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Regular Articles

Versatility of putative aromatic aminotransferases from *Candida albicans*

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ABSTRACT

Amino acids constitute the key sources of nitrogen for growth of *Candida albicans*. In order to survive inside the host in different and rapidly changing environments, this fungus must be able to adapt *via* its expression of genes for amino acid metabolism. We analysed the *ARO8*, *ARO9*, *YER152C*, and *BNA3* genes with regards to their role in the nutritional flexibility of *C. albicans*. *Ca*Aro8p is undoubtedly the most versatile enzyme among the aminotransferases investigated. It is involved in the catabolism of histidine, lysine, and aromatic amino acids as well as in L-Lys, Phe and Tyr biosynthesis. *Ca*Aro9p participates in the catabolism of aromatic amino acids and lysine at high concentrations of these compounds, with no biosynthetic role. Conversely, the *Ca*Yer152Cp catalytic potential for aromatic amino acid catabolism observed *in vitro* appears to be of little importance *in vivo*. Neither biosynthetic nor catabolic roles of *Ca*Ban3p were observed for any proteinogenic amino acid. Finally, none of the analysed aminotransferases was solely responsible for the catabolism of a single particular amino acid or its biosynthesis.

1. Introduction

In recent years, the number of immunocompromised patients suffering from various infectious diseases, such as those with cancer who have been subjected to extensive chemotherapy and organ transplantation, has increased substantially (Miceli et al., 2011). In particular, invasive fungal infections continue to be a major cause of morbidity and mortality in immunocompromised or severely ill patients (Groll and Lumb, 2012). The main etiological factor of opportunistic fungal infections (63–70% of cases) is *Candida albicans* (López-Martínez, 2010).

During the course of infection, *C. albicans* cells face many different and rapidly changing host environments. The ability to use as many nutrient sources as possible is essential for adaptation to each of these microniches. Among different catabolic processes, the degradation of amino acids, necessary for their usage as a nitrogen source, has been demonstrated to be relevant for the survival of many microbial pathogens (Martinez and Ljungdahl, 2005). In the mammalian host infected by a pathogenic fungus, amino acids are present in a free form at low, millimolar concentrations in the plasma or urine (Tan and Gajra, 2006), or can be liberated from proteins and peptides upon the actions of fungal hydrolases. Their utilisation as a nitrogen source requires the presence of a respective aminotransferase in fungal pathogen cells (Fig. 1).

Human pathogenic fungi differ in their ability to use amino acids as a sole nitrogen source. *Histoplasma capsulatum* assimilates ammonium sulphate, aspartic acid and asparagine, although cannot grow with methionine and gives only poor growth with cysteine, lysine, histidine, tryptophan and phenylalanine. *Sporotrichum schenckii* grows poorly or not at all with aspartic acid, lysine or histidine (Gilardi, 1965). *C. albicans* is able to use each of the 20 proteinogenic amino acids when grown in minimal medium with glucose as a carbon source (Brunke et al., 2014). Conversely, L-cysteine and L-lysine cannot support the growth of *Candida glabrata* or the baker's yeast, *Saccharomyces cerevisiae*, and only *C. glabrata* and *C. albicans* are able to grow on L-histidine. Specifically, the aromatic amino acid aminotransferase *Cg*Aro8p has been recently identified as an enzyme participating in histidine degradation in *C. glabrata* (Brunke et al., 2014). α-Aminoadipate, an intermediate of the lysine biosynthetic pathway in fungi, when used as the sole nitrogen source, was found as growth inhibitory and toxic to *S. cerevisiae,* whereas the human pathogenic fungi, *C. albicans*, *C. neoformans* and *A. fumigatus,* are able to use both lysine and α-aminoadipate as the sole nitrogen source. This unique metabolic property was proposed as a criterium for the identification of these pathogens (Ye et al., 1991).

In fungi, the catabolism of amino acids is complicated by the fact that some species can use them as the sole carbon source and some other as the sole nitrogen source. There are also fungal species that can use amino acids as both carbon and nitrogen source (Kinzel et al., 1983). Even within *Candida* species of the CUG clade, there is a great diversity in that ability. *C. albicans*, *C. tropicalis* and *C. lusitaniae* utilize amino acids (in the form of Casamino Acids in YNB) effectively as the carbon source, whereas the growth of *C. dubliniensis* or *C. parapsilosis*

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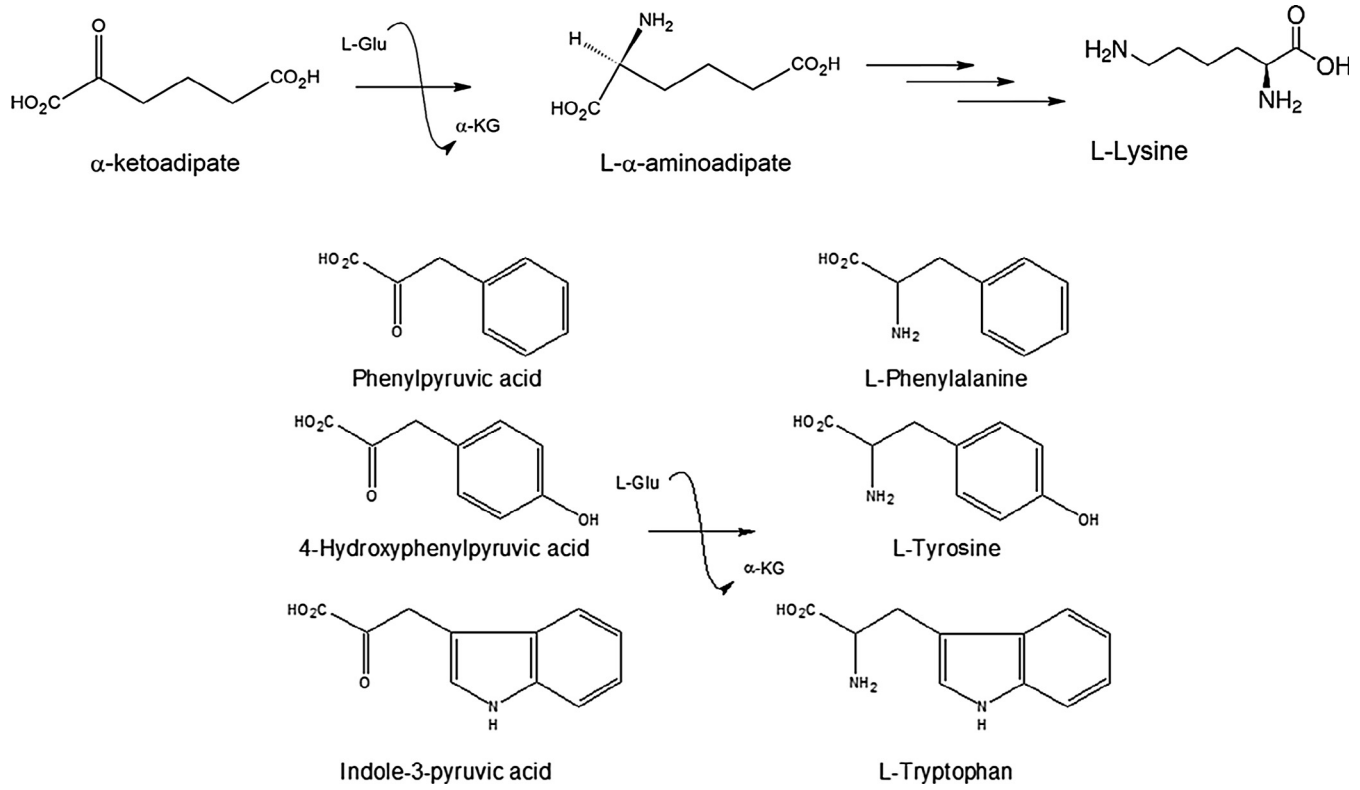

Fig. 1. The schematic overview of reactions performed by aromatic and aminoadipate transaminases for L-lysine and aromatic amino acids biosynthesis. The catabolic process is a reversal of the biosynthetic reaction.

under these conditions is much slower (Priest and Lorenz, 2015).

Enzymes catalysing particular steps of amino acid biosynthesis in fungi are considered as potential targets for antifungal therapy, especially those that are absent in mammalian hosts (Jastrzębowska and Gabriel, 2015; Kingsbury et al., 2004; Schöbel et al., 2010). Some inhibitors of such enzymes have already been identified and shown to exhibit antifungal activity (Yamaki et al., 1990; Aoki, 1996). Moderate antifungal activity was also demonstrated for compounds that are structural analogues of substrates or products of the enzymes involved in L-lysine biosynthesis pathway in *C. albicans* (Gabriel et al., 2013; Milewska et al., 2012).

In our efforts to identify an aminotransferase participating in L-Lys biosynthesis and degradation, we have focused our attention on four candidate genes: *CaARO8, CaARO9, CaYER152C*, and *BNA3*. In this work we present the results of our studies on the actual function of these genes and their products.

2. Materials and methods

2.1. Strains and growth conditions

C. albicans strains used in this investigation are listed in Table 1. The strains were routinely grown in YPG liquid medium (1% yeast extract, 1% peptone, 2% glucose) in a shaking incubator. For growth on solid media, 1.5% agar was added to the medium (YPG agar medium).

Escherichia coli TOP 10F′ strain from Invitrogen was used in all cloning procedures. *E. coli* Rosetta (DE3) pLysS strain from Novagen was used for the overproduction of wild-type *Ca*Aro8p and *Ca*Yer152Cp, and the *E. coli* BL21 Star (DE3) strain from Invitrogen was used for the overproduction of *Ca*Aro9p. *E. coli* strains were cultured at 37 °C on Luria-Bertani (LB) solid medium (1.0% (w/v) NaCl, 1.0% (w/v) tryptone, 0.5% yeast extract, and 1.5% (w/v) agar) and LB liquid medium supplemented with 100 μg mL^{-1} ampicillin and/or 34 μg mL^{-1} chloramphenicol, when required.

