antimicrobial peptides. Mol Microbiol 50:219–230
12. Balassa G (1963) Genetic transformation of *Rhizobium*: A review of the work of R. Balassa. Bacteriol Rev 27:228
13. Belachew T (2010) Intrinsic antibiotic resistance, survival of *Rhizobium leguminosarum* strains and fixation potential of pea varieties (*Pisum sativum* L.) in southeast Ethiopia. Int J Microbiol Res 2:75–79
14. Ben W, Qiang Z, Adams C, Zhang H, Chen L (2008) Simultaneous determination of sulfonamides, tetracyclines and tiamulin in swine wastewater by solid-phase extraction and liquid chromatography–mass spectrometry. J Chromatogr 1202:173–180
15. Berge O, Lodhi A, Brandelet G, Santaella C, Roncato M-A, Christen R, Heulin T, Achouak W (2009) *Rhizobium alamii* sp. nov., an exopolysaccharide-producing species isolated from legume and non-legume rhizospheres. Int J Syst Evol Microbiol 59:367–372
16. Beringer J (1974) R factor transfer in *Rhizobium leguminosarum*. J Gen Microbiol 84:188–198
17. Beynon J, Josey D (1980) Demonstration of heterogeneity in a natural population of *Rhizobium phaseoli* using variation in intrinsic antibiotic resistance. J Gen Microbiol 118:437–442
18. Bibi F, Chung EJ, Khan A, Jeon CO, Chung YR (2012) *Rhizobium halophytocola* sp. nov., isolated from the root of a coastal dune plant. Int J Syst Evol Microbiol 62:1997–2003
19. Botha WJ, Jaftha JB, Bloem JF, Habig JH, Law IJ (2004) Effect of soil bradyrhizobia on the success of soybean inoculant strain CB 1809. Microbiol Res 159:219–231
20. Brígido C, Oliveira S (2013) Most acid-tolerant chickpea mesorhizobia show induction of major chaperone genes upon acid shock. Microb Ecol 65:145–153
21. Brockman F, Bezdicek D (1989) Diversity within serogroups of *Rhizobium leguminosarum* biovar viceae in the Palouse region of eastern Washington as indicated by plasmid profiles, intrinsic antibiotic resistance, and topography. Appl Environ Microbiol 55:109–115
22. Bromfield E, Lewis D, Barran L (1985) Cryptic plasmid and rifampin resistance in *Rhizobium meliloti* influencing nodulation competitiveness. J Bacteriol 164:410–413
23. Calvori C, Frontali L, Leoni L, Tecce G (1965) Effect of rifamycin on protein synthesis. Nature 207:417–418
24. Carpenter CF, Chambers HF (2004) Daptomycin: another novel agent for treating infections due to drug-resistant gram-positive pathogens. Clin Infect Dis 38:994–1000
25. Chahboune R, Carro L, Peix A, Barrijal S, Velázquez E, Bedmar EJ (2011) *Bradyrhizobium cytisi* sp. nov. isolated from effective nodules of *Cytisus villosus* in Morocco. Int J Syst Evol Microbiol 61:2922–2927
26. Chahboune R, Carro L, Peix A, Ramírez-Bahena M-H, Barrijal S, Velázquez E, Bedmar EJ (2012) *Bradyrhizobium rifense* sp. nov. isolated from effective nodules of *Cytisus villosus* grown in the Moroccan Rif. Syst Appl Microbiol 35:302–305
27. Chang YL, Wang JY, Wang ET, Liu HC, Sui XH, Chen WX (2011) *Bradyrhizobium lablabi* sp. nov., isolated from effective nodules of *Lablab purpureus* and *Arachis hypogaea*. Int J Syst Evol Microbiol 61:2496–2502
28. Chopra I, Roberts M (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 65:232–260
29. Cole MA, Elkan G (1979) Multiple antibiotic resistance in *Rhizobium japonicum*. Appl Environ Microbiol 37:867–870
