A gene (pncA) with mutations associated with pyrazinamide (PZA) resistance in Mycobacterium tuberculosis complex was examined in 26 PZA-resistant isolates recovered from the patients of pulmonary tuberculosis in Osaka, Japan. First, the minimum inhibitory concentration (MIC) was determined by macrodilutions (4·ml) in the acidified Middlebrook 7H9 broth (pH 6.0), and PZA resistance was designated as the isolate having >100 ug/ml of MIC. Of the 26 PZA-resistant isolates included, twenty-one isolates were negative for pyrazinamidase (PZase). Of these, twenty isolates had pncA mutations that altered the primary amino acid sequence of PZase or frameshift mutations. A total of 10 previously-unreported mutations were identified, including various point mutations, nucleotide insertions and deletions. The IS6110 restriction fragment length polymorphism (RFLP) pattern analysis demonstrated various distinct IS6110 types and only two pairs of isolates were very close with each other (>90% identical pattern). In addition, we found one PZA-resistant, PZase-negative isolate which did not have any mutation throughout the pncA nucleotide sequence including the 105 bp upstream of start codon and the 60 bp downstream of stop codon. The remaining five PZA-resistant isolates were positive for PZase and had identical pncA alleles with PZA-susceptible isolates. This study demonstrates that most of the PZA resistance of the isolates is due to various mutations of pncA resulting in loss of PZase activity. Further investigation, particularly into PZase-positive but PZA-resistant isolates and a PZase-negative isolate with no mutation in pncA, is urgent.