Amino Acids (2004) 27: 169–174
DOI 10.1007/s00726-004-0111-7

Amino Acids
Printed in Austria

Production of amino acids by *Rhizobium*, *Mesorhizobium* and *Sinorhizobium* strains in chemically defined media

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Received June 20, 2004
Accepted June 29, 2004
Published online September 22, 2004;

Summary. Five strains of *Rhizobium* spp, one strain of *Mesorhizobium loti* and two strains of *Sinorhizobium meliloti* were tested for their ability to grow in chemically-defined medium lacking growth factor. Qualitative and quantitative production of aspartic acid, serine, glutamic acid, glycine, histidine, threonine, arginine, alanine, proline, cysteine, tyrosine, valine, methionine, lysine, isoleucine, leucine, and phenylalanine was determined by the use of mannitol as sole carbon source.

Strains of *Rhizobium* spp. and *Sinorhizobium* sp. produced all the amino acids analysed with the exception of cysteine and high biological levels of serine, glycine and alanine were detected after 2 days of culture in mineral medium.

Strain U226 of *M. loti* only produced small amounts of amino acids and glutamic acid, histidine, arginine, cysteine, methionine, lysine and phenylalanine was not liberated into the media.

Keywords: Amino acids – *Rhizobium* – *Mesorhizobium* – *Sinorhizobium*

Introduction

Rhizobia are useful organisms commercially because of their agricultural applications as inoculants for legumes (Buttery et al., 1992; Peix et al., 2001). Competence of inoculant bacteria with native strains, and their persistence in soil, are two major factors in the success of inoculation practices (Rodelas et al., 2002). The molecular details of the plant-bacterial interaction that leads to formation of nodules have been characterized (van Rhijn and Vanderleyden, 1995). However, the role of these interactions with regard to successful bacterial root colonization is poorly known. In this way, the influence on rhizobial root colonization of biologically-active substances presents in plant root exudates or the liberation of substances produced and released by rhizobia, such as phytohormones (Hirsch, 1992), on success of the establishment of symbiosis has been proposed by not clarified.

Amongst other compounds present in plant root exudates that can exert a positive chemotactic effect on soil microbiota, amino acids influence the proliferation of rhizobacteria in the vicinity of root systems considerably (Gaworzewska and Carlile, 1982). Amino acids in the rhizosphere are both of plant and microbial origen. The role of amino acids produced by rhizobacteria such as *Rhizobium*, *Mesorhizobium* and *Sinorhizobium* in their interaction with legumes is practically unknown, although it is well established that methionine and tryptophan act in soil as main precursors for the synthesis of plant hormones ethylene and indole-3-acetic acid, respectively (Murcia et al., 1997). Plants also show response to the exogenous application of L-methionine to soils (Arshad et al., 1993).

Production of amino acids by rhizosphere microorganisms such as *Azotobacter* and *Azospirillum* has received some attention, despite of the roles attributed to these compounds in the interaction of these bacteria with plants (Gonzalez-Lopez et al., 1999). However, very few studies have attempted to ascertain the ability of rhizobia to synthesize and release amino acids into the surrounding media. In this paper, the production of aspartic acid, serine, glutamic acid, glycine, histidine, threonine, arginine, alanine, proline, cysteine, tyrosine, valine, methionine, lysine, isoleucine, leucine and phenylalanine by five strains of *Rhizobium* spp., one strain of *Mesorhizobium*

loti and two strain of *Sinorhizobium meliloti*, selected from a set of 28 strains previously screened for ability to grow in chemically-defined media lacking growth factors, was tested and age of cultures on amino acids production was determined.

Materials and methods

Microorganisms

Microorganism used in this study were *Rhizobium leguminosarum* bv. *viceae* strain GR119, and strain 300, *R. leguminosarum* bv. *phaseoli* strain GR12, *R. leguminosarum* bv. *trifolii* strain GRZ7, *Rhizobium* sp. (Acacia) strain GRH28, *Mesorhizobium loti* strain U226, *Sinorhizobium meliloti* strain GR4B and strain 102F65. The strains were previously listed by Rodelas et al. (1998) and maintained at 4°C in yeast-extract mannitol agar slants (YMA) (Jordan, 1984).