2.2. Mutant strain construction

C. albicans SC5314 genomic DNA was isolated using a method described previously (Reuss et al., 2004). Isolation of plasmid DNA was carried out according to the protocol of the NucleoSpin Plasmid kit (Macherey-Nagel). DNA fragments were isolated from agarose gels following the standard procedure of the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel). DNA digestion with the use of restriction enzymes was performed according to the enzyme supplier's instructions.

The pSFS5 plasmid (Sasse et al., 2011) was used for the construction of deletion cassettes. Restriction enzymes and DNA polymerase were purchased from New England Biolabs. The gene knock-out cassettes were prepared by amplification of upstream and downstream regions of each particular gene: *ARO8* (orf19.2098), *ARO9* (orf19.1237), *YER152C* (orf19.1180), and *BNA3,* present in two copies as *BNA31* (orf19.5809) and *BNA32* (orf19.1589.1), from *C. albicans* SC5314 genomic DNA followed by SacI-SacII and XhoI-ApaI digestion. The primers used in this study are described in Table 2. The amplified regions were cloned on both sides of the *SAT1* flipper cassette in the pSFS5 plasmid, resulting in pARO8M1, pARO9M1, p1180M1, pBNA31M1, and pBNA32M1 plasmids. The knock-out cassettes were isolated by restriction digestion using SacI and ApaI enzymes. The entire genes were deleted using the *SAT1* flipping strategy (Reuss et al., 2004). The reintroduced strains were prepared in accordance with the above mentioned procedure. The complementation cassettes were generated using the appropriate primers as described in Table 2. The complete ORF and flanking sequences were cloned as SacI-SacII fragment and substituted for the upstream sequence in appropriate plasmids, generating pARO8K1, pARO9K1, p1180K1, pBNA31K1 and pBNA32K1. The SacI-ApaI regions were integrated into one of the alleles of homozygous mutants. Two independent mutants were constructed from their respective wild-type strain. The correct genomic integration of each construct and excision of the *SAT1* flipper cassette were confirmed by Southern hybridization with the upstream and downstream flanking sequences.

Table 1
C. albicans strains used in this study.

Strain(s)	Relevant characteristic or genotype	References
SC5314	Wild-type reference strain	Gillum et al. (1984)
SCARO8M2A and -B	*ARO8/aro8Δ::FRT*	This study
SCΔ*aro8*A and -B	*aro8Δ::FRT/aro8Δ:: FTR*	This study
SCARO8K2A and -B	*aro8Δ::FTR/ARO8::FTR*	This study
SCARO9M2A and -B	*ARO9/aro9Δ::FRT*	This study
SCΔ*aro9*A and -B	*aro9Δ::FRT/aro9Δ:: FTR*	This study
SCARO9K2A and -B	*aro9Δ::FTR/ARO9::FTR*	This study
SCYER152CM2A and -B	*YER125C/yer152CΔ::FRT*	This study
SCΔ*yer152C*A and -B	*yer152CΔ::FRT/yer152CΔ:: FTR*	This study
SCYER152CK2A and -B	*yer152CΔ::FTR/YER152C::FTR*	This study
SCBNA31M2A and -B	*BNA31/bna31Δ::FRT*	This study
SCΔ*bna31*A and -B	*bna31Δ::FRT/bna31Δ:: FTR*	This study
SCBNA31K2B	*bna31Δ::FTR/BNA31::FTR*	This study
SCBNA32M2A and -B	*BNA32/bna32Δ::FRT*	This study
SC Δ*bna32*A and -B	*bna32Δ::FRT/bna32Δ:: FTR*	This study
SCΔ*aro8* ARO9M2A and -B	*aro8Δ, ARO9/aro9Δ::FRT*	This study
SCΔ*aro8* Δ*aro9*A and -B	*aro8Δ, aro9Δ::FRT/aro9Δ:: FTR*	This study
SCΔ*aro8* ARO9K2A and -B	*aro8Δ, aro9Δ::FTR/ARO9::FTR*	This study
SC*ARO8*K2 Δ*aro9*A and -B	*aro8Δ::FTR/ARO8::FTR, aro9Δ::FRT/aro9Δ:: FTR*	This study
SC*ARO8*K2 ARO9K2A and -B	*aro8Δ::FTR/ARO8::FTR, aro8Δ, aro9Δ::FTR/ARO9::FTR*	This study
SCΔ*aro8* YER152CM2A and -B	*aro8Δ, YER152C/yer152CΔ::FRT*	This study
SCΔ*aro8* Δ*yer152C*A and -B	*aro8Δ, yer152CΔ::FRT/yer152CΔ:: FTR*	This study
SCΔ*aro8* YER152CK2A and -B	*aro8Δ, yer152CΔ::FTR/YER152C::FTR*	This study
SC*ARO8*K2 Δ*yer152C*A	*aro8Δ::FTR/ARO8::FTR, yer152CΔ::FRT/yer152CΔ:: FTR*	This study
SC*ARO8*K2 YER152CK2A and -B	*aro8Δ::FTR/ARO8::FTR, aro8Δ, yer152CΔ::FTR/YER152C::FTR*	This study
SCΔ*aro8* BNA31M2A and -B	*aro8Δ, BNA31/bna31Δ::FRT*	This study
SCΔ*aro8* Δ*bna31*A and -B	*aro8Δ, bna31Δ::FRT/bna31Δ:: FTR*	This study
SCΔ*aro8* BNA31K2A and -B	*aro8Δ, bna31Δ::FTR/BNA31::FTR*	This study
SCΔaro8 BNA31K2A and -B	*aro8Δ, BNA31::FTR/bna31Δ::FTR*	This study
SC*ARO8*K2 Δ*bna31*A	*aro8Δ::FTR/ARO8::FTR, bna31Δ::FRT/bna31Δ:: FTR*	This study
SC*ARO8*K2 BNA31K2A	*aro8Δ::FTR/ARO8::FTR, aro8Δ, bna3Δ::FTR/BNA3::FTR*	This study
SCΔ*aro8* Δ*bna31* BNA32M2A	*aro8Δ, bna31Δ, BNA32/bna32Δ::FRT*	This study
SCΔ*aro8* Δ*bna31* Δ*bna32*A	*aro8Δ, bna31Δ, bna32Δ::FRT/bna32Δ:: FTR*	This study

2.3. Cloning of ARO8, ARO9, and YER152C genes

Cloning of *ARO8* and *YER152C* was performed as previously described (Rząd and Gabriel, 2015) with the use of the pET Directional TOPO Expression Kit and the pET101/D-TOPO plasmid (Invitrogen). The same kit was used for the construction of recombinant expression plasmids encoding *ARO9*. The fragment of the *ARO9* gene was amplified from the *C. albicans* SC5314 genomic DNA by PCR. The primers used in the amplification were: ARO9.f 5′-CACCATGTCTGATCCTACTCATTTAATTTCTAAG-3′ and ARO9.r 5′-CTTAGGCTAGCTTTTTAAAACTCTAACCCATTAC-3′. Primers were

Table 2
Primers used in the construction of knock-out cassettes.

Name	Sequence	Destination
ARO8.01	ttt**GAGCTC**caccacttaaaaactgataagaaa	Amplification of *ARO8* (orf19.2098) upstream region
ARO8.02	gtat**CCGCGG**tcattatggcagtagaaa	
ARO8.03	gtaa**CTCGAG**cttgtgtgtgtcaacaagt	Amplification of *ARO8* (orf19.2098) downstream region
ARO8.04	caaa**GGGCCC**gtcgtaaagatttatctg	
ARO8.05	tgga**CCGCGG**aaagatttatctggtaatg	With ARO8.01 amplification of *ARO8* (orf19.1237) gene with upstream and downstream regions
ARO9.01	taaa**GAGCTC**acctatttagatgatgatgatg	Amplification of *ARO9* (orf19.1237) upstream region
ARO9.02	atat**CCGCGG**tgtaacaaagtttcctaagt	
ARO9.03	tggg**CTCGAG**ttttaaaaagctagcc	Amplification of *ARO9* (orf19.1237) downstream region
ARO9.07	cttattgaattcagta**GGGCCC**ttatcacc	
ARO9.09	gtaa**CCGCGG**agaaattgaatgacattgga	Amplification of *ARO9* (orf19.1237) gene with upstream and downstream regions
ARO9.11	aatg**GAGCTC**tttaatgaatctcgtgaaagt	
1180.01	aatg**GAGCTC**ttttatatggatgttgtttt	Amplification of YER152C (orf19.1180) upstream region
1180.02	ttta**CCGCGG**agtaaaaagttcatttc	
1180.03	actt**CTCGAG**tagttagtaaacagttttta	Amplification of YER152C (orf19.1180) downstream region
1180.04	aata**GGGCCC**caaaaatatatgatatgg	
1180.05	aata**CCGCGG**caaaaatatatgatatgg	With 1180.01 amplification of YER152C (orf19.1180) gene with upstream and downstream regions
5809.01	cctt**GAGCTC**tggaagaaggttgtaattc	Amplification of *BNA31* (orf19.5809) upstream region
5809.02	ggaaa**CCGCGG**tcttaacataaaagtgtaag	
5809.03	taaaa**CTCGAG**cgttgtcagagtagtcg	Amplification of *BNA31* (orf19.5809) downstream region
5809.04	cta**GGGCCC**gagttttagtacaaatacc	
5809.06	cta**CCGCGG**gagttttagtacaaatacc	With 5809.01 amplification of *BNA31* (orf19.5809) gene with upstream and downstream regions
1589.02	aatt**CCGCGG**atgatggtgagttgaaga	With 5809.01 amplification of *BNA32* (orf19.1589.1) upstream region
1589.03	gttgtcagagtagtcg**CTCGAG**catat	Amplification of *BNA32* (orf19.1589.1) downstream region
1589.04	ttacat**GGGCCC**attgaaataaaccaactctg	
1589.06	ttacat**CCGCGG**attgaaataaaccaactctg	With 1589.01 amplification of *BNA32* (orf19.1589.1) gene with upstream and downstream regions

Bolded sequences are specific regions recognized by restriction enzymes: *Sac*I (GAGCTC), *Sac*II (CCGCGG), *Xho*I (CTCGAG), *Apa*I (GGGCCC).

designed according to manufacturer instruction. The PCR products (1572 bp) were purified from an agarose gel and cloned directionally into the pET101/D-TOPO vector, thus yielding the recombinant expression plasmid pET101/D-TOPO + *ARO9* (7334 bp). The identity of the plasmid was confirmed by restriction analysis and DNA sequencing.

