Roles of Different Peptide Transporters in Nutrient Acquisition in \textit{Candida albicans}

Nico Dunkel, Tobias Hertlein, Renate Franz, Oliver Reuß, Christoph Sasse, Tina Schäfer, Knut Ohlsen, Joachim Morschhäuser

Institut für Molekulare Infektionsbiologie, Universität Würzburg, Würzburg, Germany

Fungi possess two distinct proton-coupled peptide transport systems, the dipeptide/tripeptide transporters (PTR), which enable them to utilize peptides as nutrients. In the pathogenic yeast \textit{Candida albicans}, peptide transporters are encoded by gene families consisting of two PTR genes and eight OPT genes. To gain insight into the functions and importance of specific peptide transporters, we generated mutants lacking the two dipeptide/tripeptide transporters Ptr2 and Ptr22, as well as the five major oligopeptide transporters Opt1 to Opt5. These mutants were unable to grow in media containing peptides as the sole nitrogen source. Forced expression of individual peptide transporters in the septuple mutants showed that Ptr2 and Ptr22 could utilize all tested dipeptides as substrates but differed in their abilities to transport specific tripeptides. Interestingly, several oligopeptide transporters, which are thought to transport peptides consisting of more than three amino acids, also mediate the uptake of tripeptides. Opt1 especially turned out to be a highly flexible transporter that enabled growth on all tripeptides tested and could even utilize a dipeptide, a function that has never been ascribed to this family of peptide transporters. Despite their inability to grow on proteins or peptides, the opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ ptr2Δ ptr22Δ septuple mutants had no \textit{in vivo} fitness defect in a mouse model of gastrointestinal colonization. Therefore, the nutritional versatility of \textit{C. albicans} enables it to utilize alternative nitrogen sources in this host niche, which probably contributes to its success as a commensal and pathogen in mammalian hosts.

\textit{C. albicans} possesses a family of eight OPT genes \cite{16}. Deletion of any single OPT gene did not affect the ability of \textit{C. albicans} to grow on bovine serum albumin (BSA) as the sole nitrogen source, but triple mutants lacking OPT1 to OPT3 had a severe growth defect under these conditions, which was further exacerbated after the additional deletion of OPT4 and OPT5 \cite{6}. In contrast to the wild-type parental strain, the opt1Δ opt2Δ opt3Δ triple mutants were also unable to grow in media containing specific tetra-, penta-, hepta-, and octapeptides as sole nitrogen sources. Forced expression of any of the OPT1 to OPT5 genes, but not the OPT6 to OPT8 genes, from the ADH1 promoter restored the ability of the opt1Δ opt2Δ opt3Δ triple mutants to grow on BSA. These studies demonstrated that at least OPT1 to OPT5 encode functional peptide transporters, while Opt6 to Opt8 might have a more specific, narrower substrate spectrum that is not sufficient to allow growth on peptides generated by SAP-mediated digestion of BSA \cite{6}. Indeed, OPT7 has recently been found to encode a glutathione transporter \cite{17}.

In addition to the OPT genes, \textit{C. albicans} contains two genes encoding dipeptide/tripeptide transporters of the PTR family. One of these (orf19.2583) was shown to complement an \textit{S. cerevisiae} ptr2 mutation and was therefore designated PTR2 \cite{18}. A second gene (orf19.6937) is even more similar to \textit{S. cerevisiae} PTR2 (ScPTR2) than is \textit{C. albicans} PTR2 (CaPTR2) and has been termed PTR22 \cite{6}. However, the functions of PTR2 and PTR22 in \textit{C. albicans} have not been studied so far. Therefore, we investigated the roles of these genes in the ability of \textit{C. albicans} to utilize di- and tripeptides as a nitrogen source for growth. By using mutants lack-
ing all major peptide transporters, we assessed the importance of peptides as nitrogen sources for efficient growth of *C. albicans* in the mammalian intestine.

