Soybean inoculants in Brazil: an overview of quality control

Gabriela Kalinowski de Souza¹ · Jamilla Sampaio² · Letícia Longoni² · Silvine Ferreira² · Samuel Alvarenga² · Anelise Beneduzi^{1,2}

Received: 11 May 2018 / Accepted: 6 August 2018 / Published online: 29 November 2018
© Sociedade Brasileira de Microbiologia 2018

Abstract

The bacterial strains SEMIA 587 and 5019 (*Bradyrhizobium elkanii*), 5079 (*Bradyrhizobium japonicum*), and 5080 (*Bradyrhizobium diazoefficiens*) are recommended for soybean inoculants in Brazil. In several countries, the current regulations are insufficient to induce companies for improving the quality of their products, leading to low performance and subsequent abandonment of inoculant use. From 2010 to 2014, 1086 samples coming mainly from Argentina and the southern region of Brazil were analyzed for viable cells counting, strains identification, and purity analysis according to the SDA/MAPA no. 30/2010 Normative Instruction. Most products were imported and formulated in liquid carriers with 5.0×10^9 colony-forming units (CFU)/mL. The strains most frequently used were SEMIA 5079/5080. Only 2.21% of samples had contaminants. The guaranteed concentration of viable cells in inoculants mostly ranged from 4.1×10^9 to 5.0×10^9 CFU/mL or CFU/g. The most frequently found concentration was above 1.1×10^{10} CFU/mL or CFU/g, which was higher than the product guarantee. The inoculants used for soybean crop in Brazil have excellent quality, leading the country to the leadership in taking advantage of the biological nitrogen fixation benefits for a productive and sustainable agriculture.

Keywords Biological nitrogen fixation · SEMIA 587 · SEMIA 5019 · SEMIA 5079 · SEMIA 5080

Introduction

Nitrogen (N) is one of the most crucial elements for biological systems and required in large amounts by plants, as the major constituent of nucleic acids and proteins, which are fundamental molecules in all biological processes [1]. Biological nitrogen fixation (BNF) occurs when the atmospheric nitrogen (N_2) is converted into ammonia (NH_3) by diazotrophic bacteria, and, for this reason, it is considered as extremely important for the environment and agriculture [2]. The increasing use of N fertilizers, which represents the greatest human impact on

the N cycle, raised questions about the nitrogen oxide emissions, soil acidification, and water eutrophication. The N provided by BNF is less likely to leach and volatilize because BNF is an in situ process and greatly contributes to sustainable agriculture [2].

Although BNF is not performed by eukaryotic organisms, it is widely distributed among bacteria and archaeobacteria. Diazotrophic microorganisms are found in a wide variety of habitats, including soil and water, intestines of termites, in association with legumes and grasses, and many others [2]. Among diazotrophic bacteria, rhizobia can establish symbiosis with leguminous plants. Initially, the term rhizobia were used to bacteria belonging to the *Rhizobium* genus; however, it has been used to designate all bacteria capable of forming nodules and fixating N in symbiosis with legumes [3]. Rhizobia are strictly aerobic bacteria, and the difference between rhizobia and other diazotrophic bacteria is their ability to induce a new growth structure on plant roots: the nodule. The nodule formation depends on the exchange of signals between the host plant and rhizobia, leading to the invasion of these bacteria into the plant tissue [4].

Nowadays, the Ministry of Agriculture, Livestock and Food (MAPA) from Brazil, recommends four efficient

Responsible Editor: Ieda Mendes

✉ Anelise Beneduzi
abeneduzi@seapi.rs.gov.br

¹ Universidade La Salle, Av. Vitor Barreto, 2288, Canoas, RS CEP 92010-000, Brazil

² Departamento de Diagnóstico e Pesquisa Agropecuária (ex-FEPAGRO) da Secretaria da Agricultura, Pecuária e Irrigação (SEAPI) do Rio Grande do Sul, Rua Gonçalves Dias, 570, Porto Alegre, RS CEP 90130-060, Brazil

rhizobial strains for soybean inoculation: two *Bradyrhizobium elkanii* (strains SEMIA 587 and SEMIA 5019) and two *B. japonicum* (SEMIA 5079 and SEMIA 5080) [5], with SEMIA 5080 being recently reclassified into the new species *B. diazoefficiens* [6]. To be successful, inoculation with recommended strains must persist in the soil and compete with the established native microbial community [5]. According to Hungria et al. [7], strains approved as soybean inoculants in Brazil can fix 300 kg ha⁻¹ of N or more, with the utilization efficiency of nearly to 100%. The N left in soybean residues may contribute to crops in succession, such as maize and wheat. However, the whole inoculation process may be compromised if the inoculant does not meet the quality control standards [8].