30. Cole MA, Elkan GH (1973) Transmissible resistance to penicillin G, neomycin, and chloramphenicol in *Rhizobium japonicum*. Antimicrob Agents Chemother 4:248–253
31. Courvalin P (2008) New plasmid-mediated resistances to antimicrobial agents. Arch Microbiol 189:289–291
32. Dadarwal K, Sindhu S, Garg R (1987) Effect of curing on genes controlling antibiotic resistance and symbiosis in cowpea misc. rhizobia. Ind J Micobiol 27:16–21

33. Dakora F, Keya S (1997) Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. Soil Biol Biochem 29:809–817
34. Dall'Agnol RF, Ribeiro RA, Ormeño-Orrillo E, Rogel MA, Delamuta JRM, Andrade DS, Martínez-Romero E, Hungria M (2013) *Rhizobium freirei* sp. nov., a symbiont of *Phaseolus vulgaris* that is very effective at fixing nitrogen. Int J Syst Evol Microbiol 63:4167–4173
35. Date R, Hurse L (1991) Intrinsic antibiotic resistance and serological characterization of populations of indigenous *Bradyrhizobium* isolated from nodules of *Desmodium intortum* and *Macroptilium atropurpureum* in three soils of SE Queensland. Soil Biol Biochem 23:551–561
36. Datta N, Hedges R (1972) Trimethoprim resistance conferred by W plasmids in Enterobacteriaceae. J Gen Microbiol 72:349–355
37. Datta N, Hedges R, Shaw EJ, Sykes R, Richmond M (1971) Properties of an R factor from *Pseudomonas aeruginosa*. J Bacteriol 108:1244–1249
38. Davies J, Spiegelman GB, Yim G (2006) The world of subinhibitory antibiotic concentrations. Curr Opin Microbiol 9:445–453
39. D'Costa VM, McGrann KM, Hughes DW, Wright GD (2006) Sampling the antibiotic resistome. Science 311:374–377
40. Delamuta JRM, Ribeiro RA, Ormeño-Orrillo E, Melo IS, Martínez-Romero E, Hungria M (2013) Polyphasic evidence supporting the reclassification of *Bradyrhizobium japonicum* group Ia strains as *Bradyrhizobium diazoefficiens* sp. nov. Int J Syst Evol Microbiol 63:3342–3351
41. Djedidi S, Yokoyama T, Ohkama-Ohtsu N, Risal CP, Abdelly C, Sekimoto H (2011) Stress tolerance and symbiotic and phylogenic features of root nodule bacteria associated with Medicago species in different bioclimatic regions of Tunisia. Microb Environ 26:36–45
42. Doi Y, Wachino J-I, Arakawa Y (2008) Nomenclature of plasmid-mediated 16S rRNA methylases responsible for panaminoglycoside resistance. Antimicrob Agent Chemother 52:2287–2288
43. Douthwaite S, Aagaard C (1993) Erythromycin binding is reduced in ribosomes with conformational alterations in the 23 S rRNA peptidyl transferase loop. J Mol Biol 232:725–731
44. Dowdle SF, Bohlool BB (1985) Predominance of fast-growing *Rhizobium japonicum* in a soybean field in the People's Republic of China. Appl Environ Microbiol 50:1171–1176
45. Drlica K, Malik M (2003) Fluoroquinolones: action and resistance. Curr Topic Medi Chem 3:249–282
46. Drlica K, Zhao X (1997) DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev 61:377–392
47. Durán D, Rey L, Mayo J, Zúñiga-Dávila D, Imperial J, Ruiz-Argüeso T, Martínez-Romero E, Ormeño-Orrillo E (2014) *Bradyrhizobium paxllaeri* sp. nov. and *Bradyrhizobium icense* sp. nov., nitrogen-fixing rhizobial symbionts of Lima bean (*Phaseolus lunatus* L.) in Peru. Int J Syst Evol Microbiol 64:2072–2078
48. Dworkin M, Falkow S, Rosenberg E, Schleifer K, Stackebrandt E (2006) The prokaryotes: a handbook on the biology of bacteria: proteobacteria: ecophysiology, isolation, identification, applications. Springer, New York.
