We found an inducible NADP⁺-dependent D-phenylserine dehydrogenase in a soil bacterium identified as *Pseudomonas syringae* NK-15. The enzyme catalyzed the oxidation of the 3-hydroxyl group of D-threo-3-phenylserine to yield D-2-amino-3-keto-3-phenylpropanone, which was spontaneously decarboxylated into 2-aminoacetophenone. The enzyme had an optimum activity at pH 10.4. The *Kₘ* for D-threo-3-phenylserine and NADP⁺ were 0.44 mM and 29 µM, respectively.

Although a variety of L-amino acid dehydrogenases have been extensively studied as reviewed by Ohshima and Soda, little attention has been paid to the pyridine nucleotide-dependent L-amino acid dehydrogenases. We have found NADP⁺-dependent D-threonine dehydrogenase in a crude extract of *Pseudomonas cruvicive IFO 12047.* The enzyme catalyzes the oxidation of the 3-hydroxyl group of D-threonine to yield D-2-amino-3-ketobutyrate, which is spontaneously decarboxylated into aminoacetone. The enzyme has been purified to homogeneity from a crude extract of *P. cruvicive IFO 12047.* The enzyme acted on D-threonine but not on the other isolates. In addition to D-threonine, D-threo-3-phenylserine and D-threo-3-thiophenylserine were substrates. The enzyme is formed constitutively.

This paper describes the occurrence of another inducible NADP⁺-dependent dehydrogenase that catalyzes the oxidation of the 3-hydroxyl group of D-threo-3-phenylserine in a crude extract of *Pseudomonas syringae* NK-15 isolated from soil.

D-threo-3-Phenylserine was prepared from DL-threo-3-phenylserine (Sigma Chemical Company, St. Louis) by enantiomeric assimilation with *Pseudomonas pickettii* (unpublished data). D-threo-3-Phenylserine-assimilating bacteria were isolated from soil using the medium (pH 7.2) containing 1% D-threo-3-phenylserine, 0.2% K₂HPO₄, 0.2% KH₂PO₄, 0.2% NaCl, 0.01% MgSO₄·7H₂O, and 0.01% yeast extract. The isolated bacteria were grown in a medium containing 1% peptone, 0.5% D-threo-3-phenylserine, 0.2% K₂HPO₄, 0.2 KH₂PO₄, 0.2% NaCl, 0.01% MgSO₄·7H₂O, and 0.01% yeast extract (pH 7.2). The cultures were grown at 30°C for 20 h on a reciprocal shaker. The harvested cells were washed twice with 0.85% NaCl and suspended in 0.1 M potassium phosphate buffer (pH 7.2) containing 0.01% diithiothreitol, followed by sonik disruption at 0–5°C. The supernatant obtained by centrifugation was dialyzed against 0.01 M potassium phosphate buffer (pH 7.4) containing 0.01% diithiothreitol at 4°C for 20 h and used as the crude extract. The dehydrogenase activity was determined at 30°C by measuring the rate of increase in the absorbance at 340 nm with a Shimadzu double-beam spectrophotometer UV-140-02 using a cuvette with a 1.0-cm light path. The standard reaction mixture consisted of 20 µmol of D-threo-3-phenylserine, 1 µmol of NADP⁺, 200 µmol of glycine–KCl–KOH buffer (pH 10.5), and enzyme in a final volume of 1.0 ml. Protein was measured by the method of Lowry et al., with bovine serum albumin as the standard.

We isolated 12 strains of soil bacteria that could use D-threo-3-phenylserine as the sole carbon and nitrogen source. Crude extracts of most of them showed NADP⁺-dependent D-phenylserine dehydrogenase activity. Among these bacteria, the strain NK-15 had the highest activity. The strain NK-15 was a strictly aerobic, catalase-positive, oxidase-negative, non-sporule forming, and Gram-negative bacterium. The form was a rod-shaped cell (0.75 × 1.5–2.0 µm) with polar flagella. The organism assimilated glucose, sucrose, fumarate, pyruvate, L-aspartate, L-glutamate, and L-arginine and grew in the simple synthetic medium without any growth factors. The strain produced fluorescent pigments but not pyocyanin or carotenoids. It could not grow at pH 3.6 and did not accumulate poly-β-hydroxybutyrate. The degradation of protocatechuate is of ortho-type. The tests for nitrogenase, arginine dihydrolase, lecitinase, lipase, gelatin hydrolysis, and starch hydrolysis were negative. From these morphological and physiological characteristics, the strain NK-15 seemed very similar to *Pseudomonas syringae* as described in Bergey’s Manual of Systematic Bacteriology. Therefore, we named this strain *P. syringae* NK-15.

