SHORT COMMUNICATION

L-Arabinose Metabolism in Rhizobia

By MARGARET J. DUNCAN

*Department of Microbiology and Molecular Genetics,
Harvard Medical School, 25 Shattuck Street, Boston,
Massachusetts 02115, U.S.A.*

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L-Arabinose is metabolized by different pathways in fast- and slow-growing rhizobia.

INTRODUCTION

L-Arabinose is a good carbon source for the growth of most rhizobia, and it has also been used in the growth medium which allows nitrogenase production in culture by certain strains (Pagan *et al.*, 1975). The only information about the metabolism of L-arabinose in rhizobia was the demonstration in *R. japonicum* (Pedrosa & Zancan, 1974) of a pathway first described in *Pseudomonas* MSU (Dahms & Anderson, 1969):

in which reaction 1 is catalysed by L-arabinose dehydrogenase and reaction 2 by 2-keto-3-deoxy-L-arabinonate aldolase.

In *R. meliloti*, however, we have recently shown (Duncan & Fraenkel, 1979) that L-arabinose is probably metabolized by the more common route, which has the same initial steps, but continues as follows:

in which reaction 3 is catalysed by α -ketoglutarate semialdehyde dehydrogenase. Extracts of *R. meliloti* contained the enzymes which catalyse reactions 1 and 3, and a mutant blocked at the next step [α -ketoglutarate dehydrogenase (*kgd*)] was unable to grow on L-arabinose.

I have now surveyed other strains of rhizobia for the key enzymes of these pathways and find that the metabolism of L-arabinose is indeed different in fast- and slow-growing rhizobia.

METHODS

Bacterial strains. *Rhizobium meliloti* L5-30 was from the laboratory of Dr G. Martinez-Drets, Instituto de Investigaciones Biologicas Clemente Estable, Montevideo, Uruguay; *R. japonicum* 61A76 was from the laboratory of Dr F. Ausubel, Biological Laboratories, Harvard University; *R. leguminosarum* DL-14 was from the laboratory of Dr R. Valentine, University of California, Davis, U.S.A.; *R. trifolii* T1L, *R. phaseoli* K26 and *Rhizobium* sp. 32H1 (for cowpea) were from the laboratory of Dr E. R. Signer, Massachusetts Institute of Technology, Cambridge, Mass., U.S.A.

Growth of bacteria and preparation of extracts. *Rhizobium meliloti* L5-30 was grown to stationary phase in rich LBCG medium (Lennox, 1955) which contained (per litre) 10 g Bacto-tryptone, 5 g yeast extract, 5 g NaCl, 2.5 mm-CaCl₂ and 2 g glucose. Cultures were resuspended (to give an A_{600} value of 2) in minimal

medium M63 (Cohen & Rickenberg, 1956) supplemented with carbon sources (0.4 %, w/v), arginine (25 $\mu\text{g ml}^{-1}$) and biotin (1 $\mu\text{g ml}^{-1}$). The other strains were grown in YP medium, i.e. 0.1 % (w/v) yeast extract in 7.5 mM- $\text{KH}_2\text{PO}_4/\text{KOH}$ (pH 6.3), with 0.1 % (w/v) L-arabinose or 0.1 % (w/v) glucose. All strains were grown at 30 °C. For enzyme assays, bacteria were collected by centrifuging and resuspended (1 g wet wt per 3 ml) in 50 mM- $\text{K}_2\text{HPO}_4/\text{KOH}$ buffer (pH 7.6) containing 2 mM-EDTA and 14 mM-2-mercaptoethanol.

Bacteria were disrupted ultrasonically for 1 min ml^{-1} in an ice-bath with a Bronwill Biosonik III sonicator, allowing 2 min cooling after each minute of sonication. Extracts were centrifuged at 110000 g for 2 h at 4 °C in a Beckman L5-50 ultracentrifuge.