49. Eaglesham A, Elkan G (1987) The use of intrinsic antibiotic resistance for *Rhizobium* study. Symbiotic Nitrogen Fixat Technol 185–204
50. Eliopoulos GM, Meka VG, Gold HS (2004) Antimicrobial resistance to linezolid. Clin Infect Dis 39:1010–1015
51. Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MO, Dantas G (2012) The shared antibiotic resistome of soil bacteria and human pathogens. Science 337:1107–1111
52. Frioni L, Rodrıguez A, Meerhoff M (2001) Differentiation of rhizobia isolated from native legume trees in Uruguay. Appl Soil Ecol 16:275–282
53. Gadre S, Mazumdar L, Modi V, Parekh V (1967) Interspecific transformation in *Rhizobium*. Arch für Mikrobiologie 57:388–391
54. Galimand M, Courvalin P, Lambert T (2003) Plasmid-mediated high-level resistance to aminoglycosides in Enterobacteriaceae due to 16S rRNA methylation. Antimicrob Agent Chemother 47:2565–2571
55. Garg F, Beri N, Tauro P (1985) Intrinsic antibiotic resistance in chickpea (*Cicer arietinum*) rhizobia. The J Agricult Sci 105:85–89
56. Gemell L, Roughley R (1993) Counting rifampicin-resistant rhizobia when a minor component of the soil rhizobial flora. Soil Biol Biochem 25:539–544
57. Ghosh S, LaPara TM (2007) The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. ISME J 1:191–203
58. Glynn P, Higgins P, Squartini A, O'Gara F (1985) Strain identification in *Rhizobium trifolii* using DNA restriction analysis, plasmid DNA profiles and intrinsic antibiotic resistances. FEMS Microbiol Lett 30:177–182
59. Gonzalez-Pasayo R, Martinez-Rpmero E (2000) Multiresistance genes of *Rhizobium etli* CFN42. MPMI 13:572–577
60. Graham P (1963) Antibiotic sensitivities of root nodule bacteria, vol 16. CSIRO publishing, Collingwood, p 557
61. Gu CT, Wang ET, Tian CF, Han TX, Chen WF, Sui XH, Chen WX (2008) *Rhizobium miluonense* sp. nov., a symbiotic bacterium isolated from Lespedeza root nodules. Int J Syst Evol Microbiol 58:1364–1368
62. Guerrouj K, Ruíz-Díez B, Chahboune R, Ramírez-Bahena M-H, Abdelmoumen H, Quiñones MA, El Idrissi MM, Velázquez E, Fernández-Pascual M, Bedmar EJ (2013) Definition of a novel symbiovar (sv. retamae) within *Bradyrhizobium retamae* sp. nov., nodulating *Retama sphaerocarpa* and *Retama monosperma*. Syst Appl Microbiol 36:218–223
63. Gupta B, Kleczkowska J (1962) A study of some mutations in a strain of *Rhizobium trifolii*. J Gen Microbiol 27:473–476
64. Hagedorn C (1979) Relationship of antibiotic resistance to effectiveness in *Rhizobium trifolii* populations. Soil Sci Soc Am J 43:921–925
65. Han TX, Wang ET, Wu LJ, Chen WF, Gu JG, Gu CT, Tian CF, Chen WX (2008) *Rhizobium multihospitium* sp. nov., isolated from multiple legume species native of Xinjiang, China. Int J Syst Evol Microbiol 58:1693–1699
66. Hastings P, Rosenberg SM, Slack A (2004) Antibiotic-induced lateral transfer of antibiotic resistance. Trend Microbiol 12:401–404
67. Heuer H, Schmitt H, Smalla K (2011) Antibiotic resistance gene spread due to manure application on agricultural fields. Curr Opin Microbiol 14:236–243
68. Heumann W, Pühler A, Wagner E (1971) The two transfer regions of the *Rhizobium lupini* conjugation. I. Fertility factor elimination and one way transfer. MGG 113:308–315
69. Heumann W, Pühler A, Wagner E (1973) The two transfer regions of the *Rhizobium lupini* conjugation. II. Genetic characterization of the transferred chromosomal segments. MGG 126:267–274
70. Hong W, Zeng J, Xie J (2014) Antibiotic drugs targeting bacterial RNAs. Acta Pharm Sin B 4:258–265
71. Howie WJ, Suslow T (1991) Role of antibiotic biosynthesis in the inhibition of *Pythium ultimoum* in the cotton spermosphere and rhizosphere. Mol Plant Microbe Interact 4:393–399

72. Islam MS, Kawasaki H, Muramatsu Y, Nakagawa Y, Seki T (2008) *Bradyrhizobium iriomotense* sp. nov., isolated from a tumor-like root of the legume *Entada koshunensis* from Iriomote Island in Japan. Biosci Biotech Biochem 72:1416–1429