The agricultural products named inoculants contain microorganisms that promote plant growth (BNF, among other features) when applied to the plants. Inoculant excipient must be sterilized and contaminant-free within the established levels and display support or feed function, or both, ensuring growth and survival of microorganisms and that facilitate their application [9]. The inoculant quality is extremely crucial; thus, in addition to being produced in a sterile vehicle and free of contaminants, inoculants should also contain at least 10⁹ cells/g or cells/mL of the product, and the recommended dosage should result in at least 1.2 × 10⁶ cells/seed [10].

Campos et al. [11] stated that from the economic and ecological point of view, BNF is classified as an extremely crucial process capable of satisfying all the N needs of soybean crop. N fertilizers are not recommended for this crop, as they are expensive and highly polluting [3]. The efficiency of fertilizers rarely exceeds 50%, half of which is lost as gases, thus contributing to increased greenhouse effect, and leaching, that pollutes rivers, lakes, and groundwater. The use of inoculant presents advantages such as lower cost than N fertilizers and in addition is environmentally friendly [3]. In Brazil, approximately 70% of the N fertilizers are imported, and the costs of applying mineral N are extremely high. In contrast, microbial inoculants are relatively cheap for the soybean crop. It is estimated that Brazil saves approximately US\$ 7 billion per year with BNF [1]. Inoculants are economically and ecologically pivotal for agriculture, as soybean crops in Brazil will not be economically feasible if farmers had to use all the N to fulfill soybean demands [3]. In 2016, soybean farmers in Brazil acquired 35 million doses of liquid inoculants and 11 million doses of peat-based inoculants [12].

The Laboratory of Agricultural Microbiology of the Department of Diagnosis and Agricultural Research (EX-FEPAGRO)/Secretariat of Agriculture, Livestock and Irrigation of Rio Grande do Sul performs official quality analyses for viable cells counting, identification of strain types and the presence of contaminants in soybean inoculants collected from the industries by MAPA. In addition, this laboratory is responsible for safekeeping the recommended rhizobial

strains and distributing them to inoculant producers in Brazil. The aim of this study was to perform an overview on the quality of inoculants products used for soybean crops in Brazil based on the analysis of the data obtained from fiscal analysis certificates in 2010 and 2014.

Materials and methods

A total of 1086 samples of liquid and peat-based inoculants were analyzed from 2010 to 2014. Data for viable cells countings, identification of strain type, and purity analysis were obtained from inoculants recommended for soybean crop in Brazil. The analysis followed the SDA/MAPA no. 30/2010 Normative Instruction [13] that establishes the methods for official analysis of inoculants. Before opening the samples, packages, sachets, bags, bladders, or flasks were disinfested externally with cotton or paper towel soaked in 70% ethanol. The externally disinfested samples were then opened in a laminar flow hood to avoid contamination.

The concentration of cells and purity levels of inoculants were measured by performing serial dilution with a sterile physiological solution (NaCl 0.85%). For peat (or any solid nature) inoculants, 10.0 g of inoculant was placed in a 250- to 300-mL vial and 90.0 mL of physiological solution was added to form a 10⁻¹ dilution. This vial was homogenized on an orbital shaker for 20 min. Then, an aliquot of 1.0 mL was transferred by a pipette to a tube containing 9.0 mL of saline, forming 10⁻² dilution and successively, until the final dilution that allowed countings between 30 and 300 colonies per plate. For liquid inoculants, a sample was vigorously homogenized, and an aliquot of 1.0 mL was aseptically transferred to a test tube containing 9.0 mL of saline to form the 10⁻¹ dilution. The different dilutions were formed using the serial dilution method described above. From each inoculant sample, two dilution series, named A and B, were prepared and plated in triplicate. The final number of each sample was obtained by calculating the average of these two dilution series and plating. For counting and calculating colony-forming units (CFU), the dilution which average counting of three plates was in the range of 30 to 300 CFU were considered and, if none of the successive dilutions were within the range, the mean of two dilutions closer to the range was calculated. If one of the repetitions presented a discrepancy above 50% of the average of the other two plates, the mean was considered in the calculation. The number of colony-forming units (CFU) is calculated using the following formula:

CFU/g or mL of inoculant = $f \times N$, where f = dilution factor (given by the reciprocal of the plate dilution multiplied by 10, the case of inoculation of 0.1 mL) and N = average number of CFU of the three plates in the selected dilution.