Growth conditions

The chemically-defined medium (CDM) used for all assays was a slight modification of that described by Rodelas et al. (1998) and had the following composition (g per L of distilled water): mannitol, 5.00; KNO_3, 1.0; K_2HPO_4, 0.22; $MgSO_4 \cdot 7H_2O$, 0.10; $FeCl_3$, 0.02; $CaCl_2$, 0.04; $NaMoO_4$, 0.001 (final pH 6.8). Media were buffered with 50 mmol/l MOPS/KOH (pH 6.8). Difco agar was used at 1.5% (w/v) when required for solid media.

Single colonies of each strain were transferred from fresh cultures on plates on 250 ml Erlenmeyer flasks containing 50 ml CDM amended with 0.5% mannitol; a filter-sterilized solution containing biotin, thiamin and pantothenic acid, to give final concentrations of 0.1 mg/ml of each vitamin, was then added. Flasks were incubated at 28°C in a shaker (Gallenkamp INR-200, UK) with gentle agitation (110 rev/min) for 72 h, and then subcultured (0.5 ml) to fresh medium and incubated for another 24 h. Cells from these 24 h cultures were collected, centrifuged at 3,000 g for 30 min (4°C) and then washed twice with sterile saline (0.9% NaCl). Cell density of washed cell suspensions was adjusted to 0.5 on the McFarland scale, and 0.5 ml of the standarized inoculum was added to 250 ml Erlenmeyer flasks containing 50 ml CDM amended with 0.5% mannitol. The bacterial growth was carried out in triplicate for each strain. Culture were kept at 28°C in the shaker. Flasks were examined for growth (viable counts) after, 1, 2, 3 and 7 days. Viable counts of strains in these cultures were determined by dilution and plating in tryptone-yeast extract agar plates (Beringer, 1974).

Amino acids production assay

Samples from broth cultures of rhizobial strains in CDM lacking growth factors and amended with 0.5% mannitol were taken after 1, 2, 3 and 7 d of incubation at 28°C. Aliquots were centrifuged at 5,000 g for 30 min in a Sorvall RC-5B centrifuge (Dupont Instruments, Wilmington, DE, USA) at 4°C, and the supernatant fluids were passed through 0.22 μm of Millipore filter membranes. Aliquots (100 μl) of filtrate supernatants were added to Eppendord tubes containing 200 μl of acetic acid (2 mol/l). The amino acids fraction was isolated with pre-preparated Dowex column (AG50WX8 in the H^+ form) according to Stoll et al. (1999) and the purified amino acids were used for HPLC analysis (Water ACCQ.TAG) with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivative. The solution was analysed for amino acids by injecting 5 μl into a HPLC 2690 (Water) with a Water column of 150 mm. Standard curve for amino acids was calculated from 3 concentrations (20, 50 y 100 pmol/ml of amino acid). The correlation coefficient was >0.99 (r^2 0.999527) and sensitivity of the method was in the range of 2 pmol of amino acid per ml. All runs were performed at least in triplicate.

Statistical analysis

Data obtained throughout this study were analysed by computer-assisted one-way ANOVA, using the software package STATGRAPHICS version 5.0 (STSC Inc., Rockville, Maryland, USA, 1989). Least significances (LSD) were calculated at 99% level of significance ($P < 0.01$).

Results and discussion

Figures 1 and 2 shows the results of the curve growth of 8 strains tested in minimal media lacking factors using mannitol as sole carbon source. No significant differences in the growth of these strains were found in CDM after 7 d of incubation at 28°C under aerobic conditions. Our experiments show that strains tested in this study were able to grow in chemically-defined media in the absence of growth factors. Although strains classified as *R. leguminosarum* bv. *viceae* and *trifolii* commonly require a large number of growth factors, including amino acids and water-soluble vitamins (Jordan, 1984), some rhizobic are able to growth without the addition of any growth factors (Rodelas et al., 1998). In this context, the results described here are in agreement with previous reports on the requirements of these microorganisms.