2.4. Overexpression of CaARO9 and purification of the gene product

Construction of the overexpression plasmid containing the *CaARO9* gene, conditions for *E. coli* transformation, overexpression of Ca*ARO9*, and isolation of Aro9p were the same as described previously for the *CaARO8* and *CaYER152* genes and their products (Rząd and Gabriel, 2015), except that *E. coli* BL21 Star (DE3) cells were used instead of *E. coli* Rosetta (DE3) pLysS as a host for the heterologous expression. Samples of purified protein were then held frozen (−20 °C) in 50 mM phosphate buffer pH 7.5, with pyridoxal 5′-phosphate (PLP, at slight molar excess with respect to the proteins) and 10% glycerol.

2.5. Growth in liquid medium with different nitrogen sources

C. albicans cells were grown overnight at 30 °C in YPG medium. Cells from the overnight culture were washed twice with PBS and suspended to 2×10^5 cells mL^{-1} in YNB medium w/o amino acids with 2% glucose, 100 mM phosphate buffer pH 5.8. Aliquots of 100 μL were used to inoculate the microtiter wells containing 100 μL of YNB medium w/o amino acids, containing a particular amino acid, aminoadypic acid, or ammonium sulphate to a final concentration of 10 mM, 5 mM, and 0.25%, respectively. If necessary, the aminotransferase inhibitor (aminooxy)acetate (AOA) was added (10 mM). The cell suspensions were cultivated for 48 h at 30 °C and the cell density was measured at time intervals spectrophotometrically (λ = 531 nm) with a microplate reader (Victor^3 Perkin Elmer). Additional experiments were performed for L-Lys, L-His and L-Tyr at different amino acids concentration. Serial 2-fold dilution of that amino acids were made in 100 μL YNB medium w/o nitrogen source with 2% glucose, 100 mM phosphate buffer pH 5.8 in 96-well plate. Aliquots of 100 μL containing 2×10^5 *C. albicans* cells mL^{-1} were used to inoculate microtiter wells containing 100 μL of serial dilutions of selected amino acid.

2.6. Determination of aminotransferase activity

The aminotransferase activity of isolated enzymatic proteins was determined as previously described (Rząd and Gabriel, 2015). In all assays, 2.5 μg of the purified enzyme was used. Briefly, in assays of aromatic aminotransferase activity, the product formation was measured spectrophotometrically at λ = 320 nm for phenylpyruvate (PhP), ε = 17 700 $dm^3\,cm^{-1}\,mol^{-1}$; λ = 331 nm for 3-indolepyruvate, ε = 19 900 $dm^3\,cm^{-1}\,mol^{-1}$; and λ = 338 nm for 4-hydroxyphenylpyruvate (4-hydroxyPhP), ε = 9 300 $dm^3\,cm^{-1}\,mol^{-1}$. The rate of Phe or Tyr formation was estimated by quantification of the decrease in the PhP or 4-hydroxyPhP derived absorbance, respectively.

α-Aminoadipate aminotransferase activity was followed with the use of L-glutamate and 2-oxoadipate (2-OA) as substrates. Estimation of the quantity of 2-oxoglutarate (2-OG) formed was performed using an L-glutamic dehydrogenase assay in which formation of NAD^+ was measured at λ = 340 nm; ε = 6 200 $dm^3\,cm^{-1}\,mol^{-1}$.

Catabolic reactions of α-aminoadipate or L-histidine degradation were analysed using the same method, in reaction mixtures containing α-aminoadipate/2-oxoglutarate or L-histidine/2-oxoglutarate as substrates, respectively. Formation of L-glutamate was measured spectrophotometrically with the use of the L-Glutamate Assay Kit (Sigma), according to the manufacturer's instructions.

The effect of (aminooxy)acetate (AOA) on aminotransferase activity was determined by measuring the enzyme activity in standard assay mixtures containing various concentrations of Phe, 2-OG (10 mM), and different concentrations of AOA. The level of PhP formation was estimated spectrophotometrically as mentioned above.

3. Results

Our previous studies showed that the *ARO8* gene of *C. albicans* encodes a transaminase, exhibiting aromatic and aminoadipate aminotransferase activity (Rząd and Gabriel, 2015). It has also been known that in *S. cerevisiae* aromatic aminotransferase exists in two isoforms encoded by the *ARO8* and *ARO9* genes (Iraqui et al., 1998). Apart from these two genes, the phylogenetic analysis of aromatic transaminases in *S. cerevisiae* genome performed by Hébert et al. (2011) resulted in identification of the *YER152C* ORF as a gene encoding putative aromatic amino acid aminotransferase. On the other hand, the putative aminoadipate aminotransferase activity has also been suggested for the products of *ARO8*, *BNA3* and *YER152C* from *S. cerevisiae* by King et al. (2009).

A BLAST search, using the nucleotide sequence of the respective ORFs from the *Saccharomyces* Genome Database (www.yeastgenome.org) performed on the *Candida* Genome Database (www.candidagenome.org) revealed orf19.1237, (named *ARO9*), orf19.1180 (*YER152C*) and orf19.5809 or orf19.1589.1, both associated with *BNA3* (being present in two copies, *BNA31* and *BNA32*), highly homologous to their *S. cerevisiae* counterparts (55%, 64% and 69% of similarity, respectively). The nucleotide sequences of *CaARO9*, *CaYER152C* and *CaBNA3* exhibit 31%, 24%, 23% identity and 48%, 44%, 37% similarity to *CaARO8*, respectively.

3.1. Growth in minimal media with different nitrogen sources

To investigate the functions of the aminotransferases encoded by *ARO8*, *ARO9*, *YER152C*, and *BNA3* in nitrogen utilisation by *C. albicans*, deletion mutants of the SC 5314 strain lacking either one, two, or three of these genes were constructed and tested for their ability in using ammonium sulphate, proteinogenic amino acids, and intermediates of lysine biosynthesis and breakdown as the sole nitrogen source. The results data presented in Fig. 2 indicate that all mutant strains grew equally as well as the wild-type strain in minimal medium with ammonium sulphate as the sole nitrogen source. This result suggested that all mutants were able to synthesise all amino acids necessary for protein biosynthesis; *i.e.*, none of the already identified or putative aminotransferases encoded by *ARO8*, *ARO9*, *YER152C*, or *BNA3* is essential for biosynthesis of any of the proteinogenic amino acids. The majority of the mutants were also able to use each of the 19 proteinogenic amino acids as the sole nitrogen source to support their growth. However, differences in histidine, lysine, and tyrosine utilisation were observed between the mutants lacking the *ARO8* gene and the wild-type SC 5314 cells. The difference can be better seen in Fig. 3, in which the growth kinetics are presented. The growth rates of the all *ARO8*-deficient mutants in minimal media containing L-His or L-Lys as the sole nitrogen source were much lower than that of the wild-type strain. The respective difference was lower for L-Tyr utilisation, but still evident. Moreover, a single gene disruption of *ARO9*, *YER152C*, or *BNA3* resulted in no effect on *C. albicans* growth with L-His, L-Lys, or L-Tyr, thus indicating that none of these genes is essential for the catabolism of these amino acids.

Notably, all the mutants tested, including the *ARO8*-deficient mutants, demonstrated similar ability as that of the wild-type strain to use L-α-aminoadipate (AA) as the sole nitrogen source, whereas deletion of *ARO8* apparently affected growth on L-Lys (Fig. 4). AA is an intermediate in the L-lysine catabolic pathway, specifically functioning as a direct substrate for a putative aminotransferase (Fig. 1).

The growth ability of *ARO8*-deficient mutants could be to a large extent restored by supplementation of the minimal medium with L-Lys or L-Tyr in an amino acid concentration-dependent manner (62.3 ± 4.2% and 74.0 ± 4.9% restoration, in comparison with the wild-type cells at 4 mM L-Lys or L-Tyr, respectively), as shown in Fig. 5. Conversely, supplementation with L-His apparently did not restore growth of these mutants (only 19.9 ± 3.7% of that of SC5314 at 4 mM L-His). Moreover, the growth ability was not related to the concentration of the nitrogen source.

