

# OXYGEN AND MANNITOL CONSUMPTION OF *RHIZOBIUM MELILOTI* IN RELATION TO SYMBIOTIC NITROGEN FIXATION EFFICIENCY\*

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## KEY WORDS

Alfalfa Bacteria Mannitol consumption Oxygen consumption Plant *Rhizobium meliloti* Symbiotic efficiency

## SUMMARY

The rate of oxygen and total mannitol consumption were studied with 48 strains of *Rhizobium meliloti* in relation to their symbiotic nitrogen fixation efficiency as expressed by the plants dry weight yields. The rate of oxygen consumption is positively correlated to the total mannitol consumption and significant inverse relationship between these two physiological properties and symbiotic efficiency are apparent. The possibility of using the rate of oxygen consumption as a preselection tool is discussed.

## INTRODUCTION

Strains of rhizobia differ widely in their symbiotic nitrogen fixation efficiency. The most reliable method available to distinguish between effective and ineffective strains, is the performance with the host plants, which is time consuming<sup>4</sup>. In order to find a more rapid and simple method for the evaluation of the symbiotic efficiency of rhizobia, several physiological, nutritional and cultural characteristics were studied. Effective strains express a higher dehydrogenase activity<sup>5,9</sup>, and require less oxygen for growth<sup>11</sup>. Production of organic acids is negatively correlated with efficiency<sup>8,14</sup> and this parameter can be used as a preselection criterion of large number of isolates<sup>3</sup>. No significant correlation were found between the symbiotic efficiency and nitrate reduction<sup>1,12</sup>, produc-

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tion and degradation of indol-acetic acid<sup>12</sup>, resistance to antibiotics<sup>15</sup>, and the immunological characteristics of the bacteria<sup>6</sup>.

In the present paper, the oxygen and mannitol consumption by 48 strains of *Rhizobium meliloti* were determined with the aim of correlating these two physiological properties of bacteria with their symbiotic efficiency as indicated by the dry weight of plants<sup>4</sup>.

#### MATERIAL AND METHODS

Strains of *R. meliloti* used in this study and their symbiotic efficiency were previously described<sup>4</sup>. As the second cutting of nodulated plants give the most necessary information for the exact evaluation of efficiency, the dry weight of plants obtained during this cutting were used in this study as an indication of the symbiotic efficiency of the strains<sup>4</sup>.

The bacteria were cultured in a medium containing per litre of distilled water: mannitol, 10.0 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.20 g; NaCl, 0.10 g; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.05 g; yeast extract (Difco), 0.20 g; K<sub>2</sub>HPO<sub>4</sub>, 0.522 g; KH<sub>2</sub>PO<sub>4</sub>, 0.408 g; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.004 mg; H<sub>3</sub>BO<sub>3</sub>, 2.86 mg; MnCl<sub>2</sub>·4H<sub>2</sub>O, 1.81 mg; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.22 mg; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.008 mg; H<sub>2</sub>MoO<sub>4</sub>·H<sub>2</sub>O, 0.09 mg; ferric citrate, 25.0 mg. To avoid precipitation, phosphates were prepared and sterilized in half the volume and then mixed to the other half after cooling. After sterilization, pH was 6.85. Growth was performed in 250 ml flasks containing 100 ml of medium inoculated with approximately 10<sup>6</sup> washed cells. Flasks were incubated at 26°C on an orbital shaker (125 rpm). Oxygen consumption was measured on 5-ml samples of log phase cultures (36–42 hrs) using a biological oxygen monitor (Yellow Spring Instrument, Ohio, Model 13–53) equipped with a polarographic electrode<sup>2</sup>. At saturation dissolved oxygen in the medium was determined according to the modified method of Winkler<sup>10</sup>. Oxygen consumption was measured during 10 minutes and the cells from 2-ml aliquot were separated and washed twice in phosphate saline buffer (0.01 M, pH 7.2) by centrifugation at 12000 g during 10 min at 4°C. Cellular protein was determined in the precipitate<sup>7</sup>. Supernatant and washings were combined and lyophilized after addition of 5 × 10<sup>-5</sup> mole of erythritol as internal standard. The dry residues were converted to their corresponding alditol acetates before analysis by gas chromatography<sup>13</sup>. Acetylated mannitol was measured on 0.1 µl samples injected at 225°C in a gas chromatograph (Tracor 220) equipped with glass columns (1.2 × 6.25 mm) packed with 3% ECNSS-M on Chromasorb-Q 100–200 mesh (Regis Chemicals) maintained at 160°C, and dual flame ionization detector at 275°C (carrier nitrogen at 80 cc/min, air and H<sub>2</sub> at 500 and 60 cc/min respectively). Under these conditions, mannitol was chromatographed in 20 min, while the internal standard (erythritol) appeared after 2 min. Consumed mannitol was calculated as the difference between the original concentration in the medium and that after growth.