The purity evaluation of inoculants was performed using the scattering method on culture medium in Petri dishes, using

Table 1 Results from analysis of inoculant recommended for soybean crops in Brazil, from 2010 to 2014

Inoculant product composition (SEMIA strains)	Origin	Type of inoculant	Viable cells concentration recorded in the guarantee (CFU/mL or CFU/g)	Concentration range results from analysis (CFU/mL or CFU/g)	Contamination	Number of samples
5019/5079	Imported	Liquid	1.5×10^9	1.4×10^9 to 8.2×10^9	Absence	39
5019/5079	Imported	Peat	1.5×10^9	5.8×10^9	Absence	1
5019/5079	Imported	Liquid	5.0×10^9	1.3×10^9 to 2.6×10^{10}	Absence	88
5019/5079	Imported	Peat	5.0×10^9	3.1×10^9 to 6.5×10^9	Absence	9
5019/5079	Imported	Liquid	6.0×10^9	1.8×10^{10}	Absence	1
5019/5079	National	Peat	5.0×10^9	5.1×10^9 to 1.1×10^{10}	Absence	17
5019/5080	Imported	Liquid	5.0×10^9	1.8×10^9	Absence	1
5019/587	Imported	Liquid	3.0×10^9	1.3×10^9	Presence	1
5019/587	Imported	Liquid	5.0×10^9	7.9×10^9 to 1.2×10^{10}	Absence	5
5019/587	Imported	Peat	1.05×10^9	1.0×10^9 to 2.4×10^{10}	Absence	18
5019/587	National	Peat	5.0×10^9	1.2×10^{10}	Absence	1
5079/5019	Imported	Liquid	1.5×10^9	4.2×10^9 to 8.1×10^9	Absence	7
5079/5019	Imported	Liquid	5.0×10^9	5.9×10^9 to 3.4×10^{10}	Absence	15
5079/5080	Imported	Liquid	1.4×10^9	3.3×10^9 to 8.0×10^9	Absence	11
5079/5080	Imported	Liquid	3.0×10^9	3.5×10^7 to 1.2×10^{10}	Absence	80
5079/5080	Imported	Liquid	5.0×10^9	2.5×10^8 to 3.2×10^{10}	Absence	348
5079/5080	Imported	Liquid	5.0×10^9	9.2×10^9	Presence	2
5079/5080	Imported	Liquid	6.0×10^9	1.0×10^9 to 2.6×10^{10}	Absence	20
5079/5080	Imported	Liquid	7.0×10^9	1.3×10^{10} to 4.3×10^{10}	Absence	9
5079/5080	Imported	Peat	1.5×10^9	3.0×10^9 to 8.1×10^9	Absence	6
5079/5080	Imported	Peat	2.625×10^9	1.1×10^9 to 5.9×10^{10}	Absence	13
5079/5080	Imported	Peat	2.625×10^9	1.5×10^9	Presence	1
5079/5080	Imported	Peat	3.0×10^9	3.7×10^9 to 8.1×10^9	Absence	10
5079/5080	Imported	Peat	5.0×10^9	6.0×10^9 to 8.2×10^9	Absence	5
5079/5080	Imported	Peat	5.0×10^9	1.0×10^{10}	Presence	1
5079/5080	Imported	Peat	7.2×10^9	7.8×10^{10} to 1.2×10^{10}	Absence	19
5079/5080	National	Liquid	2.625×10^9	2.8×10^9 to 8.6×10^9	Absence	2
5079/5080	National	Liquid	5.0×10^9	0 to 1.9×10^9	Presence	4
5079/5080	National	Liquid	5.0×10^9	1.3×10^7 to 2.3×10^{10}	Absence	68
5079/5080	National	Liquid	6.0×10^9	3.4×10^9 to 2.0×10^{10}	Absence	22
5079/5080	National	Liquid	7.0×10^9	2.5×10^{10}	Absence	1
5079/5080	National	Liquid	7.2×10^9	2.4×10^9 to 2.8×10^9	Absence	2
5079/5080	National	Liquid	8.4×10^9	8.2×10^9	Absence	1
5079/5080	National	Peat	1.5×10^9	3.6×10^9 to 4.2×10^9	Absence	2
5079/5080	National	Peat	2.625×10^9	8.3×10^8 to 7.0×10^9	Absence	4
5079/5080	National	Peat	5.0×10^9	1.3×10^9 to 1.4×10^{10}	Absence	17
5079/5080	National	Peat	5.0×10^9	5.3×10^9 to 8.0×10^9	Presence	5
5079/5080	National	Peat	5.5×10^9	4.4×10^9	Absence	1
5079/5080	National	Peat	6.0×10^9	4.1×10^9 to 8.9×10^9	Absence	5
5079/5080	National	Peat	7.2×10^9	5.8×10^9 to 2.9×10^{10}	Absence	80
5079/587	Imported	Liquid	1.5×10^9	5.7×10^9	Absence	1
5079/587	Imported	Liquid	3.0×10^9	1.4×10^8 to 4.2×10^{10}	Absence	54
5079/587	Imported	Liquid	3.0×10^9	7.6×10^8 to 2.4×10^9	Presence	5
5079/587	Imported	Liquid	5.0×10^9	2.4×10^9 to 4.0×10^9	Absence	2
5079/587	Imported	Liquid	7.0×10^9	1.3×10^{10}	Absence	1
5079/587	National	Liquid	5.0×10^9	3.3×10^7 to 7.2×10^9	Absence	4
587/5019	Imported	Liquid	5.0×10^9	5.1×10^9 to 2.2×10^{10}	Absence	5

