Peptidases are key molecules in parasite invasion and survival. Taenia solium cysts establish in the human nervous system and cause epilepsy and other neurological symptoms. By comparing with the non-pathogenic Taenia saginata, we assessed T. solium peptidase activity of Taenia solium and isolated two novel antigens. Peptidase activities of the excretory/secretory (E/S) antigens of Taenia solium and Taenia saginata oncospheres were assessed and compared. Oncospheres were cultured, and 24-, 48-, 72-, and 96-hr fractions of the spent media were analyzed. Activities for serine peptidases, cysteine peptidases, and aminopeptidase, were tested fluorometrically with peptides coupled to 7-Amino-4-Methylcoumarin. In both species, E/S antigens showed cysteine-, serine-, and aminopeptidase activity. While no particular peptidase had high activity in T. solium, but was absent in T. saginata, or vice versa, different patterns of activity were found. A chymotrypsin-like peptidase showed the highest activity in both parasites and had 10 times higher activity in T. solium than T. saginata. In the following step we purified a protein fraction containing 2 antigens of 24.9 and 52.5 KDa from the Taenia solium cysticercus fluid by using ethanol precipitation, exclusion and ion exchange chromatography. Activity for cysteine peptidases, specifically cathepsin L-like type was tested fluorometrically with the Z-Phe-Arg-AMC substrate as well as with gelatinolytic zymography, under a citrate buffer pH 4 condition. Cathepsin L-like activity was confirmed by inhibition with E64. We compared a western blot based on our purified proteins against the standard CDC immunoblot assay based on lentil-lectin purified cysticercus antigens by testing 150 defined human sera. The purified proteins did not correspond to any of the 7 lentil-lectin purified antigens. We tested 48 control sera, 50 positive sera associated to single cyst patients, and 50 positive sera associated to multiple cysts patients. Antibody response to the new antigens in Western Blot had a specificity of 97.9% and sensitivity of 96% for multiple cysts patients (78% for single cyst patients), using the standard lentil-lectin purified antigens as the reference. We furthermore demonstrated that the 24.9 and the 52.5 KDa antigens are highly immunogenic, by eliciting a strong immune response in a rabbit immunized with a total of 75 μg of total protein. These newly described antigens provide a new alternative for diagnosis and potentially vaccine protection.