#### RESULTS AND DISCUSSION

Rates of oxygen consumption and total mannitol consumption by strains of *R. meliloti* are shown in Table 1. The ineffective strain I<sub>1</sub> had the highest rate of oxygen consumption and the effective strain A<sub>5</sub> had the lowest rate. All the *R. meliloti* strains tested used mannitol during growth. The highest consumption of mannitol was recorded with the ineffective strain A<sub>1</sub>, while the effective strain S<sub>7</sub> showed the lowest consumption. The rate of oxygen used by *R. meliloti* during

Table 1. Oxygen and mannitol consumption of 48 strains of *R. meliloti*

Strain	Symbiotic* efficiency	Rate of O <sub>2</sub> consumption Picomoles × min <sup>-1</sup> × μg protein <sup>-1</sup>	Total mannitol consumption nanomoles × μg protein <sup>-1</sup>
S <sub>14</sub>	VE	687	475
V <sub>3</sub>	VE	578	339
A <sub>2</sub>	VE	541	430
A <sub>3</sub>	VE	521	432
A <sub>4</sub>	VE	488	440
3Doa8	VE	335	420
S <sub>1</sub>	E	772	297
S <sub>2</sub>	E	613	288
S <sub>3</sub>	E	669	321
S <sub>4</sub>	E	456	454
S <sub>5</sub>	E	696	296
S <sub>6</sub>	E	549	434
S <sub>7</sub>	E	398	245
S <sub>8</sub>	E	746	321
S <sub>9</sub>	E	554	309
S <sub>10</sub>	E	830	307
S <sub>11</sub>	E	495	254
S <sub>12</sub>	E	524	382
S <sub>13</sub>	E	629	296
S <sub>15</sub>	E	386	283
S <sub>16</sub>	E	405	302
S <sub>19</sub>	E	409	280
S <sub>20</sub>	E	474	366
S <sub>21</sub>	E	700	307
S <sub>22</sub>	E	511	246
V <sub>1</sub>	E	592	314
V <sub>2</sub>	E	480	322
4 <sub>4</sub>	E	664	296
V <sub>5</sub>	E	355	280
V <sub>7</sub>	E	590	312
D <sub>2</sub>	E	276	277
D <sub>3</sub>	E	580	332
A <sub>5</sub>	E	250	391
I <sub>3</sub>	E	743	294
R <sub>1</sub>	E	732	330
E <sub>2</sub>	E	617	440
3Doa2oa	E	342	284
54032	E	707	486
54033	E	362	279
23A	E	332	407
V <sub>6</sub>	I	536	328
D <sub>1</sub>	I	575	327
A <sub>1</sub>	I	945	2250
I <sub>1</sub>	I	1605	1385
I <sub>2</sub>	I	569	276
I <sub>4</sub>	I	500	260
E <sub>1</sub>	I	1486	2000
Alfalfa D	I	712	314

\* Symbiotic efficiency based on the dry matter yield obtained in the second cutting as reported by Bordeleau *et al.* <sup>4</sup>  
VE = very effective, E = effective, I = ineffective.

Table 2. Correlations between oxygen and mannitol consumption and dry weight of plants (symbiotic efficiency) in *R. meliloti*

	Mannitol	Efficiency
Oxygen	0.691** (47.8)	- 0.495** (24.46)
Mannitol		- 0.421** (17.73)

\*\* Significant at 1% level.

Values in parenthesis are the determination coefficients.

growth is positively correlated with mannitol utilization, and these two physiological parameters are negatively correlated with the symbiotic efficiency (Table 2).

The present study show an apparent significant and negative correlation between the symbiotic efficiency, as expressed by the dry weight of plants, and oxygen and mannitol utilization by the free living bacteria, during growth. It is then expected that a very effective strain of *R. meliloti* will use less oxygen and mannitol to produce the same amount of growth (protein), than an ineffective strain. The rate of oxygen consumption appear to be a more useful tool than mannitol. In fact the determination coefficients are 24% and 17% for oxygen and mannitol consumption respectively. It was previously showed that the final pH of *R. meliloti* cultures, was significantly correlated to the symbiotic efficiency<sup>3</sup> and this physiological parameter could be used as a preselection tool. This study also show that the rate of oxygen consumption can be used as a preselection criterion for a very large number of isolates. For example, if one consider a rate of oxygen consumption of  $550 \text{ picomoles} \times \text{min}^{-1} \times \text{ug protein}^{-1}$  as the highest acceptable rate, of the 48 strains tested, 23 strains will be retained. This step will reduce to 50% a large population of isolates. The initial population of 48 strains contained 12.5% very effective, 70.8% effective and 16.7% ineffective strains. The 23 selected strains will include 17.4% very effective, 74% effective, 74% effective and 8.6% ineffective strains. By using the rate of oxygen consumption as a criterion for preselection of a large number of isolates it is also possible to reduce almost by half the incidence of ineffective strains while slightly increasing the very effective and effective strains in the new selected population.

Some physiological characteristics of *R. meliloti* can offer promising tools to develop a more rapid and simple method for the *in vitro* selection of symbiotically effective strains.

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