The koji mold *Aspergillus oryzae* is able to produce glutaminase which converts glutamine to glutamic acid, one of the most important flavor components in soy sauce. We present here the isolation and the complete nucleotide sequence of the glutaminase-encoding gene from *A. oryzae* U212, an industrial strain used in Thailand. N-terminal and internal amino acid sequences were determined from purified glutaminase. A 700-bp fragment was amplified by PCR using oligonucleotide primers designed from partial amino acid sequences. This PCR fragment was used as a homologous probe for screening an *A. oryzae* genomic DNA library. RT-PCR showed that the gene contained seven short introns. Sequence analysis revealed an open reading frame that encodes a protein of 690 amino-acid residues with a predicted molecular mass of 76 kDa. The N-terminal and internal amino acid sequences of the deduced protein exactly matched the ones determined from the purified protein. Comparison of the amino acid sequence with glutaminase sequences from other origins showed that *A. oryzae* glutaminase shares little homology with those of bacteria, eukaryote and mammals. The *A. oryzae* glutaminase gene was expressed in *A. nidulans* to confirm the presence of a functional glutaminase gene in the cloned DNA. To our knowledge, this is the first reported glutaminase gene cloned from filamentous fungi.