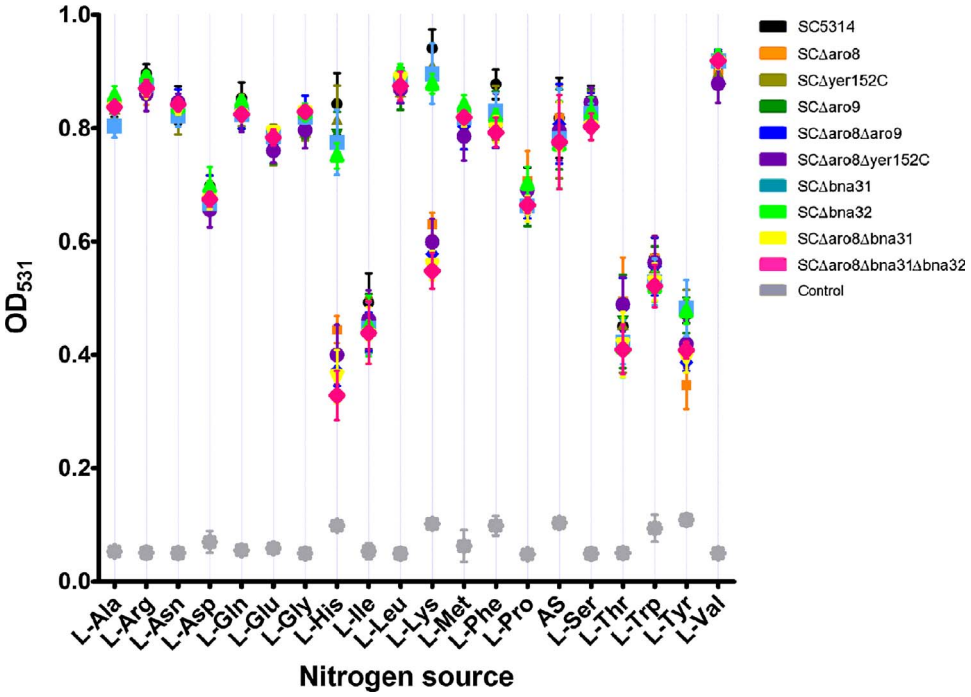

Fig. 2. Growth of *C. albicans* wild-type and deletion mutant strains in YNB media containing ammonium sulphate (AS) or particular amino acids as the sole nitrogen source. Optical density of cell suspensions and liquid media (negative control) was measured at 531 nm after incubation for 48 h at 30 °C. All data represent the means ± SD.

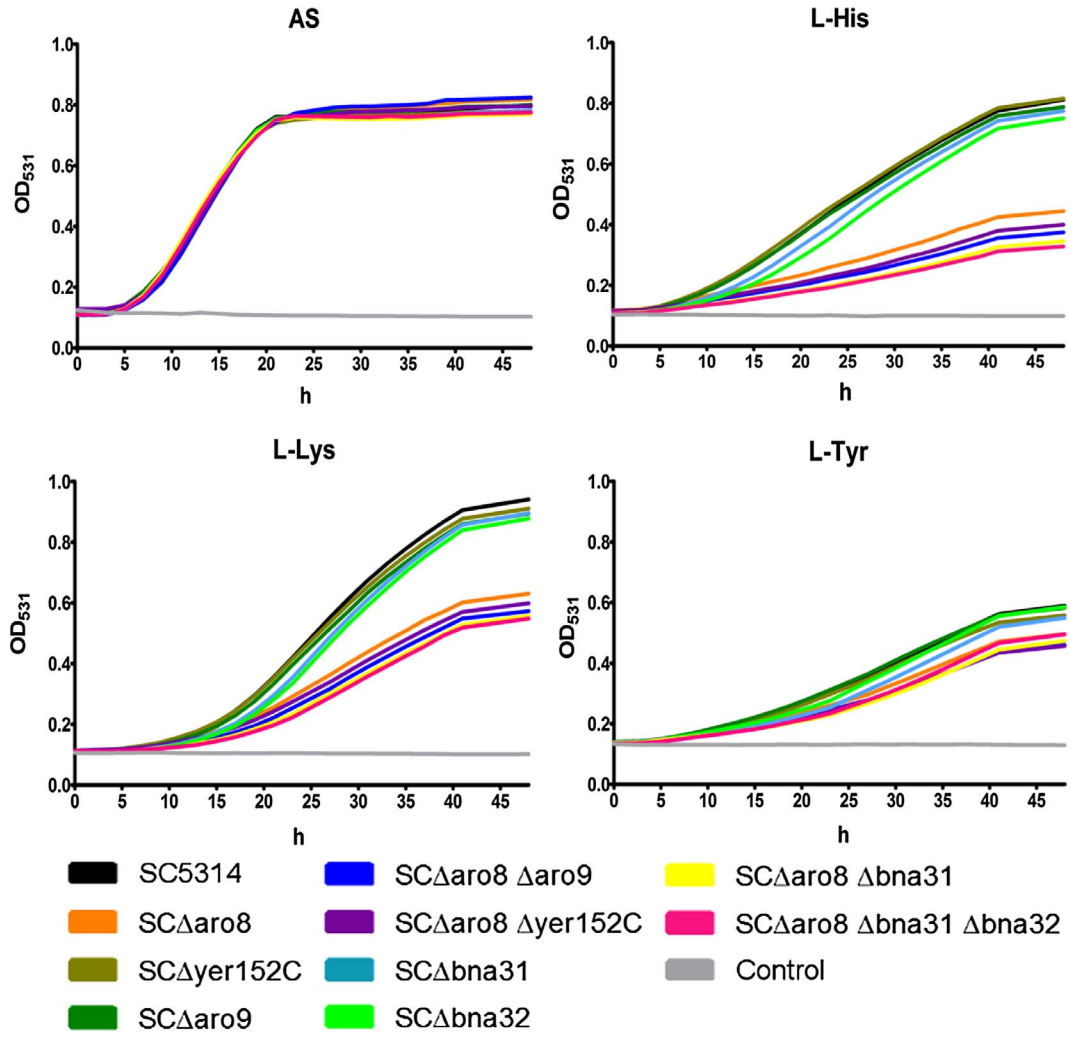

Fig. 3. Growth kinetics of *C. albicans* wild-type and deletion mutants in the YNB media containing either L-Lys, L-His, or L-Tyr as the sole nitrogen source (10 mM) or ammonium sulphate (AS). Cell density was measured at time intervals spectrophotometrically (λ = 531 nm). Optical density of liquid medium (YNB) serves as a negative control.

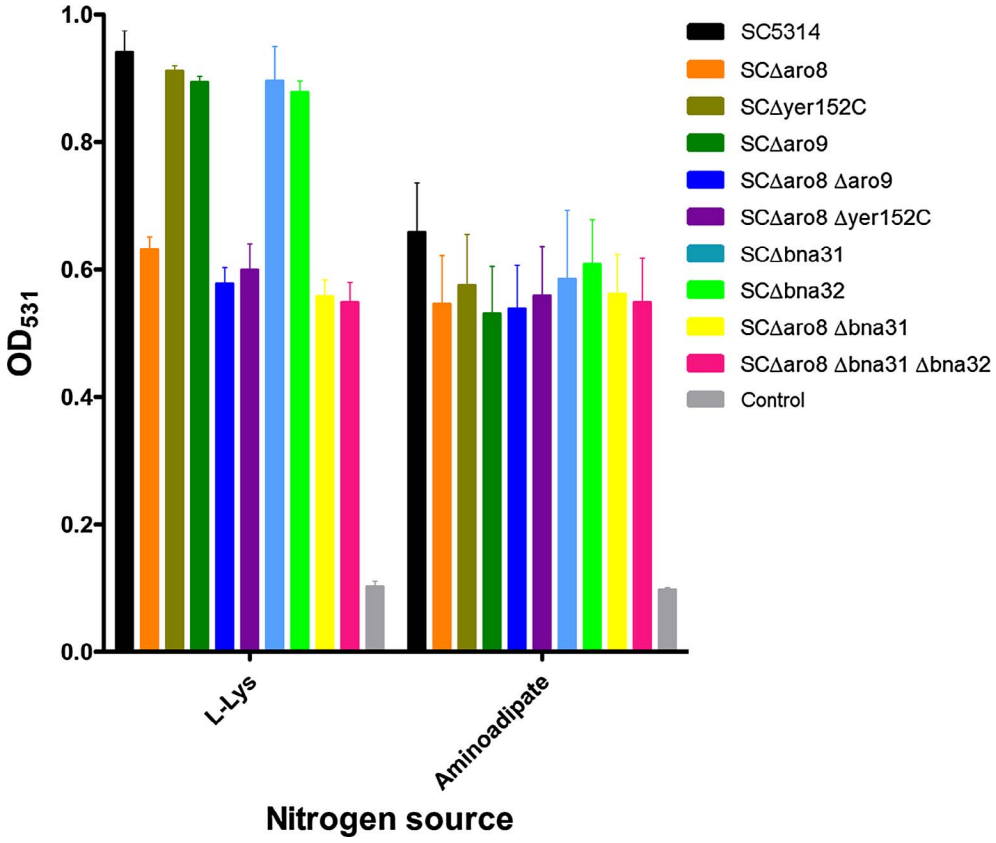

Fig. 4. Growth of *C. albicans* wild-type and deletion mutant strains in YNB media containing L-Lys or α-aminoadipate as the sole nitrogen source. Optical density of cell suspensions and liquid media (negative control) was measured at 531 nm after incubation for 48 h at 30 °C. All data represent the means ± SD.