73. Jenkins M, Bottomley P (1985) Composition and field distribution of the population of *Rhizobium meliloti* in root nodules of uninoculated field-grown alfalfa. Soil Biol Biochem 17:173–179
74. Ji X, Shen Q, Liu F, Ma J, Xu G, Wang Y, Wu M (2012) Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China. J Hazard Mater 235:178–185
75. Kaltenpoth M, Göttler W, Herzner G, Strohm E (2005) Symbiotic bacteria protect wasp larvae from fungal infestation. Curr Biol 15:475–479
76. Karcı A, Balcıoğlu IA (2009) Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. Sci Total Environ 407:4652–4664
77. King G, Murray NE (1994) Restriction enzymes in cells, not eppendorfs. Trend Microbiol 2:465–469
78. Kingsley MT, Bohlool BB (1983) Characterization of *Rhizobium* sp. (*Cicer arietinum* L.) by immunofluorescence, immunodiffusion, and intrinsic antibiotic resistance. Can J Microbiol 29:518–526
79. Kittiwongwattana C, Thawai C (2014) *Rhizobium lemnae* sp. nov., a bacterial endophyte of *Lemna aequinoctialis*. Int J Syst Evol Microbiol 64:2455–2460
80. Knapp CW, Dolfing J, Ehlert PA, Graham DW (2009) Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environ Sci Tech 44:580–587
81. Kobayashi G (1980) Actinomycetoma: the fungus-like bacteria. Microbiology Harper and Row, Philadelphia, pp 298–321
82. Kobayashi N, Nishino K, Yamaguchi A (2001) Novel Macrolide-specific ABC-type efflux transporter in *Escherichia coli*. J Bacteriol 183:5639–5644
83. Kotra L, Mobashery S (1998) β-Lactam antibiotics, β-lactamases and bacterial resistance. Bull de l'Inst Pasteur 96:139–150
84. Kowalski M (1971) Transduction in *Rhizobium meliloti*. Plant Soil 35:63–66
85. Kroiss J, Kaltenpoth M, Schneider B, Schwinger M-G, Hertweck C, Maddula RK, Strohm E, Svatoš A (2010) Symbiotic streptomycetes provide antibiotic combination prophylaxis for wasp offspring. Nat Chem Biol 6:261–263
86. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 10:597–602
87. Kumbhar C, Watve M (2013) Why antibiotics: A comparative evaluation of different hypotheses for the natural role of antibiotics and an evolutionary synthesis
88. Kuykendall L (1979) Transfer of R factors to and between genetically marked sublines of *Rhizobium japonicum*. Appl Environ Microbiol 37:862–866
89. Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10:S122–S129
90. Li D-M, Alexander M (1988) Co-inoculation with antibiotic-producing bacteria to increase colonization and nodulation by rhizobia. Plant Soil 108:211–219
91. Lin DX, Chen WF, Wang FQ, Hu D, Wang ET, Sui XH, Chen WX (2009) *Rhizobium mesosinicum* sp. nov., isolated from root nodules of three different legumes. Int J Syst Evol Microbiol 59:1919–1923
92. Linares J, Gustafsson I, Baquero F, Martinez J (2006) Antibiotics as intermicrobial signaling agents instead of weapons. Proc Nat Acad Sci 103:19484–19489
93. Liu TY, Li Y, Liu XX, Sui XH, Zhang XX, Wang ET, Chen WX, Chen WF, Puławska J (2012) *Rhizobium cauense* sp. nov., isolated from root nodules of the herbaceous legume *Kummerowia stipulacea* grown in campus lawn soil. Syst Appl Microbiol 35:415–420
94. Lorenz MG, Wackernagel W (1994) Bacterial gene transfer by natural genetic transformation in the environment. Microbiol Rev 58:563
95. Lu JK, Dou YJ, Zhu YJ, Wang SK, Sui XH, Kang LH (2014) *Bradyrhizobium ganzhouense* sp. nov., an effective symbiotic bacterium isolated from *Acacia melanoxylon* R. Br. nodules. Int J Syst Evol Microbiol 64:1900–1905
96. Lu YL, Chen WF, Han LL, Wang ET, Chen WX (2009) *Rhizobium alkalisoli* sp. nov., isolated from *Caragana intermedia* growing in saline-alkaline soils in the north of China. Int J Syst Evol Microbiol 59:3006–3011
97. Maiden MC (1998) Horizontal genetic exchange, evolution, and spread of antibiotic resistance in bacteria. Clinic Infect Dis 27:S12–S20