**MATERIALS AND METHODS**

**Strains and growth conditions.** The *C. albicans* strains used in this study are listed in Table S1 in the supplemental material. All strains were stored as frozen stocks with 15% glycerol at −80°C and subcultured on YPD agar plates (20 g peptone, 10 g yeast extract, 20 g glucose, 20 g agar per liter) at 30°C. The strains were routinely grown in YPD liquid medium at 30°C in a shaking incubator. For selection of nourseothricin-resistant transformants, 200 μg/ml nourseothricin (Werner Bioagents, Jena, Germany) was added to YPD agar plates. To obtain nourseothricin-sensitive derivatives in which the SAT1 flipper cassette was excised by FLP-mediated recombination, transformants were grown overnight in YPM liquid medium (10 g yeast extract, 20 g peptone, 20 g maltose per liter) to induce the MAL2 promoter. One hundred to 200 cells were then spread on YPD plates containing 20 μg/ml nourseothricin and grown for 2 days at 30°C. Nourseothricin-sensitive clones were identified by their small colony size. Recombinants were identified by their small colony size; transformants were grown overnight in YPM medium. The strains were routinely grown in YPD liquid medium at 30°C in a shaking incubator. For selection of nourseothricin-resistant transformants, 200 μg/ml nourseothricin (Werner Bioagents, Jena, Germany) was added to YPD agar plates. To obtain nourseothricin-sensitive derivatives in which the SAT1 flipper cassette was excised by FLP-mediated recombination, transformants were grown overnight in YPM medium (10 g yeast extract, 20 g peptone, 20 g maltose per liter) to induce the MAL2 promoter. One hundred to 200 cells were then spread on YPD plates containing 20 μg/ml nourseothricin and grown for 2 days at 30°C. Nourseothricin-sensitive clones were identified by their small colony size and confirmed by restreaking on YPD plates containing 20 μg/ml nourseothricin as described previously (19). Growth of the strains on BSA, di- and tripeptides, and amino acids was tested in YCB medium (11.7 g yeast carbon base per liter, pH 5.0) containing 0.4% BSA, 10 mM of 1 of the 20 standard L-amino acids, or peptides (Bachem, Weilheim am Rhein, Germany) at the indicated concentrations as sole nitrogen sources. Growth rate determinations and *in vitro* competition experiments were also performed in SD medium (1.7 g yeast nitrogen base without amino acids and ammonium sulfate [YNB]; Becton, Dickinson, Heidelberg, Germany, 20 g glucose per liter) containing 50 mM ammonium sulfate.

**Plasmid construction.** A PTR2 deletion construct was generated by PCR amplification of the PTR2 upstream and downstream regions from genomic DNA of strain SC5314 with the primer pairs PTR201/PTR202 and PTR203/PTR204, respectively (all primers used in this study are listed in Table S2 in the supplemental material). The PCR products were digested at the introduced SacI/SaclI and Xhol/Apal sites and inserted in place of the OPT5 flanking sequences of plasmid pOPTSM2 (6) to obtain pPTR2M2, in which the PTR2 coding sequence is replaced by the SAT1 flipper cassette. Two different constructs were made in an analogous fashion for the deletion of the two PTR22 alleles. In pPTR22M2, the SAT1 flipper cassette is flanked by the PTR22 upstream and downstream sequences, which were amplified with the primer pairs PTR221/PTR222 and PTR223/PTR224, respectively. In pPTR22M4, the SAT1 flipper cassette is flanked by N-terminal (positions +29 to +383) and C-terminal (positions +1299 to +1678) parts of the PTR22 coding region, which were amplified with the primer pairs PTR2210/PTR2211 and PTR22212/PTR2213, respectively. To express PTR2 and PTR22 from the ADH1 promoter, the coding regions of the genes were amplified with the primer pairs PTR205/PTR206 and PTR225/PTR226, respectively. The PCR products were digested at the introduced SalI and BglII sites and inserted into the Xhol/BglII-digested vector pADH1E2 (6), generating pPTR2E1 and pPTR22E1, respectively.

**C. albicans** transformation. *C. albicans* strains were transformed by electroporation (20) with the following gel-purified linear DNA fragments. The SacI-Apal fragment from pPTR2M2 was used for the sequential deletion of both PTR2 alleles in the wild-type strain SC5314 and in the opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ quintuple mutants. The SacI-Apal fragments from pPTR22M2 and pPTR22M4 were used for the deletion of the first and second PTR22 alleles, respectively, in strain SC5314, the ptr2Δ mutants, and the opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ ptr2Δ septuple mutants. The pPTR2M2 and pPTR22M4 were used to integrate OPT1, OPT2, OPT3, OPT4, OPT5, PTR2, and PTR22, respectively, under the control of the ADH1 promoter in the opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ ptr2Δ septuple mutants. The correct genomic integration of each construct and the excision of the SAT1 flipper cassette were confirmed by Southern hybridization with the upstream and downstream flanking sequences.