We investigated the culture conditions for enzyme production using *P. syringae* NK-15. D-Threonine dehydrogenase was not induced by D-threonine and D-threo-3-phenylserine, but D-phenylserine dehydrogenase production could be induced about 4 times by addition of 0.2% D-threo-3-phenylserine into 1% peptone medium. Addition of D-threonine (0.2%) to the medium did not affect the enzyme production. The highest specific and total activities were obtained by cultivating the cells in 1% peptone medium containing 0.5% D-threo-3-phenylserine at 30°C for 20 h on a reciprocal shaker.

To characterize the dehydrogenase reaction, the enzyme was partially purified as follows. The crude extract was brought to 30–60% saturation with solid ammonium sulfate. The precipitate collected by centrifugation was dissolved in 10 mM potassium phosphate buffer (pH 7.4) containing 0.01% diithiothreitol and dialyzed against the same buffer. The enzyme solution was put on a DEAE-cellulose column equilibrated with the buffer used for dialysis. After the column had been washed with the same buffer and the buffer containing 0.1 M KCl, the enzyme was eluted with the buffer containing 0.15 M KCl. All operations were done at 0–5°C. This enzyme preparation was used in the experiments described below.

When D-threo-3-phenylserine was incubated with the enzyme in the presence of NADP⁺ at pH 9.0 (Tris–HCl buffer), ammonia was not formed. This shows that the enzyme does not catalyze the deamination of D-threo-3-phenylserine. The product from D-threo-3-phenylserine was identified by thin-layer chromatography as follows. The reaction mixture (1.0 ml), containing 10 µmol of D-threo-3-phenylserine, 10 µmol of NADP⁺, 200 µmol of Na₂CO₃–NaHCO₃ buffer (pH 10.5), and 4 units of enzyme, was incubated at 30°C for 30 min. After deproteinization by

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### Table: Formation of 2-Aminoacetophenone during the d-Phenylserine Dehydrogenase Reaction

<table>
<thead>
<tr>
<th>Reaction time (min)</th>
<th>Product formed (nmol)</th>
<th>NADPH</th>
<th>2-Aminoacetophenone</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4.02</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.63</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6.75</td>
<td>7.1</td>
<td></td>
</tr>
</tbody>
</table>

![Figure](image)

**Fig.** d-Phenylserine Dehydrogenase Reaction.

Addition of 0.1 ml of 25% trichloroacetic acid, aliquots of the supernatant were examined by thin-layer chromatography on a cellulose plate (DC-plastikfolien cellulose, Merk, Art 5577) with a solvent system of n-butanol–acetic acid–water (4:1:1, by volume). In addition to d-threo-3-phenylserine (Rf, 0.44), a new compound (Rf, 0.59) was formed only in a complete system containing d-threo-3-phenylserine, NADP+, and enzyme. This new compound reacted with ninhydrin to develop a yellow color. The Rf value of this compound was very similar to that of authentic 2-aminoacetophenone (phenacylamine, Aldrich Chemical Company, St. Louis). Since aminoketones react with acetylacetone to form pyrroles, the reaction mixture was reacted with acetylacetone at 100°C for 10 min. The formed pyrrole was extracted with ether and chromatographed on a thin-layer cellulose plate with the upper phase of the mixture, n-butanol–water–25% NH₄OH (10:3:1, by volume). The pyrrole was stained by spraying the Ehrlich-Hg reagent. The Rf value of the pyrrole of the reaction product was 0.95, showing that the pyrrole had no carboxyl group. The Rf value was the same as that of 2-methyl-3-acetyl-4-phenylpyrrole made from 2-aminoacetophenone. When 2-aminoacetophenone was measured by the method of Urata and Granick, the amount of 2-aminoacetophenone increased with increasing incubation time and the ratio of 2-aminoacetophenone to NADPH formed was closely parallel (Table). These results suggest that the 3-hydroxy group of d-threo-3-phenylserine is oxidized by the enzyme to yield d-2-amino-3-keto-3-phenylpropionate, which is spontaneously decarboxylated into 2-aminoacetophenone (Fig.). This reaction is similar to that of d-threonine dehydrogenase.

D-Threonine was a poor substrate for the enzyme, but the following amino acids and hydroxy acids were inert: d-tal-e erythro-3-phenylserine, L-threonine, l-allo-threonine, d-allo-threonine, d-serine, dl-homoserine, 3-hydroxypropionate, dl-3-hydroxybutyrate, dl-phenyllactate, d-1-amino-2-propanol, l-1-amino-2-propanol, and dl-lactate. The enzyme had an optimum reactivity at about pH 10.4. The Km for d-threo-3-phenylserine and NADP+ were 0.44 mm and 29 μM, respectively.

Thus, these results provide good evidence for the occurrence of an inducible NADP+-dependent dehydrogenase catalyzing the oxidation of d-threo-3-phenylserine in the crude extract of P. syringae NK-15 isolated from soil.

Further work is needed to elucidate both the enzymological and physicochemical properties of the enzyme.

### References