98. Marger MD, Saier MH (1993) A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. Trend Biochem Sci 18:13–20
99. Mc Dermott PF, Walker RD, White DG (2003) Antimicrobials: modes of action and mechanisms of resistance. Int J Toxicol 22:135–143
100. McLaughlin W, Ahmad MH (1984) Intrinsic antibiotic resistance and streptomycin uptake in cowpea rhizobia. FEMS Microbiol Lett 21:299–303
101. McManus MC (1997) Mechanisms of bacterial resistance to antimicrobial agents. Am J Health Syst Phar 54:1420–1433
102. Mehta R, Champney WS (2002) 30S ribosomal subunit assembly is a target for inhibition by aminoglycosides in Escherichia coli. Antimicrob Agent Chemother 46:1546–1549
103. Meyer MC, Pueppke SG (1980) Differentiation of *Rhizobium japonicum* strain derivatives by antibiotic sensitivity patterns, lectin binding, and utilization of biochemicals. Can J Microbiol 26:606–612
104. Mingeot-Leclercq M-P, Glupczynski Y, Tulkens PM (1999) Aminoglycosides: activity and resistance. Antimicrob Agent Chemother 43:727–737
105. Mnasri B, Liu TY, Saidi S, Chen WF, Chen WX, Zhang XX, Mhamdi R (2014) *Rhizobium azibense* sp. nov., a nitrogen fixing bacterium isolated from root-nodules of *Phaseolus vulgaris*. Int J Syst Evol Microbiol 64:1501–1506
106. Mpepereki S, Makonese F, Wollum A (1997) Physiological characterization of indigenous rhizobia nodulating *Vigna unguiculata* in Zimbabwean soils. Symbiosis 22, Philadelphia
107. Mueller J, Skipper H, Shipe E, Grimes L, Wagner S (1988) Intrinsic antibiotic resistance in *Bradyrhizobium japonicum*. Soil Biol Biochem 20:879–882
108. Mukhtar TA, Koteva KP, Hughes DW, Wright GD (2001) Vgb from Staphylococcus aureus inactivates streptogramin B antibiotics by an elimination mechanism not hydrolysis. Biochem 40:8877–8886
109. Murray IA, Shaw WV (1997) O-Acetyltransferases for chloramphenicol and other natural products. Antimicrob Agent Chemother 41:1
110. Ochman H, Lawrence JG, Groisman EA (2000) Lateral gene transfer and the nature of bacterial innovation. Nature 405:299–304
111. Olivares J, Bedmar EJ, Sanjuán J (2013) Biological nitrogen fixation in the context of global change. Mol Plant Microb Interact 26:486–494

112. Pankhurst CE (1977) Symbiotic effectiveness of antibiotic-resistant mutants of fast-and slow-growing strains of *Rhizobium* nodulating Lotus species. Can J Microbiol 23:1026–1033
113. Pao SS, Paulsen IT, Saier MH (1998) Major facilitator superfamily. Microbiol Mol Biol Rev 62:1–34
114. Park B, Levy S (1988) The cryptic tetracycline resistance determinant on Tn4400 mediates tetracycline degradation as well as tetracycline efflux. Antimicrob Agent Chemother 32:1797–1800
115. Paterson DL, Bonomo RA (2005) Extended-spectrum β-lactamases: a clinical update. Clinic Microbiol Rev 18:657–686
116. Pattison A, Skinner F (1974) The effects of antimicrobial substances on *Rhizobium* spp. and their use in selective media. J Appl Bacteriol 37:239–250
117. Paulsen IT, Skurray RA, Tam R, Saier MH, Turner RJ, Weiner JH, Goldberg EB, Grinius LL (1996) The SMR family: a novel family of multidrug efflux proteins involved with the efflux of lipophilic drugs. Mol Microbiol 19:1167–1175
118. Peng G, Yuan Q, Li H, Zhang W, Tan Z (2008) *Rhizobium oryzae* sp. nov., isolated from the wild rice *Oryza alta*. Int J Syst Evol Microbiol 58:2158–2163
119. Petri W (1996) Antimicrobial agents: Sulfonamides, trimethoprim-sulfamethoxazole, quinolones, and agents for urinary tract infection. The pharmacological basis of therapeutics, 9th edn. Mac Millan, New York
120. Pommier Y, Leo E, Zhang H, Marchand C (2010) DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem Biol 17:421–433
121. Popowska M, Rzeczycka M, Miernik A, Krawczyk-Balska A, Walsh F, Duffy B (2012) Influence of soil use on prevalence of tetracycline, streptomycin, and erythromycin resistance and associated resistance genes. Antimicrob Agent Chemother 56:1434–1443