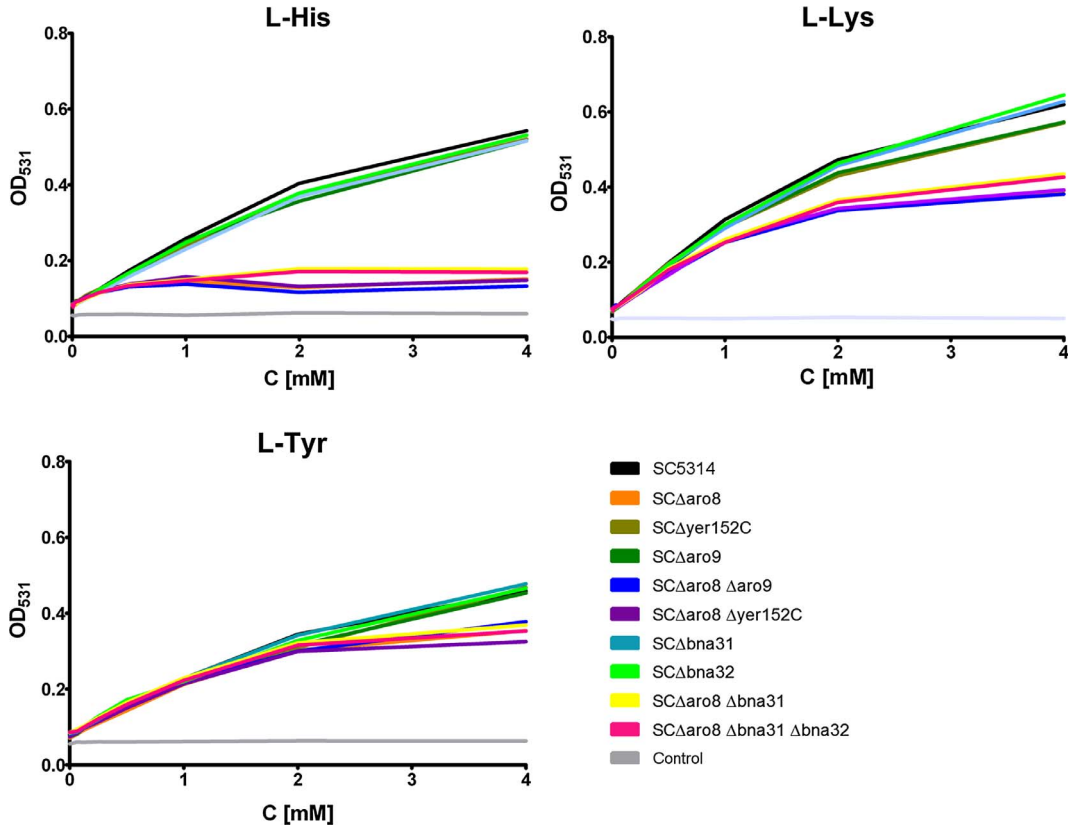

Fig. 5. Growth of *C. albicans* wild-type and deletion mutants on various concentrations of L-His, L-Lys, or L-Tyr supplementing the YNB media as the sole nitrogen source. Optical density of cell suspensions and liquid media (negative control) was measured at 531 nm after incubation for 48 h at 30 °C.

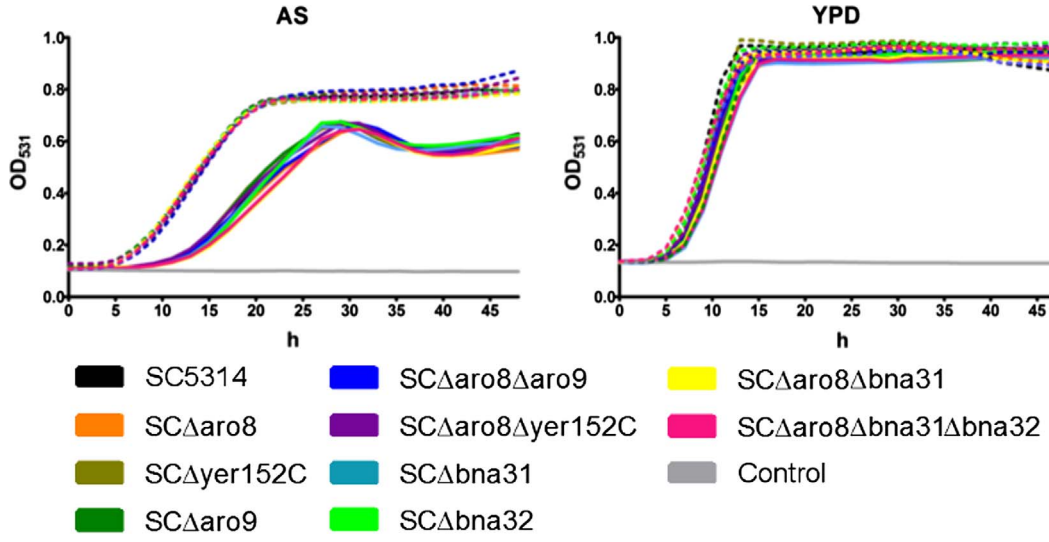

Fig. 6. Effect of (Aminooxy)acetate (AOA) on *C. albicans* wild-type and mutant growth in YNB with ammonium sulphate (AS) medium and YPD. Dashed lines, no AOA present; solid lines, 10 mM AOA. Optical density at 531 nm of the appropriate liquid medium served as a control.

3.2. Effect of aminotransferase inhibition on C. albicans growth

(Aminooxy)acetate (AOA) is a broad-spectrum inhibitor of pyridoxal phosphate-dependent enzymatic reactions, such as transaminations, which was also shown to inhibit some fungal transaminases (Brunke et al., 2014; Preuss et al., 2013; Wallach, 1961). AOA has also been reported to affect the growth of *C. glabrata* and *C. albicans* cells (Brunke et al., 2014). In our hands, presence of 10 mM AOA in cultures of *C. albicans* wild-type and mutant cells in minimal medium with AS as the sole nitrogen source resulted in growth retardation; the magnitude of this effect was roughly the same, regardless of strain type (Fig. 6). Growth retardation by AOA was not observed in the YPD complex medium, thus demonstrating that AOA is not a general toxin and that its growth inhibitory effect could be reversed by peptides and amino acids present in YPD. AOA also strongly inhibited the growth of *C. albicans* in minimal medium with selected amino acids as the sole nitrogen source in an amino acid concentration-dependent manner (Fig. 7). AOA strongly inhibited the growth of *C. albicans* SC5314 and mutant strains on 5 mM L-His as the nitrogen source. The same results were obtained by Brunke et al. (2014) for *C. albicans* SC5314 and *C. glabrata* ATCC2001. In contrast, when 10 mM L-His was used, after a long lag phase growth mostly resumed for *C. albicans* SC5314, SCΔ*yer152C*, and SCΔ*aro9*. In this instance, the main role in L-His degradation could be acquired by other remaining transaminases, such as *Ca*Aro8p or *Ca*Bna3p. Despite the fact that AOA inhibits *Ca*Aro8p and *Ca*Aro9p at micromolar concentrations *in vitro* with IC_{50} 96 ± 3.5 M, $K_{i\ L\text{-Phe}}$ 8.7 ± 1.4 M and IC_{50} 11.8 ± 3 M, $K_{i\ L\text{-Phe}}$ 3 ± 0.3 M, respectively, these or other unknown aminotransferases might not be completely inhibited *in vivo*.

The growth of strains deficient in the *ARO8* gene, including SCΔ*aro8*, SCΔ*aro8*Δ*aro9*, SCΔ*aro8*Δ*yer152C*, and SCΔ*aro8*Δ*bna31* remained reduced. For the *C. albicans* SCΔ*aro8*Δ*bna*31Δ*bna32* strain, no significant growth with any analysed concentration of L-His was detected even after 48 h in the presence of AOA. Our results, in combination with the data from the growth phenotypes of deletion mutants in minimal media with ammonium sulphate, demonstrate that L-histidine biosynthesis is not dependent on the aminotransferase activity of Aro8p, Aro9p, Yer152Cp, or Bna3p in *C. albicans*, although the presence of *Ca*Aro8p is crucial for degradation of this amino acid.

C. albicans SC5314 and mutant strain growth inability was detected with 10 mM L-Lys even after 48 h in the presence of 10 mM AOA (Fig. 7). These results demonstrate that lysine utilisation is strongly dependent on the aminotransferase activity. It is probable that the growth phenotype of the wild-type SC5314 and of SCΔ*yer152C* with L-Lys as the sole nitrogen source and AOA is the same as that for mutant strains (SCΔ*aro8*, SCΔ*aro9*, SCΔ*aro8*Δ*aro9*, SCΔ*aro8*Δ*yer152C*, SCΔ*aro8*Δ*bna31*, and SCΔ*aro8*Δ*bna*31Δ*bna32*), which are deficient in *Ca*Aro8p and/or *Ca*Aro9p aminotransferase activity, partially owing to the inhibition of both enzymes by AOA *in vivo* and the inhibition of unknown transaminases involved in L-Lys degradation. Overall, these results, in combination with the data obtained from the growth phenotypes of deletion mutants in minimal media with ammonium sulphate, demonstrate that lysine biosynthesis is not dependent on the aminotransferase activity of *Ca*Aro8p and other analysed aminotransferases. However, degradation of this amino acid is strongly dependent on *Ca*Aro8p aminotransferase activity, with an important role of transaminases not examined in this study also being probable.

L-Phe and L-Trp utilisation is also dependent on aminotransferase activity. Addition of the inhibitor AOA to *C. albicans* cultures strongly inhibited the growth of *C. albicans* SC5314 and mutant strains on 5 mM L-Phe and L-Trp as a nitrogen source. At higher concentrations of analysed amino acids, growth mostly resumed for all strains, regardless of the presence or absence of the tested genes. These results demonstrate that in *C. albicans*, L-Phe and L-Trp utilisation is not as strongly dependent on the aminotransferase activity as is L-Lys and L-His degradation. In our hands, in contrast to results obtained by Brunke et al. (2014), significant growth of *C. albicans* SC5314 in the presence of 5 or 10 mM L-Tyr and 10 mM AOA was detected. Notably, according to our results, L-Tyr utilisation is not performed mainly by aromatic aminotransferases as assumed by other authors.