**Isolation of genomic DNA and Southern hybridization.** Genomic DNA from *C. albicans* strains was isolated as described previously (19). The DNA was digested with appropriate restriction enzymes, separated on a 1% agarose gel, and, after ethidium bromide staining, transferred by vacuum blotting onto a nylon membrane and fixed by UV cross-linking. Southern hybridization with enhanced-chemiluminescence-labeled probes was performed with the Amersham ECL Direct Nucleic Acid Labeling and Detection System (GE Healthcare, Braunschweig, Germany) according to the instructions of the manufacturer.

**Growth rate determinations.** To determine the growth rates of the wild-type parental strain SC5314, the genetically marked derivative SCADH1R1, and the opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ ptr2Δ ptr22Δ septuple mutants SCOPT12345PTR222M4A and -B in YPD and SD media, overnight cultures of the strains were diluted 10−3 in 100 μl fresh medium in 96-well microtiter plates and grown at 30°C in an Infinite F200 Pro microplate reader (Tecan, Crailsheim, Germany) with shaking at 280 rpm. The optical densities of the cultures were measured every 10 min, and the generation times in log phase were calculated from three independent cultures of each strain.

**In vitro competition experiments.** Strains were grown overnight in YPD or SD medium at 30°C. The wild-type strain SC5314 and the opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ ptr2Δ ptr22Δ septuple mutants SCOPT12345PTR222M4A and -B were mixed in a 1:1 ratio with the red fluorescent protein gene (*RFP*)-labeled strain SCADH1R1A in fresh medium at a starting optical density of 0.002 and grown for 24 h at 30°C. A dilution series was prepared from each coculture at the beginning and at the end of the co-inoculation, spread on YPD plates, and grown for 2 days at 30°C. The percentages of red colonies (strain SCADH1R1A) and white colonies (test strains) were determined after storage of the plates for a few days at 4°C (which enhanced the color of the *RFP*-expressing colonies). The relative fitness of a strain is defined as the ratio of its proportions in the coculture at the end and beginning of the experiment. A value of 1 indicates wild-type fitness, and values below or above 1 indicate decreased and increased fitness, respectively, compared to SCADH1R1A. The two-tailed Student’s *t* test was used to compare the relative fitness of the mutants with that of the wild-type strain SC5314.

**In vivo fitness tests.** For infection experiments, female BALB/c mice were fed with 1 mg/ml tetracycline, 2 mg/ml streptomycin, and 0.1 mg/ml gentamycin in their drinking water, starting from day 4 prior to infection. *C. albicans* strains were grown overnight in YPD medium at 30°C, washed twice in phosphate-buffered saline (PBS), and adjusted to a density of 107 cells per ml. Each of the test strains (OPT12345PTR222M4A and its wild-type parent, SC5314) was mixed in a 1:1 ratio with the *RFP*-labeled strain SCADH1R1A. Fifty microliters containing approximately 5 × 107 cells of the mixed suspensions was orally applied to the mice (five mice per strain pair). After 24 h and on the following days, the feces of the mice were collected and homogenized in 0.5 ml sterile water. A dilution series was prepared and spread on YPD plates containing 50 μg/ml chloramphenicol. The proportions of reference and test strains in the inoculum and in the populations recovered on each following day from the feces were determined as described for the *in vitro* competition experiments.

**RESULTS**

**Growth of peptide transporter mutants on peptides as the sole nitrogen source.** To investigate the functions of PTR2 and PTR22 in nitrogen utilization by *C. albicans*, we constructed mutants of the wild-type strain SC5314 lacking either one or both of these genes. The absence of the dipeptide/tripeptide transporters did not affect the ability of the strains to grow when BSA, which is extracellularly degraded to smaller peptides by secreted aspartic proteases, was the sole nitrogen source in the medium (data not shown). Even the ptr2Δ ptr22Δ double mutants grew as well as the wild type in YCB-BSA medium, in contrast to opt1Δ opt2Δ opt3Δ...
opt4Δ opt5Δ quintuple mutants lacking the five major oligopeptide transporters, which were unable to grow under these conditions. This result demonstrated that Ptr2 and PTR22 are not required for the efficient utilization of peptides produced by the proteolytic degradation of BSA.

