ASSIMILATION OF AMMONIA IN AZOSPIRILLUM BRASILENSIS TARRAND ET AL.

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In all organisms the assimilation of nitrogenous compounds and their utilization in macromolecular synthesis are inevitable features in metabolism1. In nitrogen fixing diazotrophs, the atmospheric nitrogen is fixed in the cells as ammonia which is subsequently assimilated. Earlier studies with strict bacteria like E. coli and Aerobacter have revealed that although quite a few enzymes seem to be involved in the process of ammonia assimilation, glutamine synthetase (GS) plays a very pivotal role3.

However, in most nitrogen fixing bacteria, ammonia assimilation proceeds through the coupled reactions of the dual enzymes, viz., glutamine synthetase (GS) (E.C. 6.3.1.2) and glutamate synthase (GOGAT) (E.C. 2.6.1.52)2. Other enzymes like glutamate dehydrogenase (GDM) (E.C. 1.4.1.4) and glutamate aspartate amino transferase (E.C. 2.6.1.12) have also been implicated in organisms like Rhizobium, Bacillus polymyxa, Klebsiella4 etc. There appears to be little information available on ammonia assimilation in Azospirillum, the associative symbiotic nitrogen fixing diazotroph and the present study is an attempt in this direction.

Pure cultures of Azospirillum brasilense Tarrand et al. isolated from the root tissues of maize (Zea mays L.) and maintained on malate semisolid medium5 was transferred to 100 ml of the malate broth taken in 250 ml Erlenmeyer flasks. Malate medium containing (i) 0.1% sodium nitrate, (ii) 0.1% ammonium sulphate were also used.

The sterilised media were inoculated with a standardised cell suspension (ca. 10⁸ cells/ml) of Azospirillum and the flasks were incubated at static conditions for seven days at 28 ± 2°C. The cells were harvested by centrifugation at 10,000 × g for 15 min and the pellets were suspended in 5-0 ml of 0.05 M phosphate buffer (pH 7-0). The cells were sonicated at 0°C and the cell lysate was obtained by centrifugation at 40,000 × g for 10 min in a refrigerated centrifuge. The supernatant served as the enzyme source4. The cells at the time of harvest had sufficiently high nitrogenase activity (1620-2280 n moles of C₂H₄/mg protein/hr).

The enzymes, glutamine synthetase, glutamate synthase and glutamate dehydrogenase were determined by following the procedures of Shapiro and Stadtman6 and Vandeasteel et al.7. All assays were performed for one hour at 37°C. The dependency of NADH or NADPH was also tested for the enzyme GOGAT. The results are presented in Table 1.

The results have indicated that Azospirillum cells grown in malate medium without any nitrogen source recorded the maximum activity of glutamine synthetase. Also, the NADH and NADPH dependent glutamate synthase activity was evidently high. In nitrate and ammonia grown cell also the activity of GS and GOGAT was quite appreciable. However, no GDH activity could be detected in malate grown cells, while it was appreciable in nitrate and ammonia grown cells.

Studying the mechanism of ammonia assimilation, Tempest et al.8 recently elucidated the operation of another pathway in microorganisms. It involves the amidation of endogenous glutamate to glutamine and subsequently reductive transfer of glutaminamide nitrogen to the second position of 2-oxoglutarate producing a net synthesis of two moles of glutamate. Recently, Nagatani et al.9, also presented evidence that glutamine synthetase occurs in diazotrophs in a predominantly poorly adetylated form characterised by a low km value for ammonia. In this form the enzyme is ideally suitable for nitrogen fixing organisms as it assures efficient assimilation of fixed nitrogen and prevents the accumulation of ammonia which inhibits
**Table I**

<table>
<thead>
<tr>
<th>Source</th>
<th>Glutamine synthase*</th>
<th>Glutamate dehydrogenase</th>
<th>NADH NADPH NADH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (mulate) grown cells</td>
<td>42.5</td>
<td>119.0</td>
<td>148.7 Trace</td>
</tr>
<tr>
<td>Nitrate grown cells</td>
<td>33.8</td>
<td>96.7</td>
<td>38.2 23.6</td>
</tr>
<tr>
<td>Ammonia grown cells</td>
<td>15.4</td>
<td>78.4</td>
<td>10.3 14.5</td>
</tr>
</tbody>
</table>

* Expressed in mg of glutamic acid γ-monohydroxamate formed/min/mg protein.
** Expressed in n moles of NADH/NADPH oxidised min/mg protein.

Nitrogenase synthesis. A comparison of glutamate dehydrogenase and glutamate synthase activities in Klebsiella, Azotobacter and Clostridium indicated that glutamate synthase played a prominent role in glutamate formation rather than the enzyme, glutamate dehydrogenase. It appears from the results that in Azospirillum brasilense the chief ammonia assimilating enzymes are glutamine synthetase and glutamate synthase. We have recently demonstrated in the case of Azotobacter chroococcum the predominance of glutamine synthetase and glutamate synthase as the ammonia assimilating enzymes.

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**INHIBITION OF PURPLE SEEDCOAT COLOUR IN GROUNDNUT**

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Purple seedcoat colour, P, is dominant (rose and red) in groundnut (*Arachis hypogaea*, L.) Hammons, in a review, reported that the purple seedcoat was incompletely dominant to rose and flesh colours and showed monogenic control. Although the red colour gene, R, is not required for the purple phenotype, it modified the expression of P gene. This communication describes other loci for purple coat colour which were observed in the cross between two Spanish varieties, *Chico* and Trombay Groudnut (TG)-17.

TG-17, having flesh coloured seedcoat, was crossed as female parent to *Chico* with light rose colour, *F₁* hybrids and *F₂* progenies were grown during 1979 and 1980. Two of the *F₂* families were grown in the *F₃* generation to confirm phenoletic and genotypic segregations. The data obtained were statistically analysed and the chi-square values are given in Table I. Seedcoat colour was studied after sun-drying the pods.

The seedcoat following hybridization had flesh colour as in the maternal parent, TG-17. Similarly the *F₁* kernels also resembled TG-17, indicating dominance of flesh colour over light rose. However, in *F₂* and *F₃* generations, wide variations in seedcoat colours, viz., light rose, rose, light flesh, flesh, pink, dark pink and purple were observed. With a view to emphasizing the inheritance of purple colour, plants were classified into those carrying non-purple and purple seedcoat colours. The segregation data are presented in Table I.

In the *F₃* there were 87 plants with non-purple seedcoat colour and 7 with purple colour which gave a good fit to 15 : 1 phenotypic ratio suggesting duplicate recessive genes governing the purple colour. In the *F₃* there were 12 heterozygous progenies showing 789 plants with non-purple and 22 plants with purple seedcoat exhibiting again a good fit to the dihybrid ratio. In addition, 13 progenies segregated into 332 and 99 plants without and with purple colour, respectively indicating monohybrid segregation which was expected. The remaining 19 plants progenies were homozygous for non-purple colour. All the four purple progenies also bred true. The genotypic segregation in *F₄* for purple colour showed an excellent fit to the expected ratio 7 : 4 : 4 : 1 (χ² = 3.607, P = 0.50).

These results demonstrated that the purple seedcoat colour was governed by duplicate recessive genes and both were necessary for its expression.