Acetylene Reduction by Bacteroids Isolated from Stem Nodules of *Sesbania rostrata*. Specific Role of Lactate as an Energy-yielding Substrate

By J. C. TRINCHANT AND J. RIGAUD*

Laboratoire de Biologie végétale, UA CNRS 1114, Faculté des Sciences et des Techniques,
Parc Valrose, 06034 Nice Cedex, France

(Received 11 February 1986; revised 14 July 1986)

Bacteroids isolated from stem nodules of *Sesbania* were able to reduce C_2H_2 when glucose or succinate was the energy-yielding substrate. To be efficient, glucose always required low O_2 concentrations, both with free O_2 and with O_2 delivered by leghaemoglobin extracted from French-bean nodules. Lactate detected in the stem nodule cytosol supported C_2H_2 reduction by bacteroid preparations, but the O_2 tensions required for optimal activity were always higher than those defined for glucose, as predicted by the model of Bergersen & Trinchant (J. theor. Biol. 115, 93–102, 1985). In contrast, lactate was only present at very low concentrations in the cytosol of French-bean nodules and was unable to support C_2H_2 reduction by bacteroids isolated from these nodules. Lactate dehydrogenase (EC 1.1.1.27) extracted from stem nodule bacteroids and free-living cells of *Rhizobium* sp. (*Sesbania*) exhibited a higher affinity for lactate than did the enzyme from *Rhizobium meliloti*, *R. leguminosarum* biovar *phaseoli* or *Bradyrhizobium* and their symbiotic forms. Lactate appears to be a specific energy-yielding substrate supporting N_2 fixation by *Sesbania* bacteroids and could play a major role when fermentative processes take place in the nodules.

INTRODUCTION

The annual tropical legume *Sesbania rostrata*, which grows in the Sahel region of West Africa, is characterized by profuse nodulation along the stem and on the roots (Dreyfus & Dommergues, 1980). The stem nodules, caused by specific fast-growing *Rhizobium*, exhibit a very high capacity for N_2 fixation (Dreyfus & Dommergues, 1981). In comparison to the root nodules, their carbon supply is increased by photosynthesis in chloroplasts present in their cortex. Indeed photosynthates are undoubtedly the main products oxidized by bacteroids as sugars, cyclitols or organic acids (Reibach & Streeter, 1983) to provide energy and reducing power for nitrogenase. The common assumption that bacteroids are unable to oxidize glucose (see review by Stowers, 1985) might be correlated with enzymes of sugar catabolism having a lower activity in *R. leguminosarum* (Glenn *et al.*, 1984). An alteration of the sugar transport system during the bacteroid preparation might also play a part in hexose inefficiency (Dilworth & Glenn, 1984). However, glucose acts as an energy-yielding substrate for bacteroids isolated from French-bean and soybean nodules (Trinchant *et al.*, 1981, 1983) with O_2 tensions lower than those required with organic acids such as succinate; the O_2 requirement appears to be directly related to the stimulation by these substrates of bacteroid respiration (Bergersen & Trinchant, 1985).

In *Sesbania* stem nodules, photosynthetic activity produces O_2 and provides other carbon sources for respiration. On the other hand, *Rhizobium* sp. (*Sesbania*) preferentially uses lactate in place of glucose or mannitol in the culture medium and strain ORS 571 exhibits characteristics of fast-growing bacteria under these conditions (Elmerich *et al.*, 1982).

In this paper, we have investigated C_2H_2 reduction by isolated bacteroids from *Sesbania* stem

Abbreviations: LDH, lactate dehydrogenase; ADH, alcohol dehydrogenase.

nodules with respect to the carbon and oxygen supply. The specific role of lactate, generated in the cytosol by an active lactate dehydrogenase (LDH; EC 1.1.1.27), is discussed.