Table 1 (continued)

Inoculant product composition (SEMIA strains)	Origin	Type of inoculant	Viable cells concentration recorded in the guarantee (CFU/mL or CFU/g)	Concentration range results from analysis (CFU/mL or CFU/g)	Contamination	Number of samples
587/5019	Imported	Peat	5.0×10^9	3.3×10^9 to 8.6×10^9	Absence	11
587/5019	Imported	Peat	5.0×10^9	3.0×10^9	Presence	1
587/5019	Imported	Peat	5.2×10^9	1.9×10^9	Absence	1
587/5019	Imported	Peat	1.5×10^9	1.5×10^9 to 1.5×10^{10}	Absence	15
587/5019	National	Liquid	5.0×10^9	9.1×10^9 to 1.7×10^{10}	Absence	5
587/5019	National	Peat	5.0×10^9	3.7×10^9 to 1.7×10^{10}	Absence	10
587/5019	National	Peat	5.0×10^9	9.6×10^9	Presence	1
587/5079	Imported	Liquid	5.0×10^9	6.0×10^9 to 1.6×10^{10}	Absence	8
587/5079	Imported	Peat	2.5×10^9	6.0×10^9	Absence	1
587/5079	National	Liquid	5.0×10^9	7.9×10^6 to 7.8×10^9	Absence	11
587/5079	National	Peat	6.0×10^9	6.6×10^9 to 9.3×10^9	Absence	5
587/5079	National	Peat	6.0×10^9	6.6×10^9	Presence	3
Total samples						1086

0.1 mL aliquots of successive A and B dilutions in triplicate, starting at 10^{-5} . Rhizobia were incubated at 28 °C to 30 °C, and the culture medium was Congo Red Yeast Mannitol Agar (or Medium 79): 5.0 g/L Mannitol (Nuclear), 10.0 g/L Agar (Himedia), 0.4 g/L yeast extract (Himedia), 0.1 g/L NaCl (Vetec), 0.5 g/L K_2HPO_4 (Merck), 0.2 g/L $MgSO_4 \cdot 7H_2O$ (Merck), and 10.0 mL Congo red dye solution (2.5 g/L Congo red dye, Vetec). The pH was adjusted in the range of 6.8 to 7.0, using 0.1 mol L⁻¹ NaOH or 0.1 mol L⁻¹ HCl. Generally, rhizobia do not absorb Congo red dye, and the appearance of red colonies is considered indicative of contamination [13].

For strain identification by direct agglutination, the SDA/MAPA no. 30/2010 Normative Instruction [13] states that the laboratory must have the authorized strains for inoculant manufacturing and possess their specific sera (antisera). The test was read as follows: in case of agglutination (lumps formation at the bottom of the plate), the reaction was considered positive; in case of a white dot at the bottom of the plate, the reaction was considered negative. Positive (antigen

suspensions of pure strains) and negative controls (negative serum and saline solution) were used [13].

