A highly thermostable recombinant β-glucosidase from *Thermotoga petrophila* RKU-1, over-expressed in mesophilic expression host *Escherichia coli* BL21 CodonPlus (RIPL) was used in the present study for the production and characterization of free and immobilized enzyme in different support materials. Optimal β-glucosidase expression and production was achieved at 0.6 OD_{600nm}, 22°C temperature and 200 rpm with reduced concentration of 0.5 mM IPTG. Recombinant β-glucosidase was partially purified by heat treatment and subjected to SDS-PAGE analysis with a prominent band of 51.50 kDa molecular mass. Partially purified β-glucosidase has displayed maximum activity at 90°C and pH 7.0 with 10 minutes incubation using para-nitrophenyl-β-D-glucopyranoside (pNPG) as substrate. Enzyme stability was 8 hours at 70°C and stable over a broad pH range of 7.0 to 9.0. No obvious effect was found with different metal ions and other inhibitors except Hg^{2+} and SDS; less than 10% residual activity was observed in the presence of 40% (v/v) alcohols. Enzyme exhibited high affinity with para-nitrophenyl substrates and no activity was observed with 2% CMC and 1% starch. Free enzyme exhibited remarkable storage stability as 100% residual activity was found after being stored for 62 days at 4°C. Partially purified β-glucosidase was entrapped in calcium-alginate and polyacrylamide gel and characterized using pNPG as substrate. Enzyme entrapped in calcium alginate showed optimal activity at pH 6.0 to 7.0, whereas polyacrylamide entrapped enzyme exhibited optimal activity over the range of pH 5.0 to 8.0. Immobilized enzyme in both supports displayed improved pH stability at 5.0 to 9.0. Optimal activity was found at 90 to 95°C with 10 min. of incubation for polyacrylamide entrapped enzyme, whereas, 3 minutes incubation for enzyme entrapped in alginate beads. No significant influence was observed on entrapped enzyme with metal ions except Hg^{2+}. Inhibition with Hg^{2+} was less pronounced on immobilized enzyme as compared to free enzyme; whereas, entrapped enzyme showed more than 20% residual activity with 50 % (v/v) alcohols. Reusability of beads and gel showed that 25% of residual activity was retained after 5 cycles. Entrapped enzyme in beads and gel displayed 100% activity up to 31 days and 34 days at 4°C, respectively.