122. Raina J, Modi V (1972) Deoxyribonucleate binding and transformation in *Rhizobium japonicum*. J Bacteriol 111:356–360
123. Ramirez ME, Israel DW, Wollum A (1997) Phenotypic and genotypic diversity of similar serotypes of soybean bradyrhizobia from two soil populations. Soil Biol Biochem 29:1539–1545
124. Ramírez-Bahena M-H, Chahboune R, Peix A, Velázquez E (2013) Reclassification of *Agromonas oligotrophica* into the genus *Bradyrhizobium* as *Bradyrhizobium oligotrophicum* comb. nov. Int J Syst Evol Microbiol 63:1013–1016
125. Ramírez-Bahena MH, Peix A, Rivas R, Camacho M, Rodríguez-Navarro DN, Mateos PF, Martínez-Molina E, Willems A, Velázquez E (2009) *Bradyrhizobium pachyrhizi* sp. nov. and *Bradyrhizobium jicamae* sp. nov., isolated from effective nodules of *Pachyrhizus erosus*. Int J Syst Evol Microbiol 59:1929–1934
126. Ramos MLG, Magalhães NF, Boddey RM (1987) Native and inoculated rhizobia isolated from field grown *Phaseolus vulgaris*: effects of liming an acid soil on antibiotic resistance. Soil Biol Biochem 19:179–185
127. Ribeiro RA, Rogel MA, Lopez-Lopez A, Ormeno-Orrillo E, Barcellos FG, Martinez J, Thompson FL, Martinez-Romero E, Hungria M (2012) Reclassification of *Rhizobium tropici* type A strains as *Rhizobium leucaenae* sp. nov. Int J Syst Evol Microbiol 62:1179–1184
128. Rincón-Rosales R, Villalobos-Escobedo JM, Rogel MA, Martinez J, Ormeño-Orrillo E, Martínez-Romero E (2013) *Rhizobium calliandrae* sp. nov., *Rhizobium mayense* sp. nov. and *Rhizobium jaguaris* sp. nov., rhizobial species nodulating the medicinal legume *Calliandra grandiflora*. Int J Syst Evol Microbiol 63:3423–3429
129. Rivas R, Willems A, Palomo JL, García-Benavides P, Mateos PF, Martínez-Molina E, Gillis M, Velázquez E (2004) *Bradyrhizobium betae* sp. nov., isolated from roots of *Beta vulgaris* affected by tumour-like deformations. Int J Syst Evol Microbiol 54:1271–1275
130. Roughley R, Wahab FA, Sundram J (1992) Intrinsic resistance to streptomycin and spectinomycin among root-nodule bacteria from Malaysian soils. Soil Biol Biochem 24:715–716
131. Saïdi S, Ramírez-Bahena M-H, Santillana N, Zúñiga D, Álvarez-Martínez E, Peix A, Mhamdi R, Velázquez E (2014) *Rhizobium laguerreae* sp. nov. nodulates Vicia faba on several continents. Int J Syst Evol Microbiol 64:242–247
132. Saier M, Tam R, Reizer A, Reizer J (1994) Two novel families of bacterial membrane proteins concerned with nodulation, cell division and transport. Mol Microbiol 11:841–847
133. Salian S, Matt T, Akbergenov R, Harish S, Meyer M, Duscha S, Shcherbakov D, Bernet BB, Vasella A, Westhof E (2012) Structure-activity relationships among the kanamycin aminoglycosides: role of ring I hydroxyl and amino groups. Antimicrob Agent Chemother 56:6104–6108
134. Sarmah AK, Meyer MT, Boxall AB (2006) A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65:725–759
135. Schifano JM, Edifor R, Sharp JD, Ouyang M, Konkimalla A, Husson RN, Woychik NA (2013) Mycobacterial toxin MazF-mt6 inhibits translation through cleavage of 23S rRNA at the ribosomal A site. Proc Nat Acad Sci 110:8501–8506
136. Schlünzen F, Zarivach R, Harms J, Bashan A, Tocilj A, Albrecht R, Yonath A, Franceschi F (2001) Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature 413:814–821
137. Scholla MH, Elkan GH (1984) *Rhizobium fredii* sp. nov., a fast-growing species that effectively nodulates soybeans. Int J Syst Bacteriol 34:484–486
138. Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A (2004) Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiol Rev 28:519–542
139. Sharma P, Anand R, Lakshminarayana K (1991) Construction of Tn5 tagged mutants of *Rhizobium* spp (Cicer) for ecological studies. Soil Biol Biochem 23:881–885
