

Investigations into Enzymes of Nitrogen Metabolism of the Ectomycorrhizal Basidiomycete, *Suillus bovinus*

Norbert Grotjohann^a, Wolfgang Kowallik^{a,*}, Yi Huang^b, Andrea Schulte in den Bäumen^a

^a Lehrstuhl für Stoffwechselphysiologie, Fakultät für Biologie, Universität Bielefeld, Postfach 100131, D-33501 Bielefeld, Germany. Fax: 0521–106–6039.

E-mail: W.Kowallik@Biologie.Uni-Bielefeld.DE

^b Department of Urban and Environmental Sciences, Peking University, Beijing, 100 871, P. R. China

* Author for correspondence and reprint requests

Z. Naturforsch. **55c**, 203–212 (2000); received November 24/December 15, 1999

Dedicated to Professor André Pirson on the occasion of his 90th birthday

Suillus bovinus, Glutamate Dehydrogenase, Glutamine Synthetase/Glutamate Synthase, Aminotransferases, Urease

Axenic mycelia of the ectomycorrhizal basidiomycete, *Suillus bovinus*, were grown in liquid media under continuous aeration with compressed air at 25 °C in darkness. Provided with glucose as the only carbohydrate source, they produced similar amounts of dry weight with ammonia, with nitrate or with alanine, 60–80% more with glutamate or glutamine, but about 35% less with urea as the respectively only exogenous nitrogen source.

In crude extracts of cells from NH₄⁺-cultures, *NADH-dependent glutamate dehydrogenase* exhibited high aminating ($688 \text{ nmol} \times \text{mg protein}^{-1} \times \text{min}^{-1}$) and low deaminating ($21 \text{ nmol} \times \text{mg protein}^{-1} \times \text{min}^{-1}$) activities. Its K_m -values for 2-oxoglutarate and for glutamate were 1.43 mM and 23.99 mM, respectively. pH-optimum for amination was about 7.2, that for deamination about 9.3. *Glutamine synthetase* activity was comparatively low ($59 \text{ nmol} \times \text{mg protein}^{-1} \times \text{min}^{-1}$). Its affinity for glutamate was poor ($K_m = 23.7 \text{ mM}$), while that for the NH₄⁺ replacing NH₂OH was high ($K_m = 0.19 \text{ mM}$). pH-optimum was found at 7.0. *Glutamate synthase* (= GOGAT) revealed similar low activity ($62 \text{ nmol} \times \text{mg protein}^{-1} \times \text{min}^{-1}$), K_m -values for glutamine and for 2-oxoglutarate of 2.82 mM and 0.28 mM, respectively, and pH-optimum around 8.0. *Aspartate transaminase* (= GOT) exhibited similar affinities for aspartate ($K_m = 2.55 \text{ mM}$) and for glutamate ($K_m = 3.13 \text{ mM}$), but clearly different K_m -values for 2-oxoglutarate (1.46 mM) and for oxaloacetate (0.13 mM). Activity at optimum pH of about 8.0 was $506 \text{ nmol} \times \text{mg protein}^{-1} \times \text{min}^{-1}$ for aspartate conversion, but only $39 \text{ nmol} \times \text{mg protein}^{-1} \times \text{min}^{-1}$ at optimum pH of about 7.0 for glutamate conversion. Activity ($599 \text{ nmol} \times \text{mg protein}^{-1} \times \text{min}^{-1}$), substrate affinities (K_m for alanine = 6.30 mM, for 2-oxoglutarate = 0.45 mM) and pH-optimum (6.5–7.5) proved *alanine transaminase* (= GPT) also important in distribution of intracellular nitrogen.

There was comparatively low activity of the obviously constitutive enzyme, *urease*, ($42 \text{ nmol} \times \text{mg protein}^{-1} \times \text{min}^{-1}$) whose substrate affinity was rather high ($K_m = 0.56 \text{ mM}$). *Nitrate reductase* proved substrate induced; activity could only be measured after exposure of the mycelia to exogenous nitrate.

Routes of entry of exogenous nitrogen and tentative significance of the various enzymes in cell metabolism are discussed.