METHODS

Nodules. Seeds of *S. rostrata* were treated for 30 min with concentrated H_2SO_4 , washed 10 times with sterile distilled water and pregerminated at 30 °C in Petri dishes (Dreyfus & Dommergues, 1980). After 24 h the germinated seeds were transferred to a vermiculite/sand mixture (2:1, v/v), inoculated with *Rhizobium* sp. (*Sesbania*) strain ORS 571, and grown in a glasshouse as described by Rigaud & Puppo (1975). After 3 weeks, the stems of the plants (15 cm high) were inoculated with the same strain of *Rhizobium* by spraying. Inoculation was repeated each week as elongation of the stem proceeded. Nodules emerged 5 d after inoculation and were collected 25–30 d after their appearance on the roots or stems.

French-beans (*Phaseolus vulgaris* L., cv. Contender), inoculated with *R. leguminosarum* biovar *phaseoli* strain 9-6, were grown as above. Root nodules were collected 30–35 d after sowing (nodule age, 25–30 d).

Bacteroid preparations. *Sesbania* stem nodules (15–20 g fresh wt) and *Phaseolus* root nodules (10–15 g dry wt) were crushed anaerobically and bacteroids were washed twice with sodium phosphate buffer (50 mM, pH 7.4), containing 2 mM- MgSO_4 and 0.3 M-sucrose. After centrifugation, chloroplasts sedimented at the upper part of the pellet of *Sesbania* bacteroids were scraped away. The two types of bacteroids were finally resuspended in sodium phosphate buffer (25 mM, pH 7.4) to a final concentration of about 40 mg dry wt ml^{-1} .

Laboratory cultured bacteria. *Rhizobium* strain ORS 571 was grown at 30 °C under air (0.3 l min^{-1}) in a 6 l Biolafitte fermenter (Poissy, France) containing 4 l YLS medium (Elmerich *et al.*, 1982). The inoculum was 50 ml of exponentially growing culture (OD_{600} 1.2) and the stirring speed was 300 r.p.m. Bacteria were collected after 24 h growth, yielding 4.5 g wet wt of cells l^{-1} . *R. leguminosarum* biovar *phaseoli* strain 9-6 and *Bradyrhizobium* strain 1809 were grown under the same conditions on a medium described by Kennedy *et al.* (1975). Cultures were harvested after 72 h, yielding 2 g wet wt l^{-1} .

N_2 fixation assays. Nitrogenase activity was measured by a C_2H_2 reduction assay using a gas chromatograph (Rigaud, 1976). All bacteroid incubations were done in duplicate. The reaction mixtures (1 ml) were placed in rubber capped vials (7 ml) under a gas phase of C_2H_2 (5%, v/v) and O_2 at different partial pressures in Ar. Assays using purified leghaemoglobin were done in the 3 ml chamber of an O_2 electrode (Rank) at low concentrations of dissolved O_2 and without a gas phase. At intervals during the depletion of O_2 by bacteroids, samples were slowly withdrawn from the reaction mixture with a syringe and injected into 15 ml 'Venोजect' vacuum tubes. The evolved gas was then analysed by gas chromatography.

Incubations (3 ml) conducted in glass cuvettes (10 mm light path) closed with rubber serum stoppers were used to follow the deoxygenation of oxyleghaemoglobin by bacteroids. This was done spectrophotometrically at 562 and 576 nm, which made possible the determination of free dissolved O_2 concentrations from the oxygenation state of leghaemoglobin. The constants used in the calculations were as described by Bergersen & Turner (1979).

Bacteroid O_2 consumption. Bacteroid respiration was measured in duplicate in incubation media containing different dissolved O_2 concentrations in the 4 ml chamber of an O_2 electrode. There was no gas phase (Trinchant & Rigaud, 1979).

