Poly-β-hydroxybutyrate and glycogen metabolism in *Rhizobium leguminosarum*

E.M.Lodwig[1], D.Allaway[1], T.Wheeler[2], A.J.Downie[3], P.S.Poole[1]

[1]School of AMS, University of Reading, Whiteknights, Reading, RG6 6AJ, UK; [2]Dept. of Agriculture, University of Reading, Earley Gate, Reading, RG6 6AT, UK; [3]John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK
Poster available at http://www.rdg.ac.uk/AcaDepts/sa/MicPhys/posters.htm; Correspondence Email: e.m.lodwig@reading.ac.uk

Introduction

Rhizobium mutants unable to synthesise poly-β-hydroxybutyrate (PHB) enhance nitrogen fixation in some symbioses. Similarly, mutants unable to produce glycogen increase fixation in bean (Downie, pers. comm.), but the effect in pea is not known. To determine how altering carbon partitioning effects the symbiosis, the PHB & glycogen synthase genes (*phaC* & *glgA*) of two strains (8401.pRL1JI & 8002) of *R. leguminosarum* have been mutated. These strains are isogenic, except for differing in Sym plasmid, enabling the effect of the mutations to be compared in pea and bean symbioses respectively. We show that *phaC* mutants of *Rhizobium leguminosarum* have no effect in bean but are reduced in symbiotic efficiency in pea.

Construction of PHB mutants

A fragment of *phaC* was amplified from 8002 and an omega cassette (Sp^r) was cloned into a *Psh*AI site at 930bp. The disrupted fragment was recombined into 8401.pRL1JI & 8002. The mutants RU1328 (infects pea) & RU1329 (infects bean) were confirmed by Southern blot. The mutants were unable to produce PHB (Table 1).

Pea nodules of PHB mutants show reduced nitrogenase activity

Pea (*Pisum sativum* L. cv. Avola) and bean (*Phaseolus vulgaris* L. cv. Tendergreen) seeds inoculated with the respective WT and PHB mutants were harvested at flowering. Nitrogenase activity (acetylene reduction) of the PHB mutant was 50% less than that of the WT in pea whereas there was no difference in bean (Table 2).

Pea shoot weights are reduced in RU1328

There was no difference in biomass accumulation by bean at flowering (46 d) or at pod filling (54 d). There was no difference in pea at flowering (35 d) but plants inoculated with RU1328 were stunted in growth (Fig 2) and had a 40% reduction in dry weight at maturity (53 d) (Fig 3). As a significant effect was seen in the pea symbiosis, free living RU1328 was characterised physiologically.

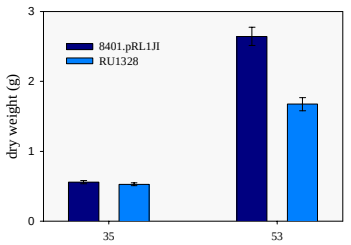

Fig 3. Biomass accumulation by pea inoculated with *R. leguminosarum* strains 8401.pRL1JI & RU1328. Values shown are means of 40 plants plus & minus the standard error of the mean. P<0.001 at 53 days.

Fig 1. Greenhouse environment for pea

Table 1. PHB content of WT & mutants grown on 10mM fructose/10mM NH_4Cl (μg mg protein^{-1})

8401.pRL1JI	65.0
RU1328	Not detectable
8002	50.4
RU1329	Not detectable

Table 2. Ethylene production by WT & mutants (μmoles plant^{-1}hr^{-1}). Values shown are means of 40 plants plus & minus the standard error of the mean

8401.pRL1JI	5.93 ± 0.59
RU1328	2.99 ± 0.17
P<0.001	
8002	9.05 ± 0.92
RU1329	10.97 ± 1.11
P>0.05	

Fig 2. Representative pea plants at 53d growth

Table 3. Activity of malate dehydrogenase (MDH), citrate synthase (CS) & isocitrate dehydrogenase (ICDH) in *R. leguminosarum* strains 8401.pRL1JI & RU1328 grown on 10mM malate/10mM NH_4Cl (μmol min^{-1}(mg protein)$^{-1}$). Values shown are means of 3 experiments plus & minus the standard error of the mean

	8401.pRL1JI	RU1328
MDH	3.604 ± 0.809	2.780 ± 0.774
CS	0.057 ± 0.001	0.043 ± 0.007
ICDH	0.645 ± 0.040	0.481 ± 0.038

Growth rate is not affected in RU1328

Mean doubling times of *R. leguminosarum* strains 8401.pRL1JI & RU1328 grown on 10mM malate/10mM NH_4Cl were 3.46 hrs & 3.24 hrs respectively indicating there is no gross effect in physiology during log phase.

TCA enzyme activities are affected in RU1328

PHB is synthesised from acetyl CoA and it was considered that preventing PHB synthesis may affect carbon flux through the TCA cycle. To indicate whether a change in flux occurred various TCA enzyme activities were assayed. There was a minor decrease in activity of malate dehydrogenase, citrate synthase and isocitrate dehydrogenase in RU1328 suggesting carbon flux through the TCA cycle may be reduced (Table 3).

TCA cycle activity is slowed in RU1328

To confirm the reduction in carbon flux we looked at the release of carbon dioxide from ^{14}C-labelled malate over a 15 minute time course. Initial studies show a significant reduction in the rate of CO_2 release from RU1328 (Fig 4) indicating decarboxylation of malate by the TCA cycling is impaired in the mutant.

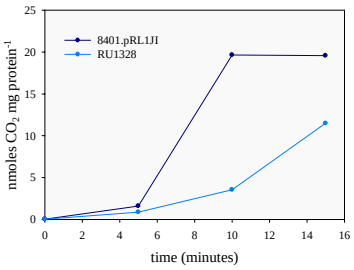

Fig 4. CO_2 release from L-[1,4(2,3)-^{14}C]malic acid by *R. leguminosarum* strains 8401.pRL1JI & RU1328 grown on 10mM malate/10mM NH_4Cl. Values shown are means of 2 experiments.

Future work

Unlike previous studies we report that a PHB mutant has a deleterious effect on symbiosis. Further characterisation of this strain (RU1328) will include measuring reductant levels and O_2 consumption. The experiments will be repeated using Percoll purified bacteroids to determine if metabolism is similarly impaired during symbiosis, and to establish whether the reduction in metabolism can be correlated with decreased nitrogen fixation.

Furthermore, mutants in *glgA* (glycogen synthase) have been constructed in 8002 and 8401.pRL1JI to investigate the effect of this mutation in symbiosis. The effect of both *phaC* and *glgA* mutants on partitioning of carbon between PHB and glycogen will be determined by measuring levels of the storage pools in free living bacteria and bacteroids.