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BRIEF COMMUNICATION

Commensal fungi and oxalate degradation: is there a link?

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Oxalic acid is one of the most common low-molecular-weight organic acids produced by living organisms, which diversify in their strategies of oxalate use and disposal (Smith 2002). For instance, plants may accumulate oxalate intracellularly for charge balance, calcium regulation, and defense, whereas oxalate secretion by fungi has been associated with pathogenicity, as reviewed in Palmieri *et al.* (2019). Conversely, bacteria can use oxalate as an energy and carbon source (Herve *et al.* 2016). In this scenario, humans fall in a gray area. Indeed, in man as well as many nonhuman animals, oxalate is an end product of glyoxylate metabolism (Ermer *et al.* 2023), since the enzymatic repertoire lacks oxalate-degrading enzymes and its physiological functions are uncertain (Palmieri *et al.* 2019). However, the widespread presence and use of oxalate in nature argues against this reductive vision of oxalate in humans. Indeed, humans cannot be considered apart from the structured communities of microorganisms, or microbiota, that colonize all the surfaces of the body exposed to the external environment, including the gut, which represents an additional source of oxalate coming from the diet. It is estimated that, in healthy individuals, the diet and the endogenous synthesis contribute equally to the levels of urinary oxalate (Mitchell *et al.* 2019). The microbiota includes, among others, bacteria and fungi that likely integrate the host metabolic pathways providing enzymes for the synthesis and degradation of oxalate that overall contribute to maintain its homeostatic levels. This is particularly important considering the pathological downsides of oxalate accumulation by either genetic or environmental causes, termed primary (PH) and secondary (SH) hyperoxaluria, respectively, which results in the formation of oxalate stones in the kidneys and, in the most severe cases, systemically (Ermer *et al.* 2023). Indeed, it is becoming increasingly clear that alterations in the microbiota may contribute to accumulation of oxalate and vice versa; thus, modulation of microbial composition may be envisioned as a therapeutic target in hyperoxaluria. This holistic approach has been pursued with the study of *Oxalobacter formigenes*, a specialist oxalotroph that uses oxalate as an exclusive carbon source. A recent multi-omics analysis has revealed that, among oxalate degrading bacteria in the gut microbiota, *O. formigenes* likely plays a dominant role, at least transcriptionally (Liu *et al.* 2021). Therapies aiming at promoting intestinal oxalate catabolism through colonization with *O. formigenes* have proved efficacious in PH1 end-stage renal disease patients (Hoppe *et al.* 2021). However, a recent phase 3, double-blind, placebo-controlled, randomized study to evaluate long-term efficacy and safety of *O. formigenes* in PH patients reported that treatment stabilized/reduced plasma oxalate compared to an increase in placebo, but without any statistically significant difference over 12 months (Ariceta *et al.* 2023). In addition, a recent study aiming at testing a causal relationship between kidney stones and gut microbiota did not find association between the genus *Oxalobacter* and kidney stones, suggesting that other taxa likely contribute (Liu *et al.* 2023). While informative on the contribution of other bacterial taxa to the regulation of oxalate levels, these studies have not addressed the role of the commensal fungal communities in this network. Even more so, a study in rats proved that microbial transplants of the entire fecal microbiota outperformed transplants of selected oxalate-degrading bacteria in oxalate degradation and persistence in the absence of dietary oxalate, further emphasizing the role of

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functional microbial communities over selected species (Miller *et al.* 2017).

Commensal fungi, collectively known as mycobiota, are numerically outnumbered by bacteria (Rowan-Nash *et al.* 2019), but their role in health and disease is increasingly being recognized (Zhang *et al.* 2022). Several studies have now accumulated to provide a comprehensive picture of the human gut mycobiota. Among the fungi commonly detected in healthy individuals (Nash *et al.* 2017), a prominent role is played by *Candida* spp. (*Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata*), as the mammalian digestive tract represent their primary niche. Common yeasts also include *Saccharomyces*, likely originating from food but still able to influence the gut microbiome, and *Malassezia*, a mammalian commensal most commonly associated with the skin but also present in feces (Hallen-Adams & Suhr 2017), while *Cladosporium* is a commonly detected filamentous fungus. So far, fungi have hit the headlines in the human oxalate field for their ability to produce oxalate crystals as pathogens, such as in pulmonary (Osholowu *et al.* 2020) and cutaneous (Meyer *et al.* 2023) aspergillosis or the presence of *Candida* in bladder stones (Takeuchi *et al.* 1989). This is in line with oxalate being one of the most common organic acids secreted by a majority of fungi (Dutton & Evans 1996). Conversely, whether commensal fungi can express oxalate-degrading enzymes and contribute to the regulation of oxalate levels remains unknown.