The liberation of amino acids by *R. leguminosarum* bv. *viceae* strain GRL19 and strain 300 in CDM is shown in Table 1. Date show that the production of those substances is influenced by the age of the culture. Thus, *R. leguminosarum* bv. *viceae* strains produced all the amino acids tested with the exception of cysteine, and large amounts of serine, glycine and alanine were produced

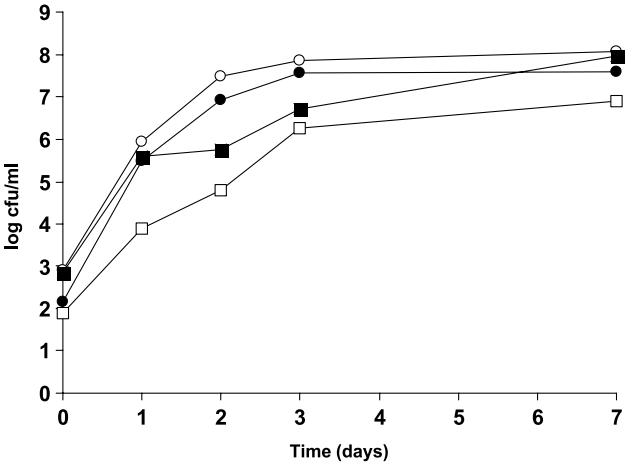

Fig. 1. Growth curves of *Rhizobium leguminosarum* bv. *viceae* strain GRL19 (●), *R. leguminosarum* bv. *viceae* strain 300 (○), *R. leguminosarum* bv. *phaseoli* strain GR12 (■) and *R. leguminosarum* bv. *trifolii* strain GRZ7 (□) in minimal medium lacking growth factors. The experiments was carried out in triplicate

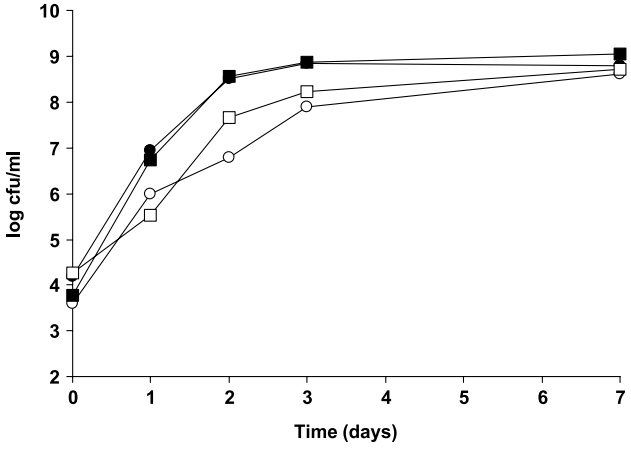

Fig. 2. Growth curves of *Rhizobium* sp (Acacia) strain GRH28 (●), *Mesorhizobium loti* strain U226 (○), *Sinorhizobium melilotii* strain GR4B (■) and *S. meliloti* strain 102F65 (□) in minimal medium lacking growth factors. The experiments was carried out in triplicate

after 2 d of incubation at 28°C. When *R. leguminosarum* bv. *phaseoli* and bv. *trifolii* were grown in CDM amended with 0.5% mannitol, all amino acids analysed with exception of cysteine was produced and high biological levels of serine, glycine and alanine were detected in mineral medium (Table 2). Similar results were obtained in the chemically-defined medium for *S. meliloti* strain GR4B, *S. meliloti* strain 102F65 and *Rhizobium* sp (Acacia). Thus in the supernatant fluids of the broth cultures grown for 2, 3 and 7 d (with the exception of cysteine) significant amounts of all amino acids tested were found (Tables 3 and 4) showed that those microorganisms are able to produce and liberate different amino acids under our experiment conditions.

