2′-*O*-Methylribonucleosides (2′-OMe-NRs) are promising raw materials for nucleic acid drugs because of their high thermal stability and nuclease tolerance. In the course of microbial screening for metabolic activity toward 2′-OMe-NRs, *Lactobacillus buchneri* LBK78 was found to decompose 2′-*O*-methyluridine (2′-OMe-UR). The enzyme responsible was partially purified from *L. buchneri* LBK78 cells by a four-step purification procedure, and identified as a novel nucleoside hydrolase. This enzyme, *Lb*NH, belongs to the nucleoside hydrolase superfamily, and formed a homotetrameric structure composed of subunits with a molecular mass around 34 kDa. *Lb*NH hydrolyzed 2′-OMe-UR to 2′-*O*-methylribose and uracil, and the kinetic constants were K_m of 0.040 mM, k_{cat} of 0.49 s^{-1}, and k_{cat}/K_m of 12 mM^{-1} s^{-1}. In a substrate specificity analysis, *Lb*NH preferred ribonucleosides and 2′-OMe-NRs as its hydrolytic substrates, but reacted weakly with 2′-deoxyribonucleosides. In a phylogenetic analysis, *Lb*NH showed a close relationship with purine-specific nucleoside hydrolases from trypanosomes.

*Lb*NH, a novel nucleoside hydrolase from *Lactobacillus buchneri* LBK 78 catalyzes 1'-hydrolysis reaction toward 2'-*O*-methyluridine to form 2'-*O*-methylribose and uracil as the degradation products.