According to MetaCyc database (Caspi *et al.* 2014), six oxalate degradation pathways are present (Fig. 1).

These different pathways have been classified in two types by Liu *et al.* (Liu *et al.* 2021). In type I pathways, the oxalate carbon–carbon bond is cleaved in a single step by the activity of either an oxalate oxidoreductase (EC 1.2.7.1), oxalate oxidase (EC 1.2.3.4), or oxalate decarboxylase (EC 4.1.1.2). In type II pathways, an oxalyl-CoA intermediate is formed by the activity of either an oxalate CoA-transferase (EC 2.8.3.2), formyl-CoA transferase (EC 2.8.3.16), or oxalate-CoA ligase (EC 6.2.1.8), then converted to carbon dioxide and formyl-CoA by an oxalyl-CoA decarboxylase (EC 4.1.1.8). The decarboxylation of oxalic acid catalyzed by oxalate decarboxylase is typical for fungi, such as *Aspergillus niger* (Emiliani & Bekes 1964, Emiliani & Riera 1968), and type I oxalate-degrading pathways have been reported across different fungal classes (Liu *et al.* 2021). Interestingly, also type II oxalate-degrading pathways may be expressed by fungi. Indeed, an oxalyl-CoA synthetase has been reported in *Saccharomyces cerevisiae* (Foster & Nakata 2014) and is one of most abundant peroxisomal proteins in yeast (Burgi *et al.* 2023). By searching for sequence similarity with the oxalyl-CoA synthetase of *Arabidopsis thaliana*, the authors identified the YBR222C (PCS60) ORF with a 45% sequence identity. The corresponding protein was purified and shown to convert oxalate to oxalyl-CoA in an enzymatic assay (Foster & Nakata 2014). In addition, similar to plants, yeasts were able to degrade ^{14}C-oxalate to $^{14}CO_2$, thus implicating the presence of an oxalyl-CoA decarboxylase to produce formyl-CoA with subsequent production of formate and carbon dioxide (Foster & Nakata 2014). In support of this hypothesis, the authors identified a putative yeast homolog (YEL020C) of the *Arabidopsis* oxalyl-CoA decarboxylase with a 35% sequence identity (Foster & Nakata 2014). As previously mentioned, *S. cerevisiae* is commonly found in human feces, but it is more likely a transient commensal and colonizer of human gut mucosa. To explore whether commensal fungi may express an ortholog of the yeast enzyme, we performed a database search for nucleotide sequence similarity of the yeast ScAAE3 ORF versus *Candida* species and found a gene in *C. glabrata* (*Nakaseomyces glabrata*) with 73% sequence identity, encoding an unnamed protein product, in line with the phylogenetic closeness between the two species. At the protein level (P38137 vs Q6FMM3), identities and positives were 79% and 86%, respectively. To get more insights into the protein from *C. glabrata*, we downloaded the structure of the protein generated by AlphaFold and available in the database (Jumper *et al.* 2021). We then superimposed the monomeric 3D crystal structures of oxalyl-CoA synthetase from *A. thaliana* (Fan *et al.* 2016) and from *S. cerevisiae* (Burgi *et al.* 2023) on the structure of oxalyl-CoA synthetase from *C. glabrata* (Fig. 2) and found that the binding pocket of oxalate as well as the P-loop involved in ATP binding was conserved among the three forms, further supporting the hypothesis that the protein product from *C. glabrata* very likely works as an oxalyl-CoA synthetase.