Leghaemoglobin. In the absence of sufficient quantities of *Sesbania* stem nodules to extract and purify their specific leghaemoglobin, the haemoprotein was prepared from French-bean nodules. All purification procedures were done at 4 °C. Nodules (110 g fresh wt) were crushed in a Sorvall Omni-mixer and the red supernatant obtained was submitted to ammonium sulphate fractionation. Leghaemoglobin was precipitated between 55 and 85% ammonium sulphate saturation. The pellet was dissolved in Tris/HCl buffer (pH 7.5, 25 mM) and dialysed for 12 h. Leghaemoglobin was concentrated with a Diaflo cell equipped with a UM 10 membrane (Amicon) and then purified by passage through a G-100 Sephadex column (80 × 2.5 cm) in Tris/HCl buffer (pH 7.5, 25 mM). It was reduced by the action of sodium dithionite and the oxygenation resulting in the passage through G-15 Sephadex columns (30 × 2.5 cm) gave oxyleghaemoglobin, which was concentrated to 500–700 μM over an Amicon UM 10 membrane. Samples (1 ml) were stored in liquid N_2 until used.

Partial purification of LDH. LDH was extracted from bacteria, bacteroids and the cytosol of stem nodules. Bacteria or bacteroids (5 g wet wt) were suspended in 5 ml 0.1 M-sodium phosphate buffer (pH 7) and disrupted in a precooled Aminco French pressure cell at 110316 MPa. Crude extracts were obtained by centrifugation at 35000 g for 20 min at 4 °C. To purify LDH, crude extracts were submitted to successive precipitations by protamine sulphate and ammonium sulphate (Trinchant & Rigaud, 1974). The last pellet of precipitation was solubilized in 2 ml 0.1 M-sodium phosphate buffer (pH 7) and its LDH activity was determined. The same purification procedure was applied to the red supernatant resulting from the centrifugation of stem nodule homogenates in order to purify their cytosolic LDH. However, in order to avoid leghaemoglobin precipitation, 40–55% ammonium sulphate saturation was used instead of 40–60%.

LDH assays. All enzyme assays were done at 25 °C. A DU7 Beckman spectrophotometer was used to determine oxidation or reduction of pyridine nucleotide at 340 nm. For pyruvate reduction, the reaction volume (2 ml)

Fig. 1. C_2H_2 -reduction by bacteroids isolated from *Sesbania* stem nodules (a) or from French bean (*Phaseolus*) root nodules (b) in relation to increasing pO_2 in the gas phase. Incubation mixtures (1 ml) contained bacteroids (9 mg dry wt) and substrates (50 mM): glucose (Δ), lactate ($\blacktriangle$), succinate ($\circ$) or nil ($\bullet$) in sodium phosphate buffer (25 mM, pH 7.4). Assayed at 25 °C with shaking (140 r.p.m.).

contained 0.5–100 mM-sodium pyruvate and 0.25 mM-NADH in sodium phosphate buffer (0.1 M, pH 8). For lactate oxidation, incubation mixtures (2 ml) received 0.5 to 100 mM-lithium lactate and 0.5 mM-NAD in Tris/HCl buffer (0.1 M, pH 8.6). In all cases, assays were done with sufficient enzyme to give a change in absorbance of 0.05–0.20 min^{-1} . One unit (U) is defined as the amount of enzyme that oxidizes or reduces 1 μmol pyridine nucleotide min^{-1} ($mg\ protein^{-1}$).

Protein determination. Protein was measured by the Lowry method using Folin-Ciocalteu reagent, with BSA as a standard.

Lactate estimation. Nodules were crushed and the red supernatants obtained after centrifugation of homogenates were submitted to a 5% TCA precipitation for 2 h at 4 °C. Precipitated proteins were discarded after centrifugation (10000 g, 10 min) and the presence of lactate in the supernatant was assessed by the colorimetric method of Barker (1957) with *p*-hydroxydiphenyl as reagent.

