hydrolysates of these, performed as described above, revealed D-ribose and D-galactose in the proportions 1.0:1.0, 1.0:2.1, and 1.0:1.1, respectively. A sample (2 mg) of the oligosaccharide with the highest mobility was reduced (sodium borodeuteride) and acetylated (acetic anhydride, pyridine) and *myo*-inositol hexaacetate (1 mg) was added as internal standard. A portion (1/3) of this preparation was hydrolysed, transformed into alditol acetates, and investigated by GLC-MS.[2,3] Deuterium labelling was observed in essentially all the ribitol but not in the galactitol.

The other part of the preparation was dissolved in acetic acid (0.06 ml), chromium trioxide (6 mg) was added and the resulting suspension was agitated at 50°C in an ultrasonic bath. The reaction mixture was diluted with water (5 ml) and extracted with chloroform (3 × 5 ml). The chloroform extracts were combined and dried (magnesium sulphate) and the material was hydrolysed, transformed into alditol acetates, and analysed by GLC-MS.[2,3] The proportions of ribitol pentaacetate, galactitol hexaacetate and *myo*-inositol hexaacetate were essentially the same as in the non-oxidized sample.

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Regulation of Branched Chain Amino Acid Transaminase Formation During the Growth of *Pseudomonas fluorescens* UK-1

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The enzyme which catalyses the transfer of amino groups from branched chain amino acids (valine, leucine, and isoleucine) to 2-oxoglutarate is classified by the Enzyme Commission as EC 2.6.1.6, L-leucine:2-oxoglutarate aminotransferase, and given the trivial name leucine aminotransferase but the name branched chain amino acid transaminase seems more correct.[1]

Branched chain amino acid transaminase catalyses the terminal reaction in the biosynthesis of branched chain amino acids in several bacteria.[2-6] In the case of an organism such as *Pseudomonas fluorescens*, which can both synthesize and metabolize the branched chain amino acids, such a transaminase is required in biosynthetic as well as catabolic pathways.[7]

Previous studies indicate that *Pseudomonas fluorescens* P-2 (previously named *Pseudomonas* P-2 by Goodhue and Snell [8]) is able to utilize pantothenate as sole source of carbon and nitrogen.[8] This substance is metabolized by way of an inducible pathway to 2-oxoisovalerate,[9-13] which is the first catabolite of valine. In the present study attention was focused on the formation and control of branched chain amino acid transaminase during the growth of *Pseudomonas fluorescens* UK-1.

Organism and culture conditions. Pseudomonas fluorescens strain UK-1 was grown in a basal mineral medium containing (per litre) KH_2PO_4, 0.82 g; $MgSO_4.7H_2O$, 0.25 g; $FeSO_4.7H_2O$, 2.8 mg. The pH was adjusted to pH 6.8 with KOH. Unless otherwise stated, the concentration of carbon source was 10 mM. $(NH_4)_2SO_4$ (10 mM) was used as nitrogen source, if necessary. The cells were precultured in the glutamate (20 mM) and pantothenate media. The culture was aerated with an aquarium pump and incubated at 30°.

Cell extract and enzyme assay. Samples (about 4 mg dry weight) were withdrawn from

the cultures during the period of active growth stage of the bacteria. The cells were harvested by centrifugation at 5000 *g* for 10 min, washed with 0.05 M potassium phosphate buffer (pH 7.5) and stored at −20°. For preparation of extract, they were suspended in 2 ml of the same buffer solution, and ruptured at 0° in a sonic oscillator (MSE 60 W, Measuring and Scientific Equipment, Ltd., London). The sonic extract was spun at 5000 *g* for 10 min at 4°. The supernatant was assayed for enzyme activity using freshly prepared cell extract.

The branched chain amino acid transaminase was determined according to Raunio,[14] except that the reaction system contained 20 μmol of L-leucine, 0.08 μmol of pyridoxal-5'-phosphate and 80 μmol of potassium phosphate buffer, pH 7.8. The reaction was initiated with enzyme extract containing about 60 μg dry weight. The total volume of reaction mixture was 0.4 ml. An enzyme unit is defined as the amount of enzyme which catalyses the formation of 1 μmol/min of 2-oxoisocaproate at 30°.

Results and discussion. Since the standard curve for 2-oxoisovalerate is non-linear at low keto acid concentrations,[14] leucine was used as substrate when the enzyme activity was determined. However, the relative activities with respect to valine and leucine remained practically the same during purification, indicating that a single aminotransferase serves for both these amino acids (Table 1).