We also identified an additional unnamed protein product (Q6FJV6) in *C. glabrata* with 56% identities

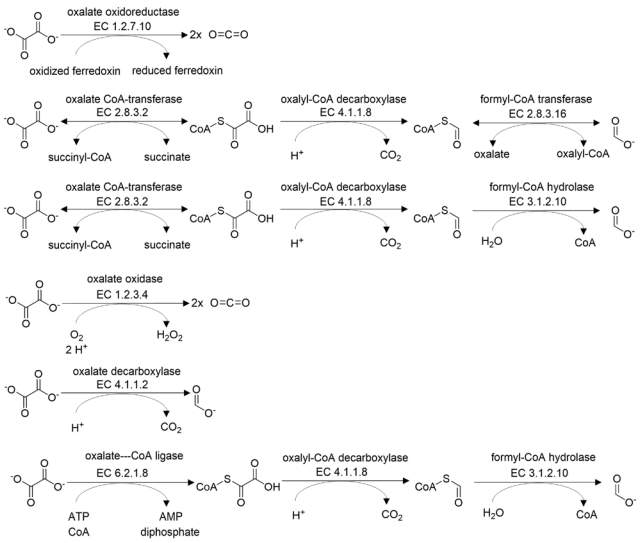

Figure 1

Oxalate-degrading pathways according to MetaCyc.

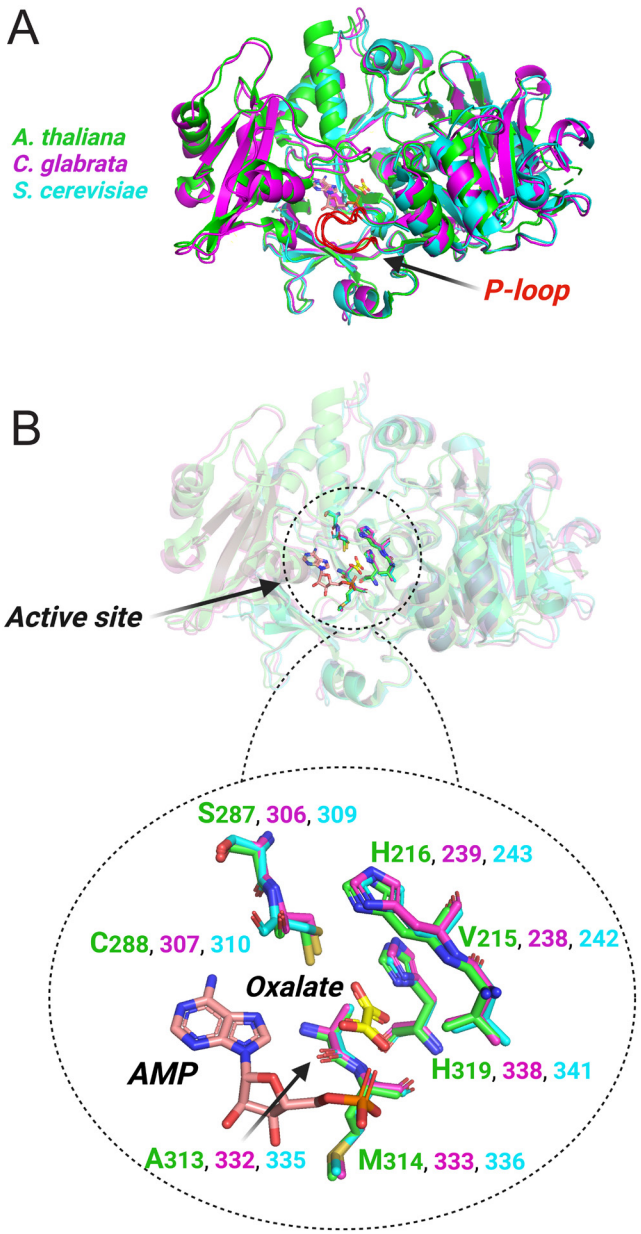

Figure 2

Ribbon representation of the superimposed monomeric 3D crystal structures of oxalyl-CoA synthetase from *A. thaliana* (PDB id: 5IE3, green), *S. cerevisiae* (PDB ID: 8ATD, cyan), and the AphaFold-generated structure of oxalyl-CoA synthetase from *C. glabrata* (purple). (A) Superimposed monomeric structures of the three enzymes. The P-loops involved in ATP binding are colored in red (residues SGTTSRP). (B) Zoom-in of most of the active site residues (represented as sticks) involved in the formation of the oxalate binding pockets for the reported crystal structures of oxalyl-CoA synthetase from *A. thaliana* and *S. cerevisiae*, and the putative oxalyl-CoA synthetase from *C. glabrata*. Carbon atoms of oxalate and AMP within the active site of *A. thaliana* oxalyl-CoA synthetase crystal structure are represented as yellow and pink sticks, respectively, while oxygen and nitrogen atoms are colored in red and blue. The images have been created by using PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.