Mesorhizobium loti strain U226 produced small amounts of serine, glycine, threonine, alanine, proline, tyrosine, valine, isoleucine and leucine. Therefore, the liberation of these amino acids were generally affected by the age of the culture (Table 4). Glutamic acid, histidine, arginine, cysteine, methionine, lysine and phenylalanine were not produced by *M. loti* in chemically-defined medium amended with 0.5% mannitol. In this context, these amino acids were not detected in the supernatant of bacterial culture growth for 1 to 7 days.

The chemical composition and processes in the rhizosphere have recently gained much attention from students of soil-plant interactions but the main interest has focused on aspects other than amino acids (Broughton et al., 2003). Our results show that culture supernatants of *Rhizobium* and *Sinorhizobium* contained at least 16 amino acids. However, the production of amino acids was influenced by the duration of incubation. Similar results were reported in *Azotobacter* and other nitrogen-fixer microorganisms (Gonzalez-Lopez et al., 1999).

Table 1. Production (pmol/ml) of amino acids by *Rhizobium leguminosarum* bv. *viceae* strain GRL19 and *R. leguminosarum* bv. *viceae* strain 300 in minimal media lacking growth factors

	Strain GRL19				Strain 300			
	1	2	3	7	1	2	3	7
Aspartic acid	0	42 ± 4	80 ± 8	76 ± 6	0	3 ± 1	10 ± 2	42 ± 10
Serine	0	276 ± 7	240 ± 5	258 ± 8	0	69 ± 5	89 ± 4	109 ± 6
Glutamic acid	0	30 ± 3	30 ± 6	26 ± 4	0	7 ± 2	9 ± 2	87 ± 5
Glycine	0	228 ± 5	192 ± 1	204 ± 4	2 ± 0.2	46 ± 7	76 ± 5	170 ± 9
Histidine	0	36 ± 3	71 ± 8	66 ± 7	0	4 ± 1	14 ± 2	31 ± 6
Threonine	0	58 ± 4	57 ± 1	48 ± 3	0	11 ± 3	31 ± 6	52 ± 9
Arginine	0	14 ± 4	21 ± 2	23 ± 8	0	3 ± 1	9 ± 3	16 ± 2
Alanine	0	210 ± 5	174 ± 8	176 ± 3	12 ± 2	79 ± 3	103 ± 8	133 ± 6
Proline	0	47 ± 7	63 ± 7	56 ± 5	0	41 ± 6	60 ± 7	79 ± 8
Cysteine	0	0	0	0	0	0	0	0
Tyrosine	0	32 ± 7	43 ± 1	39 ± 6	0	0	0	16 ± 3
Valine	0	52 ± 8	55 ± 3	58 ± 7	0	10 ± 2	24 ± 9	39 ± 6
Methionine	0	27 ± 9	35 ± 9	29 ± 4	0	16 ± 3	24 ± 6	30 ± 7
Lysine	0	21 ± 7	24 ± 6	30 ± 3	0	5 ± 1	25 ± 4	26 ± 8
Isoleucine	0	26 ± 2	28 ± 7	28 ± 6	0	6 ± 2	24 ± 4	26 ± 4
Leucine	0	34 ± 1	34 ± 4	30 ± 6	0	10 ± 3	39 ± 9	38 ± 6
Phenylalanine	0	16 ± 4	24 ± 5	23 ± 7	0	5 ± 1	25 ± 8	21 ± 3

Values are means ± standard error of three replicates
0 Not detected

Table 2. Production (pmol/ml) of amino acids by *Rhizobium leguminosarum* bv. *phaseoli* strain GR12 and *R. leguminosarum* bv. *trifolii* strain GRZ7 in minimal media lacking growth factors