RESULTS

Efficiency of different energy-yielding substrates in supporting C_2H_2 reduction by bacteroids

Bacteroids isolated from *Sesbania* stem nodules required the addition of carbon substrates to stimulate C_2H_2 reduction, as the endogenous reserves were only slightly active (Fig. 1a). Glucose efficiency was optimal at low pO_2 values (10 mmHg) [1 mmHg = 133.3 Pa] and no significant activity occurred at 20 mmHg. In contrast, succinate stimulated bacteroid C_2H_2 reduction over a wide range of pO_2 values (10–35 mmHg) with an optimum activity at 30 mmHg, where glucose was totally ineffective. Under these experimental conditions, lactate also supported C_2H_2 reduction at an optimum pO_2 of 20 mmHg, a value intermediate between those defined for glucose and succinate. The results of these different experiments are summarized in Table 1.

With bacteroids isolated from French-bean root nodules the stimulation exerted by glucose and succinate on C_2H_2 reduction exhibited the same characteristics as with the *Sesbania* bacteroids (Fig. 1b). In contrast, with *Phaseolus* bacteroids, lactate was unable to increase the level of activity obtained with the endogenous reserves. Similar rates of optimal C_2H_2 -reduction were reached by the bacteroids from both plants in the presence of active energy-yielding substrates: 5–5.5 $nmol\ min^{-1}\ mg^{-1}$ (Fig. 1a, b).

When O_2 was supplied to *Sesbania* bacteroids by oxyleghaemoglobin, the characteristic

Fig. 2. C_2H_2 -reduction by *Sesbania* bacteroids at low dissolved O_2 concentration. Incubations (3 ml), carried out at $25^\circ C$ without a gas phase, contained oxyleghaemoglobin ($105 \mu M$), bacteroids (2.8 mg dry wt) and substrates (10 mM): glucose (Δ), lactate ($\blacktriangle$), succinate ($\circ$) or nil ($\bullet$) in sodium phosphate buffer (25 mM, pH 7.4).

Table 1. O_2 uptake and O_2 conditions required by bacteroids isolated from *Sesbania* stem nodules for optimal C_2H_2 reduction in the presence of different energy-yielding substrates

Energy-yielding substrate	O_2 uptake [nmol min ⁻¹ (mg dry wt) ⁻¹]	pO ₂ in the gas phase for optimal C_2H_2 reduction (mmHg)	O_2 uptake with 105 μM - oxyleghaemoglobin [nmol min ⁻¹ (mg dry wt) ⁻¹]	O_2 concn for optimal C_2H_2 reduction (nM)
Endogenous	9	10	6.5	5
Glucose	15.4	10	11.2	5
Lactate	26.1	18	22.4	10
Succinate	51	30	36	18

response to each carbon substrate was maintained (Fig. 2). Glucose was efficient at low O_2 concentrations (1–20 nM) with an optimal C_2H_2 -reducing activity at 5 nM- O_2 . For succinate to be active, the highest O_2 concentrations were required and the greatest activity occurred at 18 nM- O_2 . The maximal C_2H_2 -reduction activity induced by lactate appeared at 10 nM- O_2 , a concentration intermediate between those defined for glucose and succinate as previously observed (Table 1). Lactate gave the highest rate of activity ($7 \text{ nmol min}^{-1} \text{ mg}^{-1}$) under these experimental conditions.

O₂ consumption by Sesbania bacteroids

Preliminary experiments indicated that concentrations of dissolved O_2 of up to $10 \mu M$ were necessary to avoid O_2 -limiting conditions and $25 \mu M$ was routinely used for assays receiving different substrates. Table 1 shows the existence of low respiration rates, due to the oxidation of endogenous reserves, which were significantly stimulated by the three substrates used. Succinate and lactate were 3.3 and 1.8 times more efficient, respectively, than glucose when O_2 was freely dissolved in the medium. Similar ratios between the relative efficiency of these substrates were found when O_2 was supplied from oxyleghaemoglobin.

LDH activity in bacteria and bacteroids

In crude extracts of both bacteria and bacteroids, the nucleoproteins and NADH oxidase activity were removed by a protamine sulphate precipitation without affecting the level of LDH activity. Successive ammonium sulphate precipitations showed localization of LDH in the 40–60% saturation fraction with a purification of about 50-fold, confirming previous results obtained with *R. meliloti* LDH (Trinchant & Rigaud, 1974). Activities and K_m values of different LDH preparations were determined using pyruvate and lactate as substrates; the results are summarized in Table 2. LDH extracted from *Rhizobium* sp. (*Sesbania*) exhibited very poor affinity for pyruvate but a very high one for lactate, both in the free-living forms and bacteroids.