and 71% positives with the putative yeast homolog (YEL020C) of the *Arabidopsis* oxalyl-CoA decarboxylase (OXC), thus arguing for the presence of all the components of the type II oxalate-degrading pathway in *C. glabrata*. To confirm this hypothesis, we performed a multiple sequence alignment (MSA) (Fig. 3) using the sequences of OXCs from *E. coli* (*Ec*OXC), *O. formigenes* (*Of*OXC), and *Arabidopsis thaliana* (*At*OXC), all reported to catalyze the conversion of oxalyl-CoA to formyl-CoA (type II oxalate-degrading pathway) (Berthold *et al.* 2005, Werther *et al.* 2010, Foster *et al.* 2021, Cheng *et al.* 2022). We also included the sequence of the putative *S. cerevisiae* OXC (*Sc*OXC, Uniprot ID: YEL020C). In the published crystal structure of *Of*OXC (Berthold *et al.* 2005), three residues in the active site are considered important for the catalysis, i.e. Tyr120, Glu121, and Tyr483. In the putative *C. glabrata* OXC sequence, these three residues are represented by Phe114, Gln115, and Tyr477, also present in the *At*OXC sequence and the putative *Sc*OXC (green asterisks of Fig. 3). Interestingly, other active site residues reported in the crystal structure *Of*OXC (Berthold *et al.* 2005), i.e. Gly33, Ile34, Glu56, and Met428, are conserved in all the sequences and correspond to Gly29, Ile30, Glu52, and Met421 in the putative *Cg*OXC and Gly39, Ile40, Glu62, and Met434 in the putative *Sc*OXC (Fig. 3).

The presence of homologs between *S. cerevisiae* and *C. glabrata* were not unexpected. Indeed, *Saccharomyces* and *Candida* belong to the same family Saccharomycetaceae. In addition, *C. glabrata* is more similar to *Saccharomyces* than to the other *Candida* spp. (Roetzer *et al.* 2011). This might explain why homologs of other *Candida* spp. were not revealed by our database search. However, this does not exclude that other *Candida* spp. may express an enzyme with a similar function. In this regard, the presence of this oxalate-degrading pathway may be more common in fungi than originally expected. Indeed, in their analysis of acetyl-CoA-generating pathways during *Cryptococcus neoformans* infection, Alden *et al.* found that, out of the three ACS genes, ACS3 was less related to ACSs while showing sequence similarity to the *S. cerevisiae* oxalyl CoA synthetase (Alden *et al.* 2022). By comparing the two sequences (P38137 vs CNAG_06433), we found that identities and positives were 24% and 42%, respectively. It is also possible that other oxalate metabolic pathways, if any, may have evolved during evolution of *Candida* spp. and additional investigations will be required to elucidate the different possibilities.

In conclusion, in this brief communication we have put forward the hypothesis that commensal fungi may participate in the regulation of oxalate levels by expressing oxalate-degrading pathways, suggesting that the mycobiome may represent an unrecognized player in oxalate catabolism, opening up novel windows of opportunity for the understanding and treatment of hyperoxaluria.