Results

In total, 1086 samples were analyzed in the Laboratory of Agricultural Microbiology/FEPAGRO from 2010 to 2014, and most of which were imported liquid inoculants with the concentration of 5.0×10^9 CFU/mL of the combination of strains SEMIA 5079 and 5080 (Table 1). Of the total of samples analyzed, 75.8% and 24.2% of samples were liquid and peat inoculants, respectively (Fig. 1). Most of these were imported products (75%), only 25% were national (Fig. 1). The strain combination most often found was SEMIA 5079/5080 (68% of analyzed samples), followed by SEMIA 5019/

Fig. 1 Percentage of liquid, peat, national, and imported inoculant for soybean crop in Brazil analyzed for official quality control from 2010 to 2014

Fig. 2 Percentage of SEMIA strain combinations in inoculant for soybean crops in Brazil, from 2010 to 2014

Fig. 3 Percentage of absence or presence of contamination in inoculant analyzed between 2010 and 2014, according to the strain combination

5079 (16%). SEMIA 5079/587 and SEMIA 5019/587 were found least often, 9% and 7%, respectively (Fig. 2).

According to SDA/MAPA no. 30/2010 Normative Instruction [14], no contamination should be found in the 10^{-5} or lower dilution. Regarding the presence or absence of contamination, red colonies are typically considered an indication of contamination, as the recommended strains for soybean do not completely absorb Congo red dye [14]. In total samples, only 2.21% of the samples showed contamination. SEMIA 5019/5079 was the only combination that did not show any contamination (Fig. 3). Of the inoculants containing only the strains SEMIA 5079/5080, the mostly used in this period, presented 1.8% of product contamination (Fig. 3). Inoculants containing only SEMIA 5019/587 and SEMIA 5079/587 combinations showed 4.1% and 8.4% of contamination, respectively (Fig. 3).

The concentration of viable cells recorded in the inoculants guarantees (CFU/mL in liquid inoculants or CFU/g in peat inoculants) most often ranges from 4.1×10^9 to 5.0×10^9 CFU/mL or CFU/g found in 59.1% of the samples (Table 2). The concentration ranges from 1.1×10^9 to 2.0×10^9 CFU/mL or CFU/g and 2.1×10^9 to 3.0×10^9 CFU/mL or CFU/g were found in 9.4% and 15.7% of inoculant, respectively. In 15.8%, the concentration was above 5.1×10^9 CFU/mL or CFU/g (Table 2). The concentration range was above 1.1×10^{10} CFU/mL or CFU/g in 43.3% of samples, indicating a higher value than the product guarantee. The concentration range of 1.1×10^9 to 5.0×10^9 CFU/mL or CFU/g was recorded in 16.6% of inoculants. Furthermore, the concentration range of 5.1×10^9 to 1.0×10^{10} CFU/mL or CFU/g was

recorded in 36.8% of products. The minimum concentration of rhizobia in inoculants should be 1.0×10^9 CFU/g or mL [15]. Only 3.3% of samples had concentration level below that indicated in the normative instructions or had the exact concentration level that fits the standards.

Discussion

During 2010 to 2014, the Laboratory of Agricultural Microbiology/FEPAGRO was the only one in Brazil to perform official analysis for MAPA. It is essential to clarify that not all inoculants imported or produced in Brazil were analyzed, only those collected by MAPA for official analysis. The recommended strains for soybean inoculant are SEMIA 5019, SEMIA 5079 (CPAC 15), SEMIA 5080 (CPAC 7), and SEMIA 587, typically, in a pair combination [16]. The strain combination in the inoculants is not relevant once each strain is capable of supplying N to the plant [3].

In this study, only 2.21% of the samples showed contamination. Herrmann et al. [14] reported that only 37% of the inoculants were not contaminated among the 65 samples from various private companies worldwide (USA, Argentina, UK, Australia, etc.), and 40% of the tested products did not contain any of the claimed strains, but only contaminants. In Argentina, 30% of the samples had contaminants and low rhizobial counts [15]. More studies reported alarming contamination in inoculants [17, 18]. Herridge et al. [19] showed that 5% of the Australian inoculants (70 out of 1234) had contaminants, and these strains were opportunistic pathogens for humans and other organisms. In addition, the number of rhizobia in the inoculants was inversely correlated to the number of microbial contaminants [19]. A high level of contamination will have a deleterious effect on the rhizobial numbers [20], and therefore, the nodulation and BNF efficiency will decrease, particularly under stress conditions [21]. Inoculant products should have a minimum concentration of 1.0×10^9 CFU/mL or CFU/g, with no maximum concentration restriction [22].

