A novel oxidation of D-pentonates to 4-keto-D-pentonates was analyzed with *Gluconobacter thailandicus* NBRC 3258. D-Pentonate 4-dehydrogenase activity in the membrane fraction was readily inactivated by EDTA and it was reactivated by the addition of PQQ and Ca^{2+}. D-Pentonate 4-dehydrogenase was purified to two different subunits, 80 and 14 kDa. The absorption spectrum of the purified enzyme showed no typical absorbance over the visible regions. The enzyme oxidized D-pentonates to 4-keto-D-pentonates at the optimum pH of 4.0. In addition, the enzyme oxidized D-fructose to 5-keto-D-fructose, D-psicose to 5-keto-D-psicose, including the other polyols such as, glycerol, D-ribitol, D-arabitol, and D-sorbitol. Thus, D-pentonate 4-dehydrogenase was found to be identical with glycerol dehydrogenase (GLDH), a major polyol dehydrogenase in *Gluconobacter* species. The reaction versatility of quinoprotein GLDH was notified in this study.