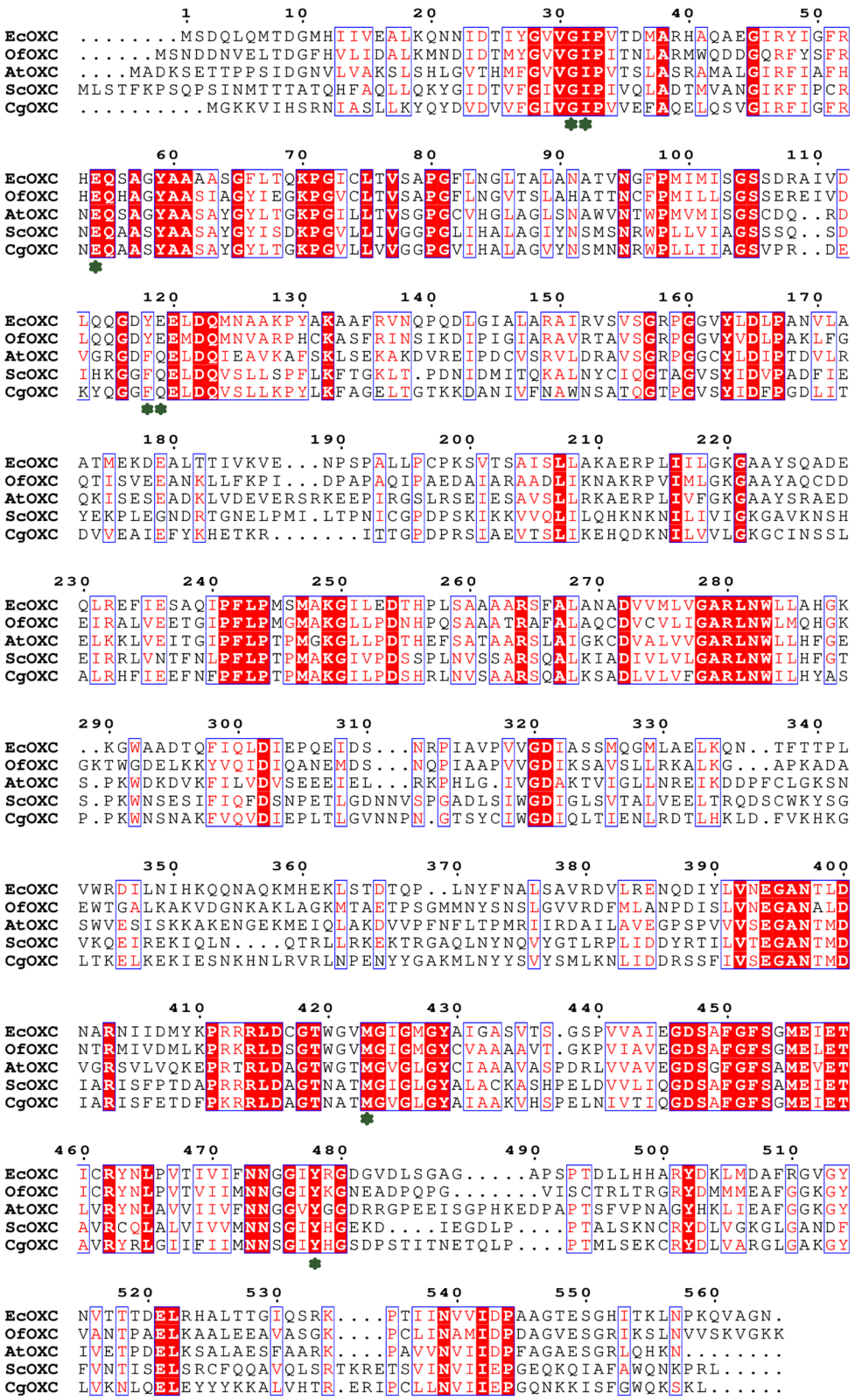

Figure 3

Multiple sequence alignment of the amino acid sequences of OXC from bacteria, plants, and fungi. Depicted is a protein sequence alignment of validated or putative OXCs from *Escherichia coli* (EcOXC), *Oxalobacter formigenes* (*Of*OXC), *Arabidopsis thaliana* (AtOXC), *Saccharomyces cerevisiae* (*Sc*OXC), and *Candida glabrata* (*Cg*OXC). Residues conserved in all proteins are shown as red blocks. Residues belonging to the active site are highlighted using green asterisks. The alignment was done using ClustalW (Thompson *et al.* 1994) and the figure was created using ESPript (Robert & Gouet 2014).

Declaration of interest
L Romani is an associate editor of *Microbiota and Host*. Luigina Romani was not involved in the review or editorial process for this paper, on which they are listed as an author. The authors declare no conflict of interest that could be perceived as prejudicing the impartiality of the article.

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Author contribution statement
CC and LR conceived the study. MD performed the analysis of Figs 2 and