Due to their highly competitive capacity, superior N-fixation efficiency, and, particularly, high competitive capacity [23], SEMIA 5019 and 587 have been used to produce commercial inoculants in Brazil since 1980. From 1968 to

Table 2 Percentage of viable cells concentration declared in inoculant products guarantees and results from inoculant products analysis, from 2010 to 2014

Concentration in inoculant's guarantee (CFU/mL or CFU/g)	Percentage	Concentration ranges from analysis (CFU/mL or CFU/g)	Percentage
1.1×10^9 to 2.0×10^9	9.4%	$\leq 1.0 \times 10^9$	3.3%
2.1×10^9 to 3.0×10^9	15.7%	1.1×10^9 to 5.0×10^9	16.6%
4.1×10^9 to 5.0×10^9	59.1%	5.1×10^9 to 1.0×10^{10}	36.8%
$> 5.1 \times 10^9$	15.8%	$> 1.1 \times 10^{10}$	43.3%

1996, SEMIA 587, which presented high efficiency and competitiveness, was recommended in combination with SEMIA 532 and 543 [24]. SEMIA 587 was recommended in combination with SEMIA 5019 from 1979 onwards [16, 25]. The low utilization of SEMIA 5019 maybe because of the difficulty in finding inoculants containing this strain in its formulation. Despite the widespread use of the combination of SEMIA 5079 and SEMIA 5080, serological characterization indicated that *B. elkanii* strains SEMIA 587 and SEMIA 5019 were more competitive and presented a higher nodular occupancy capacity than strains belonging to *B. japonicum* species in Southern Brazilian soils [26].

SEMIA 5079 is a natural variant derived from SEMIA 566 (a strain used in inoculants in the late 1960s). Furthermore, it is the most competitive of the four commercial strains used in Brazil and was selected for having a higher capacity of N₂ fixation [27]. SEMIA 5079 has been detected in every soil cropped with soybean. SEMIA 5080 is a natural variant of SEMIA 586 strain and has been selected for high efficiency of N₂ fixation and adaptation to tropical soils. This strain is highly efficient in fixing N₂ but lesser competitive than SEMIA 5079 [27]. The strains SEMIA 5079 and SEMIA 5080 are recommended for soybean inoculation in the Brazilian Cerrado, based on 22 years of study conducted by Embrapa Cerrados [16, 25, 28, 29]. Inoculation with the SEMIA 5079 strain is an effective strategy to prevent strains of low efficiency and high competitiveness from predominating in soybean nodules [30].

Generally, inoculants are produced by private industries and subjected to varying degrees of regulation of quality standards [19], and numerous new types of products are emerging in the markets worldwide [31]. In Brazil, the current regulations are inducing local and foreign companies to improve the quality of their products, as found in this study. The lack of internationally accepted regulations for legume inoculant quality and usage parameters has led, on several occasions, to inadequate inoculant performance and the subsequent abandonment of their use [32]. The inoculant of superior quality passes through an effective quality control system with a series of quality checks during and after production, which ensures that each seed after inoculation will contain a sufficient population of rhizobia to produce abundant nodulation [21]. Apart from the inoculant rhizobial strain, the most crucial aspects of inoculant quality are rhizobial counts during manufacture, the level of nonrhizobial contamination, and the shelf life of the inoculant [19]. Therefore, on a comparison of this study with other studies, the inoculant used in Brazil has a high quality. In the last decade, an increasing improvement in the quality resulted in more contaminant-free inoculants than inoculants with nonsterile carriers that, for several years, performed poorly, particularly for rhizobia [32]. Nowadays, Brazil has taken on the leadership in taking advantage of the biological N₂ fixation benefits with the soybean

crop [33]. Modern tendency in legume cultivation is increasing the use of inoculants, as they are highly efficient and cost-effective, economically and environmentally.

Conclusions

Inoculant products marketed and used in soybeans in Brazil have a high quality, coming out of the industry with more than twice the minimum label guarantee registered in the MAPA and free of contaminants, as demonstrated in this study. This high concentration of rhizobial cells provides greater safety for the producer to obtain high yields in the field. In part, this is due to current regulations in Brazil that are improving the quality of these products. It can be said that soybean cultivation in Brazil is economically viable because of the use of inoculants, which can fulfill all the N needs of plants so that they can express their genetic production capacity.