We then tested the growth of the mutants on two specific peptides, the dipeptide Arg-Lys (RK) and the tripeptide Leu-Trp-Leu (LWL). In a medium containing 1 mg/ml RK as the sole nitrogen source, the \( \text{ptr2} / H9004 \) mutants grew as well as the wild-type parental strain, whereas the growth of the \( \text{ptr22} / H9004 \) mutants was delayed (Fig. 1A and B). The \( \text{ptr2} / H9004 \text{ptr22} / H9004 \) double mutants behaved like the \( \text{ptr2} / H9004 \) single mutants, indicating that PTR22 was required for the efficient uptake of this dipeptide into the cells under the experimental conditions used. Interestingly, mutants lacking the oligopeptide transporters Opt1 to Opt5 also showed delayed growth, suggesting that one or more of these oligopeptide transporters also contribute to the utilization of the dipeptide RK. To investigate this further, we generated mutants lacking \( \text{PTR2} \) and \( \text{PTR22} \), as well as the oligopeptide transporters \( \text{OPT1} \) to \( \text{OPT5} \). Indeed, these septuple mutants had lost the ability to grow on the dipeptide RK, confirming that both types of peptide transporters can mediate the uptake of this peptide into the cells (Fig. 1A and B). When the concentration of the peptide in the growth medium was lowered from 1 mg/ml to 0.25 mg/ml, the growth of the wild-type strain was also reduced. Under these conditions, a growth defect was observed only for mutants lacking \( \text{PTR22} \), whereas the oligopep-
tide transporters OPT1 to OPT5 were not required, indicating that Ptr22 is a more efficient transporter of the dipeptide RK that enables growth at limiting concentrations of this nitrogen source (Fig. 1C).

When the tripeptide LWL served as a nitrogen source, the growth of the mutants lacking PTR2 and PTR22 was comparable to that of the wild type, whereas the opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ mutants exhibited a growth defect, indicating that oligopeptide transporters are sufficient to mediate the uptake of this tripeptide (Fig. 2). During prolonged incubation in the presence of a lower concentration of LWL, mutants lacking the dipeptide/tripeptide transporters Ptr2 and Ptr22 in addition to the oligopeptide transporters Opt1 to Opt5 exhibited a more severe growth defect than the mutants lacking only Opt1 to Opt5, demonstrating that the tripeptide LWL can also be taken up by Ptr2 and/or Ptr22 (Fig. 2B).

Transport of different di- and tripeptides by specific transporters. To investigate the abilities of individual peptide transporters to mediate the uptake of specific di- and tripeptides, we expressed each of the deleted peptide transporter genes under the control of the strong, constitutive ADH1 promoter in the opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ septuple mutants and assessed the abilities of the strains to grow on specific peptides. Control experiments demonstrated that the septuple mutants grew as well as the wild type when any of the 20 standard L-amino acids served as the sole nitrogen source (data not shown). While several oligopeptide transporters (especially Opt1) rescued the growth defect of the septuple mutants in YCB-BSA medium, forced expression of PTR2 or PTR22 did not confer the ability to grow under these conditions, confirming that Ptr2 and Ptr22 do not mediate an efficient uptake of peptides produced by proteolytic digestion of BSA (Fig. 3).
FIG 4 Growth of the wild-type strain SC5314, opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ ptr2Δ ptr22Δ septuple mutants, and derivatives expressing specific peptide transporters from the ADH1 promoter on various dipeptides as the sole nitrogen source. Overnight cultures of the strains were washed 2 times in water, diluted 10^-2 in YCB medium containing 1 mg/ml of the indicated peptides as the sole nitrogen source, and incubated at 30°C. The optical densities of the cultures were determined at the indicated times. Shown are the results from one experiment performed with two independent series of strains, and similar results were obtained in an independent repeat experiment.
the constitutive expressing one of the two dipeptide/tripeptide transporters from mutants on all dipeptides tested (Fig. 4). In most cases, strains PTR2 or PTR22 did not differ in their abilities to take up the dipeptides RK, AT, KL, LS, and HL (as well as AL and AI [data not shown]). However, some differences in the substrate preferences of the two transporters also became evident from these experiments, because cells expressing PTR22 grew better than cells expressing PTR2 on the dipeptide LW (and KP [data not shown]), while the opposite was the case when the dipeptides LL and MM served as the nitrogen source. OPT1 was the only member of the oligopeptide transporters that enabled growth on a dipeptide, and among the tested dipeptides, only RK was taken up by Opt1.

