Oligopeptidase B (opdB, EC 3.4.21.83) is a member of the prolyl oligopeptidase family of serine peptidases and unrelated to the trypsin and subtilisin families. It is a potential processing enzyme of prokaryotes to produce biologically active products, being very specific for the basic amino acid pairs of polypeptides. Bacterial oligopeptidase B cleaves globular proteins, albeit in a highly restricted fashion. While most members of this peptidase family hydrolyse peptide bonds at the C-terminal side of proline residues, oligopeptidase B exhibits a trypsin-like substrate specificity, cleaving peptides after basic residues (arginine or lysine). Oligopeptidase B was first cloned and characterized from *Escherichia coli*, and has also been described in other prokaryotes. Similar enzymes have been found in plants and some other higher organisms. We report the isolation of two different new oligopeptidase B bacterial strains producers. We identified the two genes, designated opdB1 and opdB2. The opdB genes encode a 703-residue peptide with high homology to the oligopeptidase B family in prokaryotes. The isolated opdBs gave the highest similarity score to oligopeptidase B of *Stenotrophomonas maltophilia* strain K279a (GenBank AM743169). To reveal the structural and kinetic properties of oligopeptidase B in more detail, we have cloned, expressed, and purified the enzymes to produce sufficient material to help in physical investigations, including NMR and x-ray crystallographic measurements. We also carried out a molecular characterization study of the two enzymes.