Acknowledgements The authors are grateful to Eliane Bangel for her huge efforts in the diffusion of inoculation technology.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

References

1. Hungria M, Nogueira MA, Araujo RS (2013) Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. Biol Fertil Soils 49:791–801
2. Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2:621–631
3. Willems A (2006) The taxonomy of rhizobia: an overview. Plant Soil 287:3–14
4. Giongo A, Ambrosini A, Vargas LK, Freire JRJ, Bodanese-Zanettini MH, Passaglia LMP (2008) Evaluation of genetic diversity of bradyrhizobia strains nodulating soybean [*Glycine max* (L.) Merrill] isolated from South Brazilian fields. Appl Soil Ecol 38: 261–269
5. Hungria M, Campo RJ, Mendes IC (2001) Fixação biológica do nitrogênio na cultura da soja. Brasília, Embrapa Soja
6. Delamuta JRM, Ribeiro RA, Orrillo EO, Melo IS, Romero EM, Hungria M (2013) Polyphasic evidence supporting the reclassification of *Bradyrhizobium japonicum* group Ia strains as *Bradyrhizobium diazoefficiens*. Int J Syst Evol Microbiol 63: 3342–3351
7. Hungria M, Franchini JC, Campo RJ, Crispino CC, Moraes JZ, Sibaldelli RNR, Mendes IC, Arihara J (2006) Nitrogen nutrition of soybean in Brazil: contributions of biological N₂ fixation and of N fertilizer to grain yield. Can J Plant Sci 86:927–939
8. De Paiva Barbosa L, Costa PF, Ribeiro PRA, Rufini M, Guimarães AA, De Souza Moreira FM (2017) Symbiotic efficiency and genotypic characterization of variants of *Bradyrhizobium* spp. in commercial inoculants for soybeans. Rev Bras Cienc Solo 41:e0160572