140. Shishido M, Pepper I (1990) Identification of dominant indigenous *Rhizobium meliloti* by plasmid profiles and intrinsic antibiotic resistance. Soil Biol Biochem 22:11–16
141. Sik T, Orosz L (1971) Chemistry and genetics of *Rhizobium meliloti* phage 16-3. Plant Soil 35:57–62
142. Silva FV, De Meyer SE, Simões-Araújo JL, da Costa Barbé T, Xavier GR, O'Hara G, Ardley JK, Rumjanek NG, Willems A, Zilli JE (2014) *Bradyrhizobium manausense* sp. nov., isolated from effective nodules of *Vigna unguiculata* grown in Brazilian Amazonian rainforest soils. Int J Syst Evol Microbiol 64:2358–2363
143. Simon T, Kalalova S (1996) Preparation of antibiotic resistant mutants of rhizobia and their use. Rostlinna Vyroba-UZPI (Czech Republic)
144. Sinclair MJ, Eaglesham AR (1984) Intrinsic antibiotic resistance in relation to colony morphology in three populations of West African cowpea rhizobia. Soil Biol Biochem 16:247–251
145. Singh SK, Jaiswal SK, Vaishampayan A, Dhar B (2013) Physiological behavior and antibiotic response of soybean (Glycine max L.) nodulating rhizobia isolated from Indian soils. Afr J Microbiol Res 7:2093–2102
146. Sköld O (2001) Resistance to trimethoprim and sulfonamides. Vet Res 32:261–273
147. Spratt BG (1994) Resistance to antibiotics mediated by target alterations. Science 264:388–393
148. Storm DR, Rosenthal KS, Swanson PE (1977) Polymyxin and related peptide antibiotics. Ann Rev Biochem 46:723–763

149. Subramaniam B, Babu C (1993) Intrinsic antibiotic resistance among rhizobia isolated from sub-tropical Himalayan legumes. Soil Biol Biochem 25:1057–1060
150. Tenover FC (2006) Mechanisms of antimicrobial resistance in bacteria. Am J Med 119:S3–S10
151. Thomashow LS, Weller DM, Bonsall RF, Pierson LS (1990) Production of the antibiotic phenazine-1-carboxylic acid by fluorescent Pseudomonas species in the rhizosphere of wheat. Appl Environ Microbiol 56:908–912
152. Tian CF, Wang ET, Wu LJ, Han TX, Chen WF, Gu CT, Gu JG, Chen WX (2008) *Rhizobium fabae* sp. nov., a bacterium that nodulates *Vicia faba*. Int J Syst Evol Microbiol 58:2871–2875
153. Turco R, Bezdicek D (1987) Diversity within two serogroups of *Rhizobium leguminosarum* native to soils in the Palouse of eastern Washington. Ann Appl Biol 111:103–114
154. Turpin PE, Dhir VK, Maycroft KA, Rowlands C, Wellington EM (1992) The effect of Streptomyces species on the survival of Salmonella in soil. FEMS Microbiol Lett 101:271–280
155. Valverde A, Igual JM, Peix A, Cervantes E, Velazquez E (2006) *Rhizobium lusitanum* sp. nov. a bacterium that nodulates *Phaseolus vulgaris*. Int J Syst Evol Microbiol 56:2631–2637
156. van Hoek AH, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJ (2011) Acquired antibiotic resistance genes: an overview. Front Microbiol 2:1–27
157. Vinuesa P, León-Barrios M, Silva C, Willems A, Jarabo-Lorenzo A, Pérez-Galdona R, Werner D, Martínez-Romero E (2005) *Bradyrhizobium canariense* sp. nov., an acid-tolerant endosymbiont that nodulates endemic genistoid legumes (Papilionoideae: Genisteae) from the Canary Islands, along with *Bradyrhizobium japonicum* bv. *genistearum*, *Bradyrhizobium genospecies* alpha and *Bradyrhizobium genospecies* beta. Int J Syst Evol Microbiol 55:569–575
158. Von Rechenberg M, Blake BK, Ho YSJ, Zhen Y, Chepanoske CL, Richardson BE, Xu N, Kery V (2005) Ampicillin/penicillin-binding protein interactions as a model drug-target system to optimize affinity pull-down and mass spectrometric strategies for target and pathway identification. Proteomics 5:1764–1773
159. Wang E, Van Berkum P, Beyene D, Sui X, Dorado O, Chen W, Martínez-Romero E (1998) *Rhizobium huautlense* sp. nov., a symbiont of *Sesbania herbacea* that has a close phylogenetic relationship with *Rhizobium galegae*. Int J Syst Bacteriol 48:687–699