	Strain GR12				Strain GRZ7			
	1	2	3	7	1	2	3	7
Aspartic acid	2 ± 1	17 ± 4	16 ± 3	18 ± 2	0	21 ± 4	53 ± 9	43 ± 9
Serine	4 ± 1	148 ± 6	185 ± 7	186 ± 9	77 ± 7	193 ± 12	358 ± 18	390 ± 7
Glutamic acid	0	14 ± 6	43 ± 5	40 ± 4	0	13 ± 6	48 ± 3	42 ± 8
Glycine	0	100 ± 15	112 ± 6	110 ± 7	24 ± 8	170 ± 8	268 ± 9	243 ± 13
Histidine	1 ± 0.3	23 ± 5	28 ± 3	36 ± 10	12 ± 5	19 ± 3	44 ± 9	42 ± 7
Threonine	2 ± 0.3	57 ± 3	70 ± 9	59 ± 11	0	97 ± 7	97 ± 9	73 ± 13
Arginine	0	14 ± 2	18 ± 4	26 ± 3	0	16 ± 2	79 ± 10	191 ± 10
Alanine	0	89 ± 3	109 ± 8	121 ± 6	21 ± 8	148 ± 9	219 ± 12	291 ± 7
Proline	32 ± 6	26 ± 7	28 ± 7	26 ± 5	0	0	51 ± 7	41 ± 9
Cysteine	0	0	0	0	0	0	0	0
Tyrosine	6 ± 1	42 ± 5	43 ± 5	40 ± 9	0	33 ± 8	70 ± 9	78 ± 6
Valine	0	22 ± 5	26 ± 6	33 ± 6	0	35 ± 6	57 ± 6	43 ± 5
Methionine	0	10 ± 3	14 ± 6	16 ± 6	0	0	4 ± 1	9 ± 3
Lysine	0	17 ± 5	23 ± 6	33 ± 4	0	0	32 ± 6	31 ± 7
Isoleucine	0	13 ± 3	15 ± 4	13 ± 3	0	7 ± 2	34 ± 6	28 ± 6
Leucine	0	17 ± 4	20 ± 4	23 ± 2	5 ± 1	21 ± 5	41 ± 8	45 ± 9
Phenylalanine	0	7 ± 2	9 ± 3	8 ± 3	0	9 ± 3	19 ± 2	33 ± 4

Values are means ± standard error of three replicates
0 Not detected

Table 3. Production (pmol/ml) of amino acids by *Sinorhizobium meliloti* strain GR4B and *S. meliloti* strain 102F65 in minimal media lacking growth factors

	Strain GR4B				Strain 102F65			
	1	2	3	7	1	2	3	7
Aspartic acid	3 ± 0.8	20 ± 4	37 ± 4	36 ± 9	0	0	62 ± 7	67 ± 4
Serine	102 ± 5	365 ± 21	345 ± 23	351 ± 17	0	0	383 ± 15	391 ± 9
Glutamic acid	14 ± 2	16 ± 3	37 ± 5	32 ± 8	0	48 ± 6	50 ± 5	48 ± 7
Glycine	68 ± 9	194 ± 9	184 ± 6	196 ± 8	0	0	282 ± 6	256 ± 9
Histidine	7 ± 2	9 ± 1	24 ± 6	30 ± 8	0	0	49 ± 8	59 ± 4
Threonine	40 ± 8	38 ± 6	89 ± 7	87 ± 4	0	0	37 ± 6	34 ± 7
Arginine	0	57 ± 7	39 ± 13	24 ± 7	0	0	19 ± 3	19 ± 3
Alanine	60 ± 7	187 ± 14	158 ± 16	177 ± 10	0	0	149 ± 10	164 ± 17
Proline	3 ± 1	47 ± 8	45 ± 8	30 ± 10	0	0	49 ± 8	53 ± 3
Cysteine	0	0	0	0	0	0	0	0
Tyrosine	0	22 ± 5	48 ± 7	27 ± 8	0	2 ± 0.5	18 ± 4	19 ± 9
Valine	15 ± 4	55 ± 4	58 ± 10	51 ± 3	0	7 ± 2	62 ± 12	76 ± 8
Methionine	0	5 ± 1	15 ± 5	25 ± 9	0	0	40 ± 9	32 ± 5
Lysine	5 ± 2	30 ± 6	34 ± 7	26 ± 6	0	0	28 ± 6	21 ± 7
Isoleucine	6 ± 0.6	34 ± 9	28 ± 9	22 ± 6	0	5 ± 1	44 ± 8	39 ± 4
Leucine	12 ± 4	49 ± 8	37 ± 9	36 ± 6	0	0	43 ± 6	22 ± 3
Phenylalanine	3 ± 2	17 ± 2	22 ± 4	24 ± 7	0	0	31 ± 2	47 ± 8