9. SDA/MAPA (2004) Decree n° 4.954/2004. http://www.planalto.gov.br/ccivil_03/_ato2004-2006/2004/decreto/d4954.htm. Accessed 15 February 2018
10. Hungria M, Araujo RS, Júnior S, Barbosa E, Zilli JE (2017) Inoculum rate effects on the soybean symbiosis in new or old fields under tropical conditions. *Agron J* 109:1106–1112
11. Campos BC, Hungria M, Tedesco V (2001) Eficiência da fixação biológica de N₂ por estirpes de *Bradyrhizobium* na soja em plantio direto. *Rev Bras Ciênc Solo* 25:583–592
12. Ribeiro ABM, Bruzi AT, Zuffo AM, Zambiazzi EV, Soares IO, Vilela NJD, Pereira JLAR, Moreira SG (2017) Productive performance of soybean cultivars grown in different plant densities. *Ciênc Rural* 47:e20160928
13. SDA/MAPA (2010) Normative Instruction n° 30/2010. <http://www.agricultura.gov.br/assuntos/laboratorios/legislacoes-e-metodos/fertilizantes-substratos/INSTRUONORMATIVASDAN30DE12DENOVEMBRODE2010.pdf/view>. Accessed 15 February 2018
14. Herrmann L, Atieno M, Brau L, Lesueur D (2015) Microbial quality of commercial inoculants to increase BNF and nutrient use efficiency. In: Bruijn FJ (ed) *Biological nitrogen fixation*. Wiley, Hoboken, pp 1031–1040
15. Benintende S (2010) Quality of commercial inoculants for soybean crop in Argentina: concentration of viable rhizobia and presence of contaminants. *Rev Argent Microbiol* 42:129–132
16. Freire JRJ, Vemetti FJ (1997) A pesquisa com soja, a seleção de rizóbio e a produção de inoculantes no Brasil. *Pesq Agrop Gaúcha* 5:117–121
17. Gemell LG, Hartley EJ, Herridge DF (2005) Point-of-sale evaluation of preinoculated and custom-inoculated pasture legume seed. *Anim Prod Sci* 45:161–169
18. Husen E, Simanungkalit RDM, Saraswati R (2007) Characterization and quality assessment of Indonesian commercial biofertilizers. *Indones J Agric Sci* 8:31–38
19. Herridge DF, Hartley E, Gemell LG (2014) Rhizobial counts in peat inoculants vary among legume inoculant groups at manufacture and with storage: implications for quality standards. *Plant Soil* 380:327–336
20. Catroux G, Hartmann A, Revellin C (2001) Trends in rhizobial inoculant production and use. *Plant Soil* 230:21–30
21. Lupwayi NZ, Olsen PE, Sande ES, Keyser HH, Collins MM, Singleton PW, Rice WA (2000) Inoculant quality and its evaluation. *Field Crops Res* 65:259–270
22. SDA/MAPA (2011) Normative Instruction n° 13/2011. <http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-agricolas/fertilizantes/legislacao/in-sda-13-de-24-03-2011-inoculantes.pdf>. Accessed 15 February 2018
23. Campo RJ, Hungria M (2007) Avaliação de estirpes ou combinações de estirpes de *Bradyrhizobium japonicum* e *B. elkanii* para a soja. In: Campo RJ, Hungria M (eds) *Anais da 13 Reunião da Rede de Laboratórios para Recomendação, Padronização e Difusão de Tecnologia de Inoculantes Microbianos de Interesse Agrícola (RELARE)*. Embrapa Soja, Londrina, p 32
24. Freire JRJ, Kolling J, Vidor C (1983) Sobrevivência e competição por sítios de nodulação de estirpes de *Rhizobium japonicum* para soja. *Rev Bras Ciênc Solo* 7:47–53
25. Batista JSS, Hungria M, Barcellos FG, Ferreira MC, Mendes IC (2007) Variability in *Bradyrhizobium japonicum* and *B. elkanii* seven years after introduction of both the exotic microsymbiont and the soybean host in a Cerrados soil. *Microbiol Ecol* 53:270–284
26. Bizarro MJ, Giongo A, Vargas LK, Roesch LFW, Gano KA, Sá ELS, Passaglia LMP, Selbach PA (2011) Genetic variability of soybean bradyrhizobia populations under different soil managements. *Biol Fertil Soils* 47:357–362
27. Siqueira AF, Ormeño-Orrillo E, Souza RC, Rodrigues EP, Almeida LGP, Barcellos FG, Batista JSS, Nakatani AS, Martínez-Romero E, Vasconcelos ATR, Hungria M (2014) Comparative genomics of *Bradyrhizobium japonicum* CPAC 15 and *Bradyrhizobium diazoefficiens* CPAC 7: elite model strains for understanding symbiotic performance with soybean. *BMC Genomics* 15:420
28. Peres JAR, Mendes LC, Suhel A, Vargas MAT (1993) Eficiência e competitividade de estirpes de rizóbio para soja em solos de cerrado. *Rev Bras Ciênc Solo* 17:357–363
29. Mendes IC, Vargas MAT, Peres JRR, Suhel AR, Reis-Junior FB (2007a) SEMIA 5080 (CPAC-7) e SEMIA 5079 (CPAC-15): Retrospectiva dos Experimentos conduzidos na Embrapa Cerrados. Parte I: Olhando para o passado e para o futuro. In: Campo RJ, Hungria M (eds) *Anais da 13 Reunião da Rede de Laboratórios para Recomendação, Padronização e Difusão de Tecnologia de Inoculantes Microbianos de Interesse Agrícola (RELARE)*. Embrapa Soja, Londrina, p 25
30. Mendes IC, Vargas MAT, Peres JRR, Suhel AR, Reis-Junior FB (2007b) SEMIA 5080 (CPAC-7) e SEMIA5079 (CPAC-15): Retrospectiva dos experimentos conduzidos na Embrapa Cerrados. Parte II: Fixar N eficientemente é preciso, mas, competir pelos sítios de infecção, também é. In: Campo RJ, Hungria M (eds) *Anais da 13 Reunião da Rede de Laboratórios para Recomendação, Padronização e Difusão de Tecnologia de Inoculantes Microbianos de Interesse Agrícola (RELARE)*. Embrapa Soja, Londrina, p 26
31. Herrmann L, Lesueur D (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. *Appl Microbiol Biotechnol* 97:8859–8873
32. Penna C, Massa R, Olivieri F, Gutkind G, Cassán F (2011) A simple method to evaluate the number of bradyrhizobia on soybean seeds and its implication on inoculant quality control. *AMB Express* 1:21
33. Hungria M, Campo RJ, Mendes IC (2007) A importância do processo de fixação biológica do nitrogênio para a cultura da soja: componente essencial para a competitividade do produto brasileiro. <http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/468512>. Accessed 15 February 2018