160. Wang ET, Chen WF, Sui XH, Zhang XX, Liu HC, Chen WX (2011) *Rhizobium herbae* sp. nov. and *Rhizobium giardinii*-related bacteria, minor microsymbionts of various wild legumes in China. Int J Syst Evol Microbiol 61:1912–1920
161. Wang R, Chang YL, Zheng WT, Zhang D, Zhang XX, Sui XH, Wang ET, Hu JQ, Zhang LY, Chen WX (2013) *Bradyrhizobium arachidis* sp. nov., isolated from effective nodules of *Arachis hypogaea* grown in China. Syst Appl Microbiol 36:101–105
162. Wei GH, Tan ZY, Zhu ME, Wang ET, Han SZ, Chen WX (2003) Characterization of rhizobia isolated from legume species within the genera Astragalus and Lespedeza grown in the Loess Plateau of China and description of *Rhizobium loessense* sp. nov. Int J Syst Evol Microbiol 53:1575–1583
163. Wei GH, Wang ET, Tan ZY, Zhu ME, Chen WX (2002) *Rhizobium indigoferae* sp. nov. and *Sinorhizobium kummerowiae* sp. nov., respectively isolated from Indigofera spp. and *Kummerowia stipulacea*. Int J Syst Evol Microbiol 52:2231–2239
164. Wiener P (1996) Experimental studies on the ecological role of antibiotic production in bacteria. Evolut Ecol 10:405–421
165. Wright GD (2005) Bacterial resistance to antibiotics: enzymatic degradation and modification. Adv Drug Deliver Rev 57:1451–1470
166. Wright GD (2007) The antibiotic resistome: the nexus of chemical and genetic diversity. Nat Rev Microbiol 5:175–186
167. Xavier G, Martins L, Neves M, Rumjanek N (1998) Edaphic factors as determinants for the distribution of intrinsic antibiotic resistance in a cowpea rhizobia population. Biol Fertil Soils 27:386–392
168. Xiong W, Sun Y, Zhang T, Ding X, Li Y, Wang M, Zeng Z (2015) Antibiotics, Antibiotic Resistance Genes, and Bacterial Community Composition in Fresh Water Aquaculture Environment in China. Microb Ecol 70:425–432
169. Xu L, Ge C, Cui Z, Li J, Fan H (1995) *Bradyrhizobium liaoningense* sp. nov., isolated from the root nodules of soybeans. Int J Syst Bacteriol 45:706–711
170. Xu L, Shi JF, Zhao P, Chen WM, Qin W, Tang M, Wei GH (2011) *Rhizobium sphaerophysae* sp. nov., a novel species isolated from root nodules of *Sphaerophysa salsula* in China. Antonie Van Leeuwenhoek 99:845–854
171. Yang W, Moore IF, Koteva KP, Bareich DC, Hughes DW, Wright GD (2004) TetX is a flavin-dependent monooxygenase conferring resistance to tetracycline antibiotics. J Biol Chem 279:52346–52352
172. Yao LJ, Shen YY, Zhan JP, Xu W, Cui GL, Wei GH (2012) *Rhizobium taibaishanense* sp. nov., isolated from a root nodule of *Kummerowia striata*. Int J Syst Evol Microbiol 62:335–341
173. Yu X, Cloutier S, Tambong JT, Bromfield ES (2014) *Bradyrhizobium ottawaense* sp. nov., a symbiotic nitrogen fixing bacterium from root nodules of soybeans in Canada. Int J Syst Evol Microbiol 64:3202–3207
174. Zahran HH (1999) *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63:968–989
175. Zelazna-Kowalska I (1971) Correlation between streptomycin resistance and infectiveness in *Rhizobium trifolii*. Plant Soil 35:67–71
176. Zelazna-Kowalska I, Lorkiewicz Z (1971) Transformation in *Rhizobium trifolii* IV. Correlation between streptomycin resistance and infectiveness in *Rhizobium trifolii*. Acta Microbiol Pol Ser A 3:11
177. Zhang RJ, Hou BC, Wang ET, Li Y, Zhang XX, Chen WX (2011) *Rhizobium tubonense* sp. nov., isolated from root nodules of *Oxytropis glabra*. Int J Syst Evol Microbiol 61:512–517
178. Zhang YM, Li Y, Chen WF, Wang ET, Sui XH, Li QQ, Zhang YZ, Zhou YG, Chen WX (2012) *Bradyrhizobium huanghuaihaiense* sp. nov., an effective symbiotic bacterium isolated from soybean (*Glycine max* L.) nodules. Int J Syst Evol Microbiol 62:1951–1957
179. Zilli JE, Baraúna AC, da Silva K, De Meyer SE, Farias EN, Kaminski PE, da Costa IB, Ardley JK, Willems A, Camacho NN (2014) *Bradyrhizobium neotropicale* sp. nov., isolated from effective nodules of *Centrolobium paraense*. Int J Syst Evol Microbiol 64:3950–3957