

In the yeast *Saccharomyces cerevisiae*, all strains possess a gene for the evolutionarily conserved POT family peptide transporter, Ptr2; however, the genes for a novel FOT family transporter were found only in some wine brewing strains. The substrate specificity of the POT and FOT family of transporters was compared. Among the naturally occurring oligopeptides that were tested, Lys-Leu and Arg-Phe were Ptr2-specific substrates. Artificial dipeptide aspartame was imported specifically through the FOT transporter, but the structurally similar Asp-Phe was a substrate of both FOT and Ptr2 transporters. Furthermore, only the FOT transporter was important for high sensitivity to an antibiotic puromycin. These results demonstrate that the POT and FOT family of transporters have distinct substrate preferences although both transporters import overlapping dipeptide substrates. Having POT and FOT transporters is advantageous for cells to acquire nutrients, but also detrimental when these cells are exposed to the toxic molecules of their substrates.