We tested three different tripeptides to serve as substrates for the various peptide transporters (Fig. 5). LWL was efficiently utilized only by cells expressing OPT1 or PTR22, whereas AAP also allowed growth of cells expressing PTR2. KFK was a poorer nitrogen source that required longer incubation than the other tripeptides to yield high cell densities, but it was a substrate for several additional oligopeptide transporters (mainly Opt2 to Opt4) besides Opt1 and Ptr22. Altogether, these results indicate that, of the two dipeptide/tripeptide transporters, Ptr22 may have a somewhat broader substrate spectrum than Ptr2. In addition, Opt1 appears to be a highly flexible oligopeptide transporter that utilizes not only oligopeptides of more than 3 amino acids (6, 21), but also a variety of tripeptides, and even dipeptides.

**In vivo fitness of C. albicans peptide transporter mutants.** The ability of *C. albicans* to utilize peptides as an alternative nitrogen source may enhance growth in specific host niches. To investigate whether peptides are an important nutrient when *C. albicans* grows in the mammalian intestine, we performed *in vivo* competition experiments with the *opt1Δ* *opt2Δ* *opt3Δ* *opt4Δ* *opt5Δ* *ptr2Δ* *ptr22Δ* septuple mutants and an *RFP*-expressing derivative of the wild-type strain SC5314 (strain SCADH1R1A) in a mouse model of gastrointestinal colonization that mimics the natural habitat of *C. albicans* (22, 23). This approach enabled us to determine the proportions of the mutants in mixed populations with the marked wild-type strain over time by determining the numbers of red and white colonies after plating (24) (see Materials and Methods). Cocultures of strain SCADH1R1A with the unmarked wild-type strain SC5314 were included as controls.

To examine whether the peptide transporter mutants might display a nonspecific growth defect after the multiple rounds of transformation that were necessary for their construction, we first determined their growth rates in SD medium, which contains ammonium as the sole nitrogen source, as well as in the rich medium YPD. One of the two independently constructed septuple mutants showed wild-type growth in both tested media (doubling times were 94.5 ± 0.6 min in YPD medium and 134.5 ± 1.5 min in SD medium for the wild-type strain SC5314, 95.7 ± 0.3 min and 131.7 ± 1.1 min for its marked derivative SCADH1R1A, and 95.0 ± 0.1 min and 134.1 ± 1.7 min for SCOPT12345PTR22M4A). However, the second mutant grew more slowly, especially in SD medium (doubling times in YPD and SD media were 97.3 ± 0.4 min and 148.2 ± 1.5 min, respectively, for SCOPT12345PTR22M4B). The *in vitro* growth defect of the B strain was also apparent in competition experiments with the labeled wild-type strain SCADH1R1A, whereas the A strain exhibited wild-type fitness (Fig. 6A). These control experiments demonstrated that the B mutant had a growth defect that was not related to the absence of the peptide transporters. Therefore, only the wild-type strain SC5314, indicating that the endogenous PTR2 and PTR22 genes were not sufficiently expressed to allow efficient growth under the experimental conditions used. Ptr2 and Ptr22 did not differ in their abilities to take up the dipeptides RK, AT, KL, LS, and HL (as well as AI and AL [data not shown]).
the A mutant was tested in the in vivo competition experiments. As can be seen in Fig. 6B, the proportion of the mutant SCOPT12345PTR222M4A and -B in competition experiments with the genetically marked wild-type strain SCADH1R1A. Cocultures were performed in YPD (left) and SD (right) media as described in Materials and Methods. The results are the means and standard deviations of six independent cocultures for each test strain. Significant differences from the wild-type strain SC5314 (P < 0.05) are marked with asterisks. (B) Relative in vivo fitness of the wild-type strain SC5314 (left) and the opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ ptr2Δ ptr22Δ septuple mutant SCOPT12345PTR222M4A (right) in competition with the genetically marked wild-type strain SCADH1R1A. In vivo competition experiments were performed in orally infected mice as described in Materials and Methods. The proportions of the test strains in the inoculum (time point 0; empty circles) and in samples recovered at the indicated times from the feces of the animals are given. Each individual mouse in a group is represented by a different symbol. The horizontal bars represent the mean values for each group of mice and day. Data were omitted when too few cells were recovered for a meaningful evaluation. One of the five mice infected with the SCOPT12345PTR222M4A/SCADH1R1A mixture died on day 1 and could not be used for the evaluation.

