Purification and Characterization of Tyrosinases from *Streptomyces albus*

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Z. Naturforsch. **64 c**, 724–732 (2009); received May 18/June 29, 2009

The bacterium *Streptomyces albus* has so far never been investigated for tyrosinase activity. The studies presented in this communication show that this bacterium may be a future source for larger production of tyrosinase. The enzyme was purified starting with 5,600 ml of culture filtrate. The crude enzyme was first purified by centrifugation, followed by ammonium sulfate precipitation and ultrafiltration. Then, melanin was removed applying a Servacell DEAE 52 resin, using the batch technique. Thereafter, the crude enzyme was loaded on a SEC Sephacryl S-100 column and, after ultrafiltration, 1.17 mg of purified tyrosinase were obtained. The molecular mass of the purified enzyme was determined by MALDI mass spectrometry to be 30,096 Da which corresponds to the obtained results from SDS-PAGE.

Using the diphenol L-DOPA and the monophenol L-tyrosine as substrates, the kinetic parameters for both substrates, K_m = 7.8 mM and 0.5 mM and k_{cat}/K_m = 157 $mM^{-1}\,s^{-1}$ and 23 $mM^{-1}\,s^{-1}$, respectively, were determined. Maximal activities of the purified enzyme were recorded at pH 7.0. Long-term experiments with *Streptomyces albus* tyrosinase revealed that storage of the lyophilized enzyme sample at temperatures below zero turned out to be the best. For tyrosinase in buffer containing 20% glycerol, no loss of activity was observed at 4 °C and –60 °C.

Key words: Tyrosinase, *Streptomyces albus*, Enzyme Kinetics