Table 1. Partial purification of branched chain amino acid transaminase of *P. fluorescens* UK-1, according to the method of Norton and Sokatch.[15] The cells were grown on DL-valine. Crude extract was made from the end of the exponential phase cells and enzyme activities were determined as described under *Cell extract and enzyme assay.*

Enzyme preparation	Specific activity unit/mg Leucine	Valine	Leucine/ Valine
Cell-free extract	0.65	0.28	2.3
Heat extract 62°	4.6	2.2	2.1
» » 75°	5.7	2.2	2.6
Protamined extract	5.7	2.3	2.5
$(NH_4)_2SO_4$ fraction			
0.35–0.45	14.9	6.4	2.3
0.45–0.60	9.8	4.5	2.2
0.60–1.0	—	—	—

When *P. fluorescens* UK-1 was precultured for two generations in glutamic acid medium the level of branched chain amino acid transaminase was low, about 0.1 unit per mg dry weight. If the cells were then transferred to the valine, leucine, or isoleucine medium, the specific activity of the enzyme began to increase at the time when the growth of the bacteria started. The enzyme was formed even in the presence of the end products of the reaction, 2-oxoisovalerate and 2-oxoisocaproate. The specific activity was maximal at the end of the exponential phase (Table 2).

Table 2. Effect of growth substrates on synthesis of branched chain amino acid transaminase of *P. fluorescens* UK-1. Bacteria were grown in basal mineral medium supplemented with growth substrates as indicated. Crude extracts were made from the exponential phase cells and the specific activities were determined as described under *Cell extract and enzyme assay.*

Growth substrates 10 mM	Specific activity of branched chain amino acid transaminase
Isoleucine	0.65
2-Oxoisovalerate	0.65
Valine (5 mM) + isoleucine (5 mM)	0.65
Valine	0.56
Leucine (5 mM) + isoleucine (5 mM)	0.53
Leucine	0.51
Valine + leucine + isoleucine (each 3.3 mM)	0.45
2-Oxoisocaproate	0.41
Valine (5 mM) + leucine (5 mM)	0.34
Isobutyrate	0.13
3-Hydroxyisobutyrate	0.12
Propionate	0.12
Glutamate	0.12

The data presented in Table 2 reveal that there was a marked increase in specific activity when the bacteria were grown in mixtures of valine and leucine, valine and isoleucine, or valine, leucine and isoleucine, but no increase was observed when the cells were grown in media containing isobutyrate, 3-hydroxyisobutyrate or propionate. The specific activity of branched

chain amino acid transaminase on isoleucine, 2-oxoisovalerate, and a mixture of valine and isoleucine increased almost 7-fold, and on valine, leucine and a mixture of leucine and isoleucine 5-fold. In cells grown on mixtures of leucine, isoleucine, and valine or leucine and valine, the specific activity of branched chain amino acid transaminase was roughly the same as in cells grown on 2-oxoisocaproate. The enzyme was not induced in the presence of isobutyrate, 3-hydroxyisobutyrate, propionate, or glutamate (Table 2).

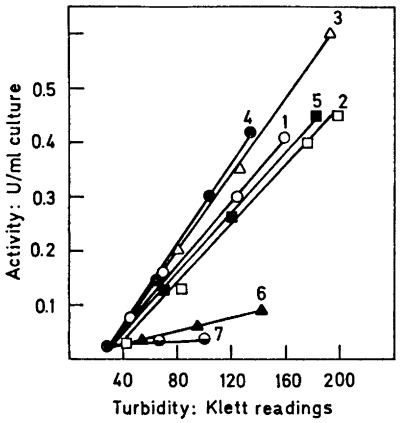

Fig. 1. Differential rate of synthesis of branched chain amino acid transaminase during growth on different carbon sources. The cells were precultured in glutamic acid medium. The samples were withdrawn from the cultures during the growth of the bacteria. Crude extracts were made and enzyme activities determined as described under *Cell extract and enzyme assay.*
1. Valine. 2. Leucine. 3. Isoleucine. 4. 2-Oxoisovalerate. 5. 2-Oxoisocaproate. 6. Isobutyrate (or 3-hydroxyisobutyrate). 7. Propionate.

Fig. 1 shows the kinetics of induction of branched chain amino acid transaminase in response to the different carbon sources. Isoleucine and 2-oxoisovalerate were the most powerful inducers among the carbon sources studied. Once induction had started, the differential rate of synthesis of branched chain amino acid transaminase remained constant in response to the inducer.

These data show that branched chain amino acid transaminase is subject to regulatory control, and the results of a brief survey of enzyme levels in cells grown on a number of carbon sources (Table 2 and Fig. 1) suggest that the branched chain amino acid transaminase is induced by branched chain amino acids and branched chain 2-oxoacids rather than repressed by isobutyrate, 3-hydroxyisobutyrate, or propionate. Branched chain amino acid transaminase exhibits characteristics of several bacterial catabolic enzymes. It is inducible, and induction begins at the same time as cell multiplication and continues throughout the exponential phase of growth.

According to Marshall and Sokatch,[16] branched chain amino acid transaminase is a constitutive enzyme in *Pseudomonas putida*, but our results indicate that the corresponding enzyme in *P. fluorescens* UK-1 is subject to regulatory control. As far as we know, it has not previously been observed that branched chain amino acids increase the activity of branched chain amino acid transaminase in bacteria.

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