**DISCUSSION**

Proteins of the OPT family are usually described as tetrapeptide/pentapeptide transporters, while di- and tripeptides are mainly transported by members of the PTR family (4). This functional distinction is supported by the findings that heterologous expression of CaOPT1 in an S. cerevisiae ptr2 mutant did not restore growth on the dipeptide KL or the tripeptide KLG and that expression of various Opt proteins from Arabidopsis thaliana also did not support growth of S. cerevisiae ptr2 mutants on di- and tripeptides (21, 25). However, work in recent years has demonstrated that oligopeptide transporters of the OPT family can also transport peptides longer than 5 amino acids (5, 6). In addition, Opt1 from C. albicans and Isp4 from Schizosaccharomyces pombe have been shown to exhibit some tripeptide transport activity, because uptake of the radiolabeled tetrapeptide KLGL was competitively inhibited to a limited extent by the tripeptide KLG (13, 21). The results of our present study provide further support for such an extended substrate spectrum of this family of transporters, as several C. albicans oligopeptide transporters also enabled growth on the tripeptide KFK, and CaOpt1 mediated the uptake of additional tripeptides (Fig. 5). In contrast, oligopeptide transporters had not been implicated in the transport of dipeptides until now. Our finding that CaOpt1 could also utilize a dipeptide as the substrate, therefore, reveals an intriguing new facet of the function of these transporters. Of the limited number of tested dipeptides, RK was the only one that was transported by CaOpt1, and CaOpt1 was the only member of the OPT family that mediated its uptake, indicating that CaOpt1 is an especially versatile representative of the oligopeptide transporters.
Investigation of the roles and importance of individual peptide transporters of *C. albicans* was complicated by the fact that they are encoded by gene families whose members have partially redundant functions. In addition, expression of at least some of the PTR and OPT genes depends on the Ssy1-Ptr3-Ssy5 (SPS) sensor and is induced by micromolar concentrations of extracellular amino acids (26), which had to be excluded from the medium in our growth assays on peptides. The phenotypes of mutants lacking a specific transporter would therefore not be expected to differ from that of the wild type under conditions in which the gene is not expressed. These obstacles were overcome by the construction of mutants lacking the two dipeptide/tripeptide transporters, as well as the five major oligopeptide transporters, which were unable to grow on any of the peptides tested, and the subsequent expression of individual peptide transporters from a constitutive promoter. Phenotypic analysis of the resulting strains clearly showed that both Ptr2 and Ptr22 promoted growth on the examined dipeptides, albeit with somewhat different efficiencies. It should be noted that, although the genes were expressed from the same promoter, the protein levels of the two transporters may not be the same, which may contribute to some of the growth differences. Overall, Ptr22 seems to have a broader substrate spectrum than Ptr2, because it also allowed growth on all three tested tripeptides, whereas Ptr2 efficiently transported only one of them. Forced expression of PTR2 or PTR22 considerably improved growth of the cells compared to that of the wild-type strain SC5314 on most of the dipeptides, implying that the endogenous copies of these genes were not adequately expressed in the test medium to allow optimal growth. In fact, the wild-type strain SC5314 grew well only on those peptides that were also taken up by Opt1 (the dipeptide RK and all tested tripeptides), indicating that OPT1 was sufficiently expressed under the experimental conditions to enable growth on its substrates.

The inability of the opt1Δ opt2Δ opt3Δ opt4Δ opt5Δ ptr2Δ ptr22Δ septuple mutants to grow on peptides allowed us to investigate whether peptides are an important nitrogen source for *C. albicans* during the colonization of the mammalian gastrointestinal tract. The mutant did not exhibit a competitive fitness defect in a mouse model of gastrointestinal colonization. This result indicates that *C. albicans* finds sufficient alternative nitrogen sources in this environment and does not depend on peptides provided by the host and its microbiota or by its own secreted proteases for optimal growth. Similarly, it was recently shown that deletion of the whole oligopeptide transporter family did not reduce the virulence of the mold *Aspergillus fumigatus* in a murine model of pulmonary aspergillosis (3). This reflects the nutritional versatility of these fungi, which can utilize many different nitrogen sources. Nevertheless, one must keep in mind that the nutritional situation in the digestive tract of mice is certainly different from that in humans, and it will also differ between individuals and change with alterations in the diet or age. It is quite possible that at certain times or in other host niches encountered during an infection peptides are a more important nutrient source, and the evolution of an arsenal of dedicated transporters may have contributed to the success of *C. albicans* as a commensal and pathogen.

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