Enzyme assays. NAD^+ -dependent L-arabinose dehydrogenase (EC 1.1.1.46) was assayed in 0.1 M $\text{KH}_2\text{PO}_4/\text{KOH}$ buffer (pH 7.5) containing 1 $\mu\text{mol NAD}^+ \text{ml}^{-1}$ and 0.7 $\mu\text{mol L-arabinose ml}^{-1}$. NAD^+ -linked α -ketoglutarate semialdehyde dehydrogenase (2,5-dioxovalerate: NAD^+ oxidoreductase; EC 1.2.1.26) and glycolaldehyde dehydrogenase (EC 1.2.1.21) were measured under the same conditions using either α -ketoglutarate semialdehyde (0.1 $\mu\text{mol ml}^{-1}$) or glycolaldehyde (2 $\mu\text{mol ml}^{-1}$) as substrates. (α -Ketoglutarate semialdehyde was a generous gift from Dr Y. F. Chang, Dental School Department of Microbiology, University of Maryland.) Spectrophotometric assays were carried out at 25 °C with a Gilford model 240 spectrophotometer. For 2-keto-3-deoxy-L-arabonate aldolase (EC 4.1.2.18) assays (Pedrosa & Zancan, 1974), the reaction mixture (0.5 ml) contained 40 mM-HEPES buffer (pH 8.0), 10 $\mu\text{mol MgCl}_2$, 7.5 μmol sodium pyruvate and 10 μmol glycolaldehyde. The reaction was carried out for 30 min at 30 °C. Controls without substrates were performed and 2-keto-3-deoxy-L-arabonate formation was detected by the thiobarbituric acid/periodate method (Weissbach & Hurwitz, 1959). Specific activity is expressed as $\text{nmol min}^{-1} (\text{mg protein})^{-1}$. Enzyme activities were measured on the same day as the extracts were prepared. Aldolase activities were stable for up to 3 d in crude sonicates kept at 4 °C, while dehydrogenase activities fell as much as 70 % in 3 d.

Protein was assayed by the biuret method with bovine serum albumin as standard.

RESULTS AND DISCUSSION

L-Arabinose dehydrogenase activity was found in all six strains tested (Table 1). 2-Keto-3-deoxy-L-arabonate aldolase activity was not detected in three of the four fast-growing strains (*R. trifolii*, *R. phaseoli* and *R. leguminosarum*) but was found in the two slow-growers (*R. japonicum* and the cowpea strain). α -Ketoglutarate semialdehyde dehydrogenase activity was high in the fast-growing strains. The two slow-growing strains showed a low dehydrogenase activity with α -ketoglutarate semialdehyde and a similar level of activity with glycolaldehyde as substrate. Thus, this activity may be due to a non-specific dehydrogenase for the glycolaldehyde product of the L-arabinose pathway in these strains.

The results with *R. meliloti* confirm our previous data (Duncan & Fraenkel, 1979), except that low, rather than zero, aldolase activity was detected. We are uncertain as to its significance, considering the much higher level of α -ketoglutarate semialdehyde dehydrogenase, and the *kdg* mutant study implicating the α -ketoglutarate pathway in L-arabinose metabolism. [Since the aldolase reaction is assayed in the backward direction, it is possible that the low apparent activity in *R. meliloti* is due to a different enzyme, such as that described for D-arabinose metabolism in *Pseudomonas saccharophila* (Palleroni & Doudoroff, 1957). Although the same reservation could apply to aldolase activity in the slow-growing strains, there is the additional evidence of pyruvate formation from L-arabinose (Pedrosa & Zancan, 1974).] Experiments with mixed extracts of *R. trifolii* and *Rhizobium* sp. 32H1 showed that the fast-grower did not contain an inhibitor for the aldolase activity of the slow-growing strain.

These results show that fast- and slow-growing rhizobia probably have different pathways of L-arabinose metabolism, and adds to the other known enzymic differences between the two groups (Martinez de Drets & Arias, 1972; Martinez de Drets *et al.*, 1974).

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Table 1. *Activities of enzymes in L-arabinose metabolism in rhizobia*All activities are expressed as nmol min⁻¹ (mg protein)⁻¹.

Strain	Growth type	Carbon source	L-Arabinose dehydro- genase	2-Keto-3- deoxy-L- arabonate aldolase	α -Keto- glutarate semi- aldehyde dehydro- genase	Glycol- aldehyde dehydro- genase
<i>R. meliloti</i> L5-30	Fast	L-Arabinose	14	15	304	6
		D-Glucose	11	23	185	13
<i>R. trifolii</i> T1L	Fast	L-Arabinose	184	< 0.5	375	< 0.5
		D-Glucose	21	< 0.5	33	< 0.5
<i>R. phaseoli</i> K26	Fast	L-Arabinose	102	< 0.5	70	10
		D-Glucose	82	< 0.5	61	4
<i>R. leguminosarum</i> DL-14	Fast	L-Arabinose	42	< 0.5	136	3
		D-Glucose	25	< 0.5	113	< 0.5
<i>R. japonicum</i> 61A76	Slow	L-Arabinose	6	54	26	31
		D-Glucose	1	61	41	33
<i>Rhizobium</i> sp. 32H1 (for cowpea)	Slow	L-Arabinose	34	42	44	25
		D-Glucose	5	34	52	46

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