3.3. Catalytic properties of CaAro8p, CaAro9p, and CaYer152Cp

The *CaARO9* gene was heterologously expressed in *E. coli* and its product, *Ca*Aro9p, was isolated and purified in several steps including ammonium sulphate precipitation, desalting, and ion exchange chromatography, under conditions previously reported for *Ca*Aro8p and *Ca*Yer152Cp (Rząd and Gabriel, 2015).

The *Ca*Aro8p, *Ca*Aro9p, and *Ca*Yer152Cp proteins were tested for their catalytic properties. The kinetic parameters of reactions catalysed by *Ca*Aro8p and *Ca*Aro9p are summarized in Table 3. *Ca*Yer152Cp catalysed the transamination of L-Phe, L-Tyr and L-Trp with 2-oxoglutarate as an amino group acceptor (kinetic parameters: K_{mPhe} = 0.24 ± 0.024 mM, V_{maxPhe} = 184 ± 4 nmol min^{-1} mg^{-1}; K_{mTyr} = 0.46 ± 0.122 mM, V_{maxTyr} = 164 ± 15 nmol min^{-1} mg^{-1}; and K_{mTrp} = 1.07 ± 0.182 mM, V_{maxTrp} = 383 ± 16 nmol min^{-1} mg^{-1}, Rząd and Gabriel, 2015). No

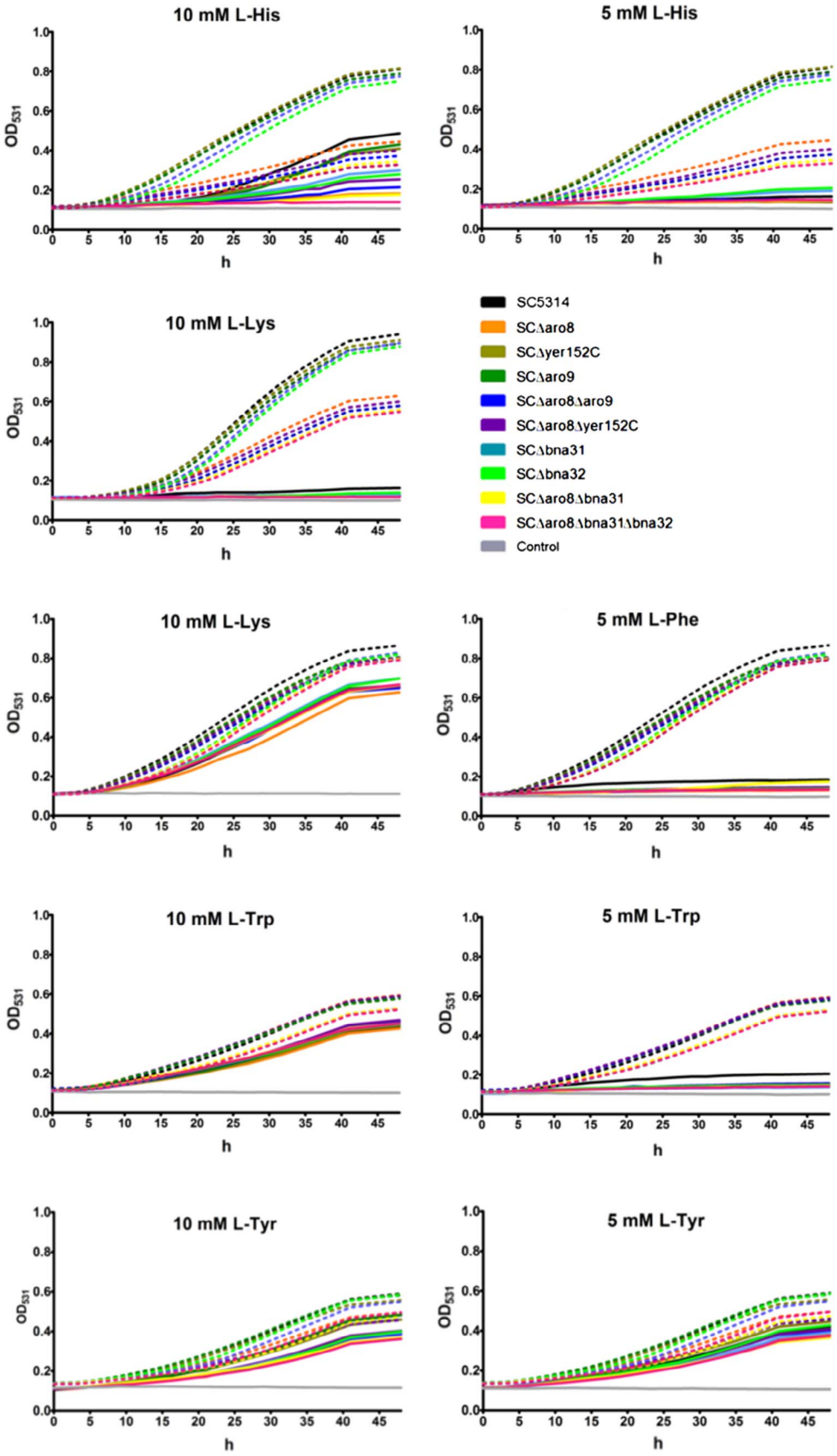

Fig. 7. Effect of (Aminooxy)acetate (AOA) on *C. albicans* wild-type and mutant growth in YNB media containing particular amino acids as the sole nitrogen source. Dashed lines, no AOA present; solid lines, 10 mM AOA. Optical density at 531 nm of the appropriate liquid medium served as a control.

activity of this enzyme was detected with L-α-aminoadipate and L-His as substrates for 2-oxoglutarate amination, nor were any transamination products formed with phenylpyruvate or 4-hydroxyphenylpyruvate as amino acceptors from L-Glu.

Analysis of the kinetic parameters of reactions catalysed by the three homologous enzymes, determined under *in vitro* conditions,

Table 3
Kinetic parameters of catabolic and anabolic reactions catalysed by *Ca*Aro8p and *Ca*Aro9p.

Aro8p			Aro9p		
Substrate	K_m [mM]	V_{max} [nmol $min^{-1}mg^{-1}$]	Substrate	K_m [mM]	V_{max} [nmol $min^{-1}mg^{-1}$]
Catabolic activity; amino group acceptor: 2-oxoglutarate					
L-Phe	0.053 ± 0.003	1200 ± 27	L-Phe	4.76 ± 1.17	7457 ± 457
L-Tyr	0.09 ± 0.008	1528 ± 34	L-Tyr	2.80 ± 0.25	6922 ± 240
L-Trp	1.68 ± 0.28	6634 ± 357	L-Trp	1.80 ± 0.18	4988 ± 168
L-AA[a]	0.016 ± 0.008	779 ± 87	L-AA	4.28 ± 1	6466 ± 511
L-His	11.5 ± 2.42	6520 ± 567	L-His	ND	ND
Anabolic activity; amino group donor: L-Glu					
PhP	0.017 ± 0.004	696 ± 68	PhP	ND	ND
4-hydroxyPhP	0.026 ± 0.004	870 ± 50	4-hydroxyPhP	ND	ND
2-oxoadipate	0.1 ± 0.033	9954 ± 2032	2-oxoadipate	0.13 ± 0.04	7743 ± 577

[a] K_m values of 2-oxoglutarate in this reaction were: 0.27 ± 0.07 mM for Aro8p and 0.41 ± 0.036 mM for Aro9p, respectively; ND – not determined, very low activity.

indicated that under *in vivo* conditions, *Ca*Aro8p is probably mainly involved in L-Phe, L-Tyr, and L-Lys catabolism when concentration of these amino acids in the cytosol is low, such as at the micromolar level, whereas at higher, millimolar concentrations, *Ca*Aro9p takes over this duty. Both enzymes exhibit similar affinity for L-Trp as an amino donor in the transamination of 2-oxoglutarate and show comparable catalytic efficiency in this reaction. The most intriguing finding is a low affinity (high K_m value) of *Ca*Aro8p for L-His as a substrate in 2-oxoglutarate transamination, assuming that *C. albicans* mutants lacking the *ARO8* gene displayed the most noticeable growth defects in minimal medium with L-His as the sole nitrogen source. Nevertheless, the high catalytic efficiency of *Ca*Aro8p in reaction with L-His, reflected by its high V_{max}, may compensate for the low affinity for this substrate. It is noteworthy therefore, that a similar K_m value was found for Aro8p from *C. glabrata*, namely 8.80 ± 1.41 mM, although the V_{max} was in that case much lower; *i.e.*, 274.9 ± 19.8 nmol min^{-1}. However, the *C. glabrata* Δ*aro8* strain was not able to grow in minimal medium with L-His as the sole nitrogen source, which identified *Cg*Aro8p as the principal transaminase involved in histidine catabolism (Brunke et al., 2014). Our results obtained for the *C. albicans* version of Aro8p (high K_m and V_{max} and growth impairment but not complete inability of the Δ*aro8* mutant) suggest that activity of this enzyme is important for L-His degradation, especially at high L-His concentration, although another, as-yet unidentified transaminase is probably operative at lower concentrations of this amino acid. The kinetic parameters determined for transaminations with L-Glu as an amino donor clearly suggest that *Ca*Aro8p but not *Ca*Aro9p can participate in the biosynthesis of aromatic amino acids (confirmed for L-Phe and L-Tyr and probably also L-Trp) and that both enzymes may be involved in L-Lys biosynthesis. In comparison, the undisturbed growth of Δ*aro8*, Δ*aro9*, and Δ*aro8*Δ*aro9* mutants in minimal medium with AS as the sole nitrogen source indicates that under *in vivo* conditions neither *Ca*Aro8p nor *Ca*Aro9p serves as the only transaminase participating in the biosynthesis of lysine and aromatic amino acids.

