Synthesis and Catabolism of Alanine in *Rhizobium leguminosarum*

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Introduction

L-Alanine dehydrogenase (AldA) is normally considered to deaminate L-alanine to pyruvate. However, the kinetics of AldA from Rhizobia indicate a much higher affinity for ammonium than those from most other organisms and that under certain physiological conditions AldA may have a role in alanine synthesis rather than in catabolism. This is of particular importance since alanine has been identified as the sole secretion product of nitrogen fixation in *Bradyrhizobium japonicum* (Waters *et al*., 1998. PNAS (USA) 95:12038-12042). In *Rhizobium leguminosarum* both alanine and ammonium are primary secretion products (data this lab). However, the genes involved in alanine synthesis in both free-living bacteria and bacteroids have yet to be determined. The objectives of this work were to identify genes required for alanine synthesis and catabolism and to determine their importance in symbiosis.

Tn*5*-*lacZ* transposon mutagenesis of *R. leguminosarum* bv *viciae* strain 3841 identified two distinct classes of mutants defective in utilising alanine

20,000 colonies from a Tn*5*-*lacZ* mutant bank were screened for specific defects in growth with alanine as sole C source. Two classes of mutant defective in alanine utilisation were defined. The first grew very poorly on alanine and had Tn*5*-*lacZ* insertions in a putative alanine permease. The second class represented by strain RU1275 did not grow at all on alanine. Sequencing from the Tn5-*lacZ* insertion site identified the interrupted gene as a leucine-responsive regulatory protein (*lrp*)-type regulator, presumably regulating transcription of genes essential for alanine catabolism.

Two classes of cosmid complement RU1275 for growth on alanine

A cosmid bank was conjugated into strain RU1275 to identify loci able to complement or suppress RU1275 for growth on alanine. From the more common class of cosmid complementing RU1275 sequencing confirmed the presence of the *lrp*-type regulator and also identified two adjacent genes with homology to the D-alanine racemase (*dad*X) and the D-amino acid dehydrogenase (*dad*A) from a number of organisms.

The *dadXA* operon represents the pathway for alanine catabolism

Genes encoding proteins required for converting L-alanine to D-alanine and its subsequent deamination to pyruvate lie adjacent to a *lrp*-like regulator essential for alanine catabolism. In *E. coli* the *dadAX* operon is essential for catabolism of L-alanine. We propose that in *R. leguminosarum* catabolism of L-alanine normally requires isomerisation to D-alanine and subsequent oxidative deamination by D-amino acid dehydrogenase and transcription of *dadXA* is regulated by *dadR*.

The second class of cosmid suppressed the *dadR* mutant. Transposon mutagenesis and screening for loss of suppression identified the interrupted gene as an L-alanine dehydrogenase (*aldA*) with a second *lrp*-type regulator (*aldR*) upstream to and divergent from *aldA*.

AldA represents the alanine anabolic pathway

An *aldA* mutant (RU1327) was constructed by integrating *aldA*::Tn*5*-*lacZ* into strain 3841. Total loss of AldA activity in RU1327 confirmed this to be the only *aldA* present in strain 3841. In symbiosis *aldA* is not essential but bacteroids are defective in alanine secretion.

In summary we have identified genes important in both alanine synthesis and catabolism. Evidence to support the hypothesis that AldA is responsible for alanine synthesis include the following: *aldA* is not required for growth with alanine as sole C source, *aldA* was only able to suppress RU1275 when present in multiple copies and *aldA* mutant bacteroids are defective in alanine secretion. Future work includes determining the regulatory effects of the two *lrp*-type proteins on transcription of the *aldAR* and *dadAXR* operons and to study further the effects of AldA activity on the pea-*R. leguminosarum* symbiosis.