Values are means ± standard error of three replicates
0 Not detected

Serine, glycine and alanine were produced by *Rhizobium* and *Sinorhizobium* in large amounts. In this context different amino acids are also produced in large amounts by certain bacteria isolated from rhizosphere. In this sense, soil microorganisms may enhance mycorrhizas formation, possibly by supplying amino acids and vitamins to the rhizosphere (Strzelczyk and Leniarska, 1985). However, the possibility that plant growth could be

Table 4. Production (pmol/ml) of amino acids by *Rhizobium* spp (Acacia) strain GRH28 and *Mesorhizobium loti* strain U226 in minimal media lacking growth factors

	Strain GRH28				Strain U226			
	1	2	3	7	1	2	3	7
Aspartic acid	0	23 ± 7	35 ± 8	39 ± 4	0	0	0	0
Serine	13 ± 2	272 ± 10	377 ± 20	421 ± 13	56 ± 3	79 ± 9	82 ± 6	57 ± 9
Glutamic acid	0	4 ± 1	25 ± 4	42 ± 7	0	0	0	0
Glycine	0	195 ± 7	265 ± 13	275 ± 5	12 ± 1	14 ± 2	19 ± 3	30 ± 7
Histidine	0	30 ± 7	44 ± 7	50 ± 4	0	0	0	0
Threonine	0	55 ± 8	141 ± 9	102 ± 12	4 ± 0.7	7 ± 2	6 ± 2	17 ± 3
Arginine	0	27 ± 9	24 ± 10	24 ± 6	0	0	0	0
Alanine	6 ± 2	95 ± 8	213 ± 9	172 ± 16	0	27 ± 6	40 ± 4	66 ± 6
Proline	17 ± 3	61 ± 2	54 ± 5	38 ± 6	0	0	8 ± 3	6 ± 2
Cysteine	0	0	0	0	0	0	0	0
Tyrosine	0	40 ± 9	67 ± 6	91 ± 5	11 ± 4	19 ± 3	19 ± 4	16 ± 2
Valine	0	30 ± 4	49 ± 5	82 ± 6	0	0	0	17 ± 2
Methionine	0	74 ± 9	74 ± 10	79 ± 4	0	0	0	0
Lysine	0	30 ± 2	31 ± 8	58 ± 9	0	0	0	0
Isoleucine	0	18 ± 2	28 ± 5	38 ± 6	0	0	0	20 ± 5
Leucine	0	29 ± 7	37 ± 2	56 ± 10	0	0	1 ± 0.3	2 ± 0.8
Phenylalanine	0	22 ± 2	21 ± 6	33 ± 6	0	0	0	0

Values are means ± standard error of three replicates
0 Not detected

improved by inoculation with amino acids or vitamins producing bacteria has received little attention.