Despite the high degree of *Ca*Yer152Cp sequence similarity to *Ca*Aro8p (44%), the *YER152C* gene product exhibited a narrower substrate spectrum (aromatic amino acids only) and relatively low catalytic efficiency (low V_{max} values) in catabolic transaminations, with apparently no anabolic activity. These properties are in contrast to those of the *S. cerevisiae YER152C* gene product, which was identified as an L-α-aminoadipate aminotransferase (King et al., 2009). Taking into account that the K_m values of *Ca*Yer152Cp for L-Phe and L-Tyr transamination with 2-oxoglutarate as an amino acceptor are much higher than those of *Ca*Aro8p and *Ca*Aro9p and the V_{max} values are much lower, it appears that the role of this enzyme in the catabolism of aromatic amino acids is of secondary importance. This assumption is supported by the fact that a single gene disruption of *YER152C* did not affect any deficiency of *C. albicans* growth in minimal media with L-Phe, L-Tyr or L-Trp as the only nitrogen source (Fig. 2). However, the possibility that this protein exhibits any other catalytic activity, not detected in our studies, cannot be excluded.

Although cloning of the *BNA3* gene was performed and the construction of recombinant expression plasmids was completed successfully, we were not able to obtain overproduction of the *Ca*BNA3p in the *E. coli* cells used as a host for heterologous expression.

4. Discussion

Nutrient assimilation is a central and fundamental prerequisite for the growth and survival of all living organisms. Among the most important nutrients, nitrogen and carbon are required for almost all biosynthetic processes and must be assimilated in large quantities. The major fungal pathogens display different lifestyles, and, consequently, their metabolic flexibility has been shaped by different evolutionary pressures. *C. albicans* in particular faced especially diverse living conditions. This facultative pathogen can exist primarily as a commensal of the oral cavity as well as the gastrointestinal and urogenital tracts but can also persist within other extracellular (blood, tissues) and intracellular microenvironments, such as inside damaged epithelial and endothelial cells (Brock, 2009).

The unique unlimited ability of *C. albicans* to use different amino acids as the sole nitrogen source may, at least in part, be due to the versatility of its transaminases. In the hemiascomycete *C. glabrata*, histidine degradation was shown to be associated with an aromatic amino acid aminotransferase, Aro8p (Brunke et al., 2014). In the present study, we identified three genes in *C. albicans* of moderate homology to *CaARO8,* encoding putative aminotransferases with possible aminoadipate/aromatic aminotransferase activity, namely *ARO9, YER152C*, and *BNA3*. Our results indicated that *C. albicans* differs significantly from *S. cerevisiae* and *C. glabrata* in its ability to survive the lack of Aro8p or Aro9p activity. In contrast to *S. cerevisiae* (Iraqui et al., 1998; Urrestarazu et al., 1998), *C. albicans* Δ*aro8*Δ*aro9* double mutants appeared not to be auxotrophic for aromatic amino acids. Moreover, the mutant strain behaviour was completely unique in respect to its growth ability when phenylalanine, tyrosine, or tryptophan comprised the only nitrogen source. Neither the Δ*aro8* nor the Δ*aro9* mutant exhibited growth impairment, whereas only minor changes were observed for growth of the Δ*aro8* mutant on tyrosine. In this regard, *C. albicans* differs considerably from *C. glabrata*, for which a severe growth defect of the Δ*aro8* mutant in tryptophan-containing medium and significantly reduced growth in Phe and Tyr-containing media were observed. *C. glabrata* Δ*aro9* mutant was only slightly reduced in growth. The Δ*aro8* mutant also showed a severe growth defect with histidine as the sole nitrogen source (Brunke et al., 2010, 2014). Notably, this is the only amino acid that *C. glabrata* can use for growth, but *S. cerevisiae* cannot (Brunke et al., 2010). Only cysteine and lysine cannot support the

growth of both *C. glabrata* and *S. cerevisiae* (Brunke et al., 2014). The capability of using L-Lys as nitrogen source is unique exclusively for *C. albicans* and according to our results, it appears that the *Ca*Aro8p aminotransferase contributes significantly to the growth ability of *C. albicans* on lysine as the sole nitrogen source.

The *S. cerevisiae* Δ*aro8* mutant was not auxotrophic but totally failed to grow when the nitrogen source was tryptophan, tyrosine, phenylalanine, or a mixture of these three, whereas the Δ*aro8*Δ*aro9* double mutants were auxotrophic for both phenylalanine and tyrosine and totally failed to grow when tryptophan or methionine were the nitrogen source (Iraqui et al., 1998; Urrestarazu et al., 1998). These authors postulated that Aro8p from *S. cerevisiae* plays an essentially biosynthetic role with regards to phenylalanine and tyrosine formation. In comparison, they consider that Aro9p is mainly involved in tryptophan degradation and is an inducible catabolic enzyme that nevertheless ensures the formation of Phe and Tyr in *S. cerevisiae* Δ*aro8* mutants.

In baker's yeast, tryptophan is metabolised by two distinct routes: *via* the Ehrlich pathway to indole-3-ethanol (common for methionine, aromatic and branched-chain amino acids, in which the first step is catalysed by Aro8p and Aro9p) (Hazelwood et al., 2008) or *via* the kynurenine pathway to NAD^+ (Panozzo et al., 2002). It was postulated that in *S. cerevisiae*, the aromatic aminotransferase Aro8p participates in L-Trp biosynthesis and Aro9p is involved principally in its degradation. Presumably, the latter constitutes an inducible catabolic enzyme that is normally absent under anabolic growth conditions (Urrestarazu et al., 1998; Iraqui et al., 1998).

The results of our studies indicate that in *C. albicans*, tryptophan degradation is not mainly performed by the aromatic amino acid aminotransferases *Ca*Aro8p or *Ca*Aro9p in the first pathway, as the *ARO8* and *ARO9* deficient mutants did not show any growth defect in minimal medium containing Trp as the sole nitrogen source. Moreover, although the *in vitro* studies of catalytic properties of *Ca*Aro8p and *Ca*Aro9p revealed that both enzymes use L-Trp as an amino donor in the transamination of 2-oxoglutarate, the kinetic parameters, especially relatively high K_m values for L-Trp, suggest that in *C. albicans* there must be another aminotransferase that utilises L-Trp as a substrate, operating at lower concentrations of this amino acid. Therefore, we decided to investigate the importance of the second pathway with regards to tryptophan degradation.

In *S. cerevisiae*, the kynurenine pathway is a recognized route for *de novo* NAD^+ biosynthesis; in this pathway, the *BNA1* to *BNA6* genes encoding enzymes catalysing the conversion of tryptophan to nicotinic acid mononucleotide have been identified. However, whereas *BNA1*, *BNA2*, *BNA4*, *BNA5*, and *BNA6* have orthologues in the genomes of *Debaryomyces hansenii*, *C. albicans*, and *Yarrowia lipolytica*, these all are absent in *C. glabrata*, *Kluyveromyces lactis*, and *Schizosaccharyomyces pombe*. Conversely, *BNA3* is present in every genome (Li and Bao, 2007). Although *BNA3* was initially presumed to encode N-formylkynurenine formamidase (FKF), catalysing the second step of the kynurenine pathway (Panozzo et al., 2002), subsequent studies demonstrated that Bna3p instead most likely functions as the kynurenine aminotransferase, which converts kynurenine to kynurenic acid (Wogulis et al., 2008). Notably, we have found that in the *C. albicans* genome, the *BNA3* gene is present in two copies, *BNA31* and *BNA32*, stressing the presumed importance of a putative *Ca*Bna3p aminotransferase. However, knock-out of both *BNA31* and *BNA32* in *C. albicans* resulted in no growth impairment with L-Trp as a sole nitrogen source. Moreover, a similar effect was obtained when *CaARO8, CaBNA31*, and *CaBNA32* genes were simultaneously disrupted.

Finally, it appears that in *C. albicans*, Aro8p and Aro9p likely participate in tryptophan degradation at millimolar concentrations of this amino acid but another, as-yet unidentified aminotransferase is operative when the concentration is lower. This unidentified aminotransferase may also be involved in the biosynthesis of L-Trp.

It is known that *S. cerevisiae* is unable to use histidine as the sole nitrogen source (Large, 1986), while in *C. glabrata,* histidine degradation was shown to be exclusively associated with *Cg*Aro8p (Brunke et al., 2014). Conversely, Our results indicate that in *C. albicans*, *Ca*Aro8p serves as a principal but not the only enzyme involved in histidine catabolism, as the growth of the Δ*aro8* mutant was severely hampered but not completely abolished. The products of three other genes homologous to *ARO8,* namely *ARO9, YER152C*, and *BNA3* are apparently not involved in histidine catabolism. Because it is well known that hemiascomycetes, including *C. albicans*, do not possess any gene encoding histidine ammonia lyase (histidinase) (Brunke et al., 2014), which is a principal enzyme catabolising histidine in bacteria and in most Eukaryota, we hypothesise that in *C. albicans* some other aminotransferase, not investigated in the present study, may be able to catalyse the amino transfer from L-His to an α-ketoacid.