Table 2. Activity and K_m of LDH extracted from different rhizobia and their corresponding bacteroids, and from the cytosol of stem nodules of *Sesbania rostrata*

LDH source	Activity (U)*		K_m (mM)	
	Pyruvate	Lactate	Pyruvate	Lactate
Bacteria				
<i>Rhizobium</i> sp. (<i>Sesbania</i>)	0.042	0.650	66	0.082
<i>Rhizobium leguminosarum</i> biovar <i>phaseoli</i>	0.750	0.033	0.095	60
<i>Bradyrhizobium</i>	0.718	0.038	0.090	72
Bacteroids isolated from:				
<i>Sesbania rostrata</i>	0.049	0.668	58	0.075
<i>Phaseolus vulgaris</i>	0.702	0.040	0.120	80
<i>Glycine max</i>	0.710	0.039	0.102	56
<i>Sesbania</i> stem nodule cytosol	0.310	0.021	2.5	98

* 1 U is the amount of enzyme that oxidizes or reduces 1 μ mol pyridine nucleotide min^{-1} (mg protein) $^{-1}$.

In contrast, in the other two species of *Rhizobium* and in their corresponding bacteroids, LDH proved capable of reducing pyruvate very quickly, whereas its affinity for lactate remained low.

LDH activity in stem nodule cytosol

LDH activity was detected in the cytosol of *Sesbania* stem nodules and a 50-fold purification was achieved, as with the bacteria. The plant LDH exhibited a higher affinity for pyruvate (K_m 2.5 mM) than for lactate (K_m 98 mM) (Table 2), which is probably related to the significant amount of lactate determined in the cytosol of stem nodules (108 ± 10 nmol g^{-1} fresh wt; mean $\pm$ SEM). In contrast, the concentrations of lactate detected, under the same conditions, in the cytosol of French-bean and soybean root nodules were low: 15 ± 2.5 and 18 ± 3.2 nmol (g fresh wt) $^{-1}$ respectively.

DISCUSSION

Bacteroids isolated from *Sesbania* stem nodules exhibited the same ability to reduce C_2H_2 when utilizing glucose or succinate as observed with French-bean (Fig. 1*b*) or soybean bacteroids (Trinchant *et al.*, 1981). The strict O_2 requirement with glucose as substrate was also confirmed when O_2 was either freely dissolved or supplied by oxyleghaemoglobin. It could be responsible for the commonly reported inefficiency of glucose in supporting C_2H_2 reduction, which may be explained by inadequate conditions of incubation as previously suggested (Trinchant & Rigaud, 1979; Trinchant *et al.*, 1981).

A new finding concerns the specific role of lactate, unable to support C_2H_2 reduction by either French-bean bacteroids (Fig. 1*b*) or by soybean bacteroids (results not given), but acting as an energy-yielding substrate for *Sesbania* bacteroids. It supported C_2H_2 reduction with a similar rate to that observed with glucose or succinate in the absence of an O_2 carrier and was particularly efficient when O_2 was provided at very low concentrations by oxyleghaemoglobin. The O_2 tensions required for optimal efficiency with lactate could be predicted by the model of Bergersen & Trinchant (1985), which takes into account results obtained under experimental conditions similar to those used in the present study where O_2 was also supplied to either soybean or French-bean bacteroids, free or bound to leghaemoglobin. It made it possible to determine the required O_2 concentration around a bacteroid, for optimal C_2H_2 reduction. This was shown to be directly related to the rate of O_2 consumption for a given substrate. For example, bacteroid O_2 uptake was about 3.3 times higher with succinate than with glucose, and a similar ratio of 3:1 was observed between the O_2 tensions inducing optimal C_2H_2 reduction with these substrates. Since O_2 uptake by bacteroids was 1.8 times higher with lactate than with glucose, the model predicted an O_2 tension of 18 mmHg to ensure optimal C_2H_2 reduction in the

presence of lactate. Experimental data pointed to about 20 mmHg. A similar relationship between O_2 consumption and O_2 concentration for optimal bacteroid nitrogenase activity also occurred when O_2 was delivered by oxyleghaemoglobin with glucose, lactate or succinate acting as substrates.