Mesorhizobium loti strain U226 produced lower amounts of amino acids compared to *Rhizobium* spp. and *Sinorhizobium* spp. Thus culture supernatants of *Mesorhizobium* contained 9 amino acids after 7 days of incubation at 28°C. Therefore the concentration of serine, glycine and alanine in the *Mesorhizobium* culture supernatants was significantly lower than those detected for *Rhizobium* and *Sinorhizobium*. However, these results may reflect the influence of the nutritional conditions utilized in our experiments, nature of carbon sustrate, C and N concentration and ratios, pH, temperature and time incubation. In this sense, has been reported for many other genera of diazotrophic soil bacteria that the production of amino acids is directly affected by the growth conditions applied (Murcia et al., 1997).

Rhizobium, Sinorhizobium and *Mesorhizobium* bacteria obtain the energy for N_2 fixation from the plant but in addition to this major necessity, they also depend on the host for other nutrients such as amino acids and vitamins, which may not always be available in sufficient quantities under natural conditions. Thus, could be suggested that amino acids liberation by rhizobial strains may well be an advantage in competition for nodulation and root colonization it is concluded that amino acids production by rhizobial strains, and the possible involvement of these compound in the colonization and nodulation of legumes by rhizobia deserves more attention.

References

Arshad M, Hussain A, Javed M, William T (1993) Effect of soil applied L-methionine on growth, nodulation and chemical composition of *Albicia lebbeck*, L. Plant Soil 148: 129–135

Beringer JE (1974) R factor transfer in *Rhizobium leguminosarum*. J Gen Microbiol 84: 188–189

Broughton WJ, Zhang F, Perret X, Staehelin C (2003) Signals exchanges between legumes and *Rhizobium*: agricultural uses and perspectives. Plant Soil 252: 129–137

Buttery BR, Parj SJ, Hume DJ (1992) Potential for increasing nitrogen-fixation in grain legumes. Canadian J Plant Sci 72: 323–349

Gaworzewska ET, Carlile MJ (1982) Positive chemotaxis of *Rhizobium leguminosarum* and other bacteria towards root exudates from legumes and other plants. J Gen Microbiol 128: 1179–1188

Gonzalez-Lopez J, Martinez-Toledo MV, Rodelas B, Salmeron V (1999) Effect of some herbicides on the production of lysine by *Azotobacter choococcum*. Amino Acids 17: 165–173

Hirsch AM (1992) Developmental biology of legume nodulation. New Phytologyst 122: 211–237

Jordan DC (1984) Genus I *Rhizobium* Frank 1889, 338^{AL}. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 1. Williams & Wilkins, Baltimore, pp 235–242

Murcia R, Rodelas B, Salmeron V, Pozo C, Gonzalez-Lopez J (1997) Effect of simazine on the production of lisine and methionine by *Azotobacter chroococcum* and *Azotobacter vinelandii*. Amino Acids 12: 249–255

Peix A, Rivas-Boyero AA, Mateos PF, Rodriguez-Borrueco C, Martinez-Molina E, Velazquez E (2001) Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediter-*

raneum under growth chamber conditions. Soil Biol Biochem 33: 103–110

Rodelas B, Gonzalez-Lopez J, Salmeron V, Martinez-Toledo MV, Pozo C (1998) Symbiotic effectiveness and bacteriocin production by *Rhizobium leguminosarum* bv. *viceae* isolated from agricultural soils in Spain. Appl Soil Ecol 8: 51–60

Rodelas B, Mora L, Pozo C, Martinez-Toledo MV, Gonzalez-Lopez J (2002) Molecular basis of bacteriocin production by *Rhizobium*. Recent Res Devel Mol Microbiol 1: 19–31

Stoll B, Burrin DG, Henry JF, Jahoor F, Reeds PJ (1999) Dietary and systemic phenylalanine utilization for mucosal and hepatic constitutive protein synthesis in pigs. Am J Physiol 276: 49–57

Strzelczyk E, Leniarska U (1985) Production of B-group vitamins by mycorrhizal fungi and actinomycetes isolated from the root of pine (*Pinus sylvestris*, L.). Plant Soil 86: 387–394

Van Rhijn P, Vanderleyden J (1995) The *Rhizobium*-plant symbiosis. Microbial Rev 59: 124–142

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