Our results indicate that the role of aminotransferases in lysine metabolism in *C. albicans* is to some extent unique in comparison with other hemiascomycetes. As in the other fungi, L-Lys is synthesized in *C. albicans via* the seven-step AA pathway (Xu et al., 2006). The fourth step of this pathway is catalysed by the PLP-dependent α-aminoadipate aminotransferase. Although Aro8p from *S. cerevisiae* was considered to be a putative α-aminoadipate aminotransferase on the basis of structural studies (Bulfer et al., 2013) and the kinetic parameters of *Ca*Aro8p-catalysed transamination with AA as an amino donor confirmed the biosynthetic potential of this enzyme, our Δ*aro8* mutant was not auxotrophic for lysine. The same was true for the Δ*aro9* mutant and for the Δ*aro8*Δ*aro9* double mutant. In comparison, in our previous studies we showed that disruption of the *CaLYS21* and *CaLYS22* genes encoding isoforms of homocitrate synthase (Gabriel and Milewski, 2016) or the *LYS4* gene coding for homoaconitase; *i.e.*, enzymes catalysing two initial steps of the AA pathway, resulted in auxotrophy of *C. albicans* cells for lysine (Kur et al., 2010; Gabriel et al., 2014). Further analysis indicated that the Δ*lys*21Δ *lys22* and Δ*lys4* mutant cells were able to utilize AA, an intermediate of the lysine biosynthesis pathway, to rescue this auxotrophy. In the present study, we found that AA as the sole nitrogen source supported growth of the *ARO8* deficient mutants and that no statistically significant differences in growth kinetics were observed. These results differ significantly from that obtained for *C. albicans* Δ*aro8* mutant cells in the presence of L-Lys as the sole nitrogen source. Therefore, our results indicate that *Ca*Aro8p constitutes the principal but not the only transaminase involved in lysine biosynthesis in *C. albicans*.

The Δ*aro8* mutant demonstrated general growth impairment in minimal media with lysine as the sole nitrogen source, although this effect was weaker than that observed in L-His-containing minimal medium. The kinetic parameters of *Ca*Aro8p, especially the high affinity to AA reflected by the low K_m value, confirm its potential principal role in lysine degradation. In contrast, the much higher K_m for AA of *Ca*Aro9p is associated with the high V_{max} for this substrate, although the Δ*aro9* mutant did not show any growth defect in lysine-supplemented minimal medium. The most plausible interpretation of these data supposes a principal role of CaAro8p in lysine degradation and auxiliary participation of CaAro9p in this process, when *C. albicans* cells are overloaded with lysine. As previously reported, *C. glabrata* and *S. cerevisiae* were not able to grow in minimal medium with L-Lys as the sole nitrogen source (Brunke et al., 2014), despite the presence of *ARO8* genes in the genomes of both species. This indicates the unique role of Aro8p in *C. albicans*.

In fungal microorganisms possessing all enzymes catalysing the complete Ehrlich pathway, for example in *S. cerevisiae*, L-methionine comprises one of the substrates for Aro8p/Aro9p catalysing the first committed step of this pathway. Another possibility for methionine utilisation is *via* the methionine salvage pathway, in which the α-amino group of methionine does not serve as a nitrogen source for the cells but is incorporated into polyamines, spermine, and spermidine. In this cyclic pathway, methionine is finally reconstructed by the transamination of 4-methylthio-2-oxobutyrate catalysed by Aro8p, Aro9p, and the branched-chain amino acid aminotransferases Bat1p and Bat2p

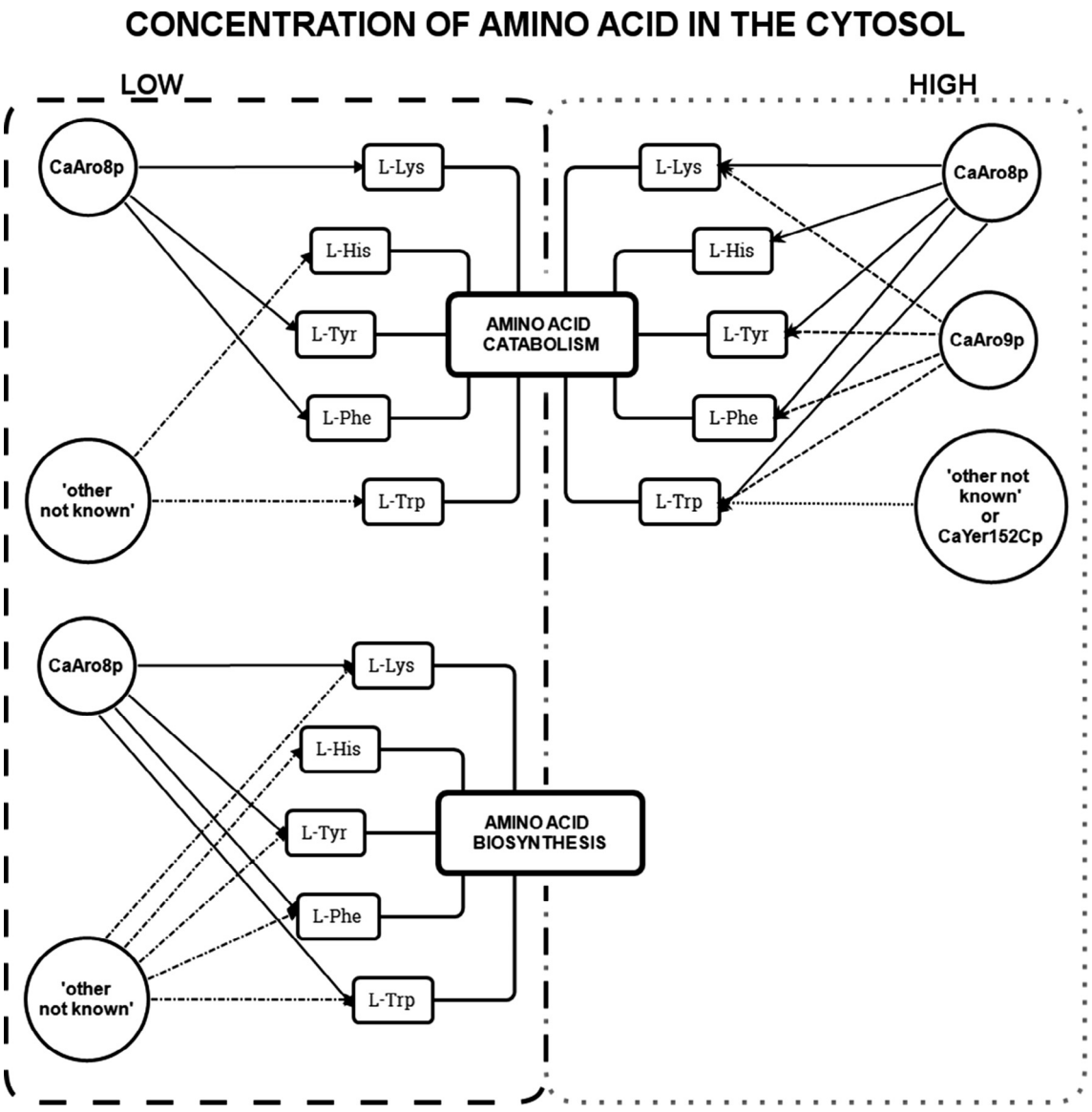

Fig. 8. A proposed model of *Ca*Aro8p, *Ca*Aro9p and *Ca*Yer152Cp role in *C. albicans* L-Lys, L-His and aromatic amino acids metabolism.

(Pirkov et al., 2008). This pathway clearly does not have any nutritional role. Nevertheless, if methionine is catabolised by Aro8p or Aro9p, the *ARO8* and *ARO9* deficient mutants should exhibit substantial growth impairment in minimal medium containing methionine as the sole nitrogen source. Accordingly, growth of the *C. glabrata* mutant lacking Aro8p activity was significantly reduced under this condition (Brunke et al., 2010). In our hands, the growth phenotype of the *C. albicans* Δ*aro8* mutant was comparable to that of the wild-type SC5314 cells. On the basis of previously published results, among five enzymes proposed to be important for L-Met catabolism in the group of hemiascomycetes, namely Bat1p, Bat2p, Aro8p, Aro9p, and Yer152Cp, only two are present in all species: Aro8p and Yer152Cp (Hébert et al., 2011). In our studies, the growth profile of the *C. albicans* SCΔ*aro8*Δ*yer152C* mutant strain in minimal media with ammonium sulphate or L-Met as a nitrogen source was comparable to that of the wild-type SC5314. Therefore, it may be confidently concluded that none of the four aminotransferases investigated in the present study is involved in methionine catabolism in *C. albicans*.

In summary, our results indicate that *Ca*Aro8p is undoubtedly the most versatile enzyme among the aminotransferases investigated. It is involved in the catabolism of histidine, lysine, and aromatic amino acids as well as in the biosynthesis of L-Lys, Phe and Tyr. *Ca*Aro9p appears to be an auxiliary enzyme, participating in the catabolism of aromatic amino acids and lysine at high concentrations of these compounds, with no biosynthetic role. The catalytic potential of *Ca*Yer152Cp for the catabolism of aromatic amino acids deduced from the results of *in vitro* studies appears to be of little importance *in vivo*. The principal activity of this aminotransferase thus remains to be discovered. Finally, *Ca*Ban3p is likely involved in the kynurenine pathway of tryptophan conversion into NAD^+ but does not take part in the biosynthesis of any proteinogenic amino acids. None of the aminotransferases *Ca*Aro8p, *Ca*Aro9p, *Ca*Yer152Cp, or *Ca*Ban3p is solely responsible for the catabolism of a single particular proteinogenic amino acid or for its biosynthesis. Having a broad set of catabolic pathways for the specific nutrient sources in the host is clearly advantageous for pathogenic fungi (Brock, 2009). The results of our studies confirm the versatility of *C. albicans* aminotransferases, which constitute a basis for the nutritional flexibility of this human pathogenic fungus. Based on our data, we suggest a model of *Ca*Aro8p, *Ca*Aro9p and *Ca*Yer152Cp role in *C. albicans* L-Lys, L-His and aromatic amino acids metabolism (Fig. 8).

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