The efficiency of lactate in supporting C_2H_2 reduction proved to be in direct relation to the presence of an active LDH in the bacteroids of *Sesbania* stem nodules. We previously detected and purified this enzyme from free living *R. meliloti* (Trinchant & Rigaud, 1974) and it is present in other species of *Rhizobium*. However, the reduction of pyruvate was in general favoured in *R. meliloti*, *R. leguminosarum* biovar *phaseoli* and *Bradyrhizobium* (free-living bacteria and symbiotic forms) rather than lactate oxidation, as evidenced by the low K_m values for pyruvate. In contrast, LDH extracted from *Rhizobium* sp. (*Sesbania*) and stem nodule bacteroids exhibited a high affinity for lactate, confirming the use of this molecule as a carbon source in the culture medium of bacteria (Elmerich *et al.*, 1982). Moreover, the presence of significant quantities of lactate in the cytosol of the host cells of stem nodules makes possible its utilization as an energy source by bacteroids *in vivo*. Its synthesis resulted in the existence of an active LDH in the nodule cytosol, exhibiting a high affinity for pyruvate. It was favoured by a high NADH : NAD ratio when fermentative processes took place in the nodules subjected to O_2 limitation. This situation can easily occur, since *Sesbania* often thrives in temporarily flooded areas during the rainy season. Lactate was not detected in soybean nodules where malate, fumarate and succinate were largely represented (Reibach & Streeter, 1983) and existed only at low concentrations in French-bean nodules. Moreover, the poor affinity of bacteroid LDH for lactate in these nodules accounts for its inability to play a part in supporting N_2 fixation (Fig. 1b). The existence of another fermentative process, which was enhanced when plants were grown in waterlogged soils has been reported by De Vries *et al.* (1980) and LaRue *et al.* (1984) in soybean nodules. It involves the reduction of acetaldehyde to ethanol by an alcohol dehydrogenase (ADH) which is present in the cytosol. The ethanol generated under O_2 -limiting conditions was used by the bacteroids to support N_2 fixation (Peterson & LaRue, 1981; Tajima & LaRue, 1982). This possibility did not arise in *Sesbania* stem nodules, since ADH activity was not detected in their cytosol, but could be replaced by the lactic fermentation pathway as suggested by the present study.

We wish to thank Dr Y. R. Dommergues for providing *S. rostrata* seeds and *Rhizobium* strain ORS 571. Also, we are indebted to Miss G. Van de Sype for skilful technical assistance. This research was supported by a grant from Ministère de la Recherche et de la Technologie: 'Esson des Biotechnologies'.

REFERENCES

- BARKER, S. B. (1957). Preparation and colorimetric determination of lactic acid. *Methods in Enzymology* **3**, 241–246.
- BERGERSEN, F. J. & TRINCHANT, J. C. (1985). Flux of O_2 affects apparent optimal concentration of O_2 in suspensions of bacteria respiring microaerobically. *Journal of Theoretical Biology* **115**, 93–102.
- BERGERSEN, F. J. & TURNER, G. L. (1979). Systems utilizing oxygenated leghaemoglobin and myoglobin as sources of free dissolved O_2 at low concentrations for experiments with bacteria. *Analytical Biochemistry* **96**, 165–174.
- DE VRIES, G. E., IN 'T VELD, P. & KIJNE, J. W. (1980). Production of organic acids in *Pisum sativum* root nodules as a result of oxygen stress. *Plant Science Letters* **20**, 115–123.
- DILWORTH, M. J. & GLENN, A. R. (1984). How does a legume nodule work? *Trends in Biochemical Sciences* **12**, 519–523.
- DREYFUS, B. & DOMMERGUES, Y. (1980). Non-inhibition de la fixation d'azote atmosphérique par l'azote combiné chez une légumineuse à nodules caulinaires, *Sesbania rostrata*. *Comptes rendus de l'Académie des sciences* **291**, 767–770.
- DREYFUS, B. & DOMMERGUES, Y. (1981). Nitrogen-fixing nodules induced by *Rhizobium* on the stem of the tropical legume *Sesbania rostrata*. *FEMS Microbiology Letters* **10**, 313–317.
- ELMERICH, C., DREYFUS, B. L., REYSSET, G. & AUBERT, J. P. (1982). Genetic analysis of nitrogen fixation in a tropical fast-growing *Rhizobium*. *EMBO Journal* **1**, 499–503.
- GLENN, A. R., MCKAY, I. A., ARWAS, R. & DILWORTH, M. J. (1984). Sugar metabolism and the symbiotic properties of carbohydrate mutants of *Rhizobium leguminosarum*. *Journal of General Microbiology* **130**, 239–245.
- KENNEDY, I. R., RIGAUD, J. & TRINCHANT, J. C. (1975). Nitrate reductase from bacteroids of *Rhizobium japonicum*: enzyme characteristics and possible interaction with nitrogen fixation. *Biochimica et biophysica acta* **397**, 24–35.

- LARUE, T. A., PETERSON, J. B. & TAJIMA, S. (1984). Carbon metabolism in the legume nodule. In *Advances in Nitrogen Fixation Research*, pp. 437–443. Edited by C. Veege & W. E. Newton. The Hague: M. Nijhoff & W. Junk.
- PETERSON, J. B. & LARUE, T. A. (1981). Utilization of aldehydes and alcohols by soybean bacteroids. *Plant Physiology* **68**, 489–493.
- REIBACH, P. H. & STREETER, J. G. (1983). Metabolism of ^{14}C -labelled photosynthate and distribution of enzymes of glucose metabolism in soybean nodules. *Plant Physiology* **72**, 634–640.
- RIGAUD, J. (1976). Effet des nitrates sur la fixation d'azote par les nodules de haricot (*Phaseolus vulgaris* L.). *Physiologie végétale* **14**, 297–308.
- RIGAUD, J. & PUPPO, A. (1975). Indole-3-acetic acid catabolism by soybean bacteroids. *Journal of General Microbiology* **88**, 223–228.
- STOWERS, M. D. (1985). Carbon metabolism in *Rhizobium* species. *Annual Review of Microbiology* **39**, 89–108.
- TAJIMA, S. & LARUE, T. A. (1982). Enzymes for acetaldehyde and ethanol formation in legume nodules. *Plant Physiology* **70**, 388–392.
- TRINCHANT, J. C. & RIGAUD, J. (1974). Lactate dehydrogenase from *Rhizobium*. Purification and role in indole metabolism. *Physiologia plantarum* **32**, 394–399.
- TRINCHANT, J. C. & RIGAUD, J. (1979). Sur les substrats énergétiques utilisés, lors de la réduction de C_2H_2 , par les bactéroïdes extraits des nodosités de *Phaseolus vulgaris* L. *Physiologie végétale* **17**, 547–556.
- TRINCHANT, J. C., BIROT, A. M. & RIGAUD, J. (1981). Oxygen supply and energy-yielding substrates for nitrogen fixation (acetylene reduction) by bacteroid preparations. *Journal of General Microbiology* **125**, 159–165.
- TRINCHANT, J. C., BIROT, A. M., DENIS, M. & RIGAUD, J. (1983). C_2H_2 reduction, oxygen uptake and cytochrome-*c* reduction by bacteroids isolated from French-bean nodules. *Archives of Microbiology* **134**, 182–186.