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WANIDA KHAMPHA: SCREENING FOR L-PHENYLGLYCINE AMINOTRANSFERASE PRODUCING MICROORGANISM, ENZYME PURIFICATION AND CHARACTERIZATION. THESIS ADVISORS: VITHAYA MEEVOOTISOM, Ph.D., SUTHEP WIYAKRUTTA, Ph.D., BHINYO PANIJPAN, Ph.D., 189P. ISBN 974-663-489-5

Pseudomonas putida SC-501 was newly isolated from Thai soil under the screening method designed to search for microorganisms capable of producing an enzyme L-phenylglycine aminotransferase (L-PhgAT). The bacterium was induced to produce the enzyme in the minimal medium containing L-phenylglycine as the sole carbon and nitrogen sources. Cell homogenate was prepared and the enzyme was purified by ammonium sulfate precipitation, isocratic hydrophobic interaction, SP-Sepharose Fast Flow cation exchange, phenyl agarose hydrophobic interaction, and Superdex gel filtration chromatography. The purified enzyme revealed homogeneity by SDS-PAGE analysis. Molecular weight of the native enzyme was estimated to be 67,000. The enzyme was composed of two identical subunits, each with a molecular weight of 35,400. N-terminal amino acid sequence of the enzyme was similar to that of branched-chain amino acid aminotransferases from *Helicobacter pylori* and *Haemophilus influenzae* at about 50% and 46%, respectively. The isoelectric point (p*I*) of the native enzyme was 6.0. The enzyme was most active at pH 9.0. Optimum temperature for enzyme activity was 40-45 °C. The enzyme was found to be stable at up to 45 °C. Substrate specificity was found to be broad with the decreasing preference with L-leucine, L-phenylglycine L-isoleucine, L-lysine, L-methionine, L-alanine, L-aspartate, L-valine, L-p-hydroxyphenylglycine, and L-phenylalanine. No D-amino acid including D-phenylglycine was used as a substrate. The enzyme preferred L-leucine as well as L-phenylglycine as the best amino donor in which 2-oxoglutarate was an amino group acceptor. The apparent K_M values for L-phenylglycine and for 2-oxoglutarate at 30 °C, pH 9.0 were 0.75 mM and 0.18 mM, respectively. The enzyme was strongly inhibited by typical inhibitors of pyridoxal-5′-phosphate-dependent enzymes. In summary L-PhgAT found in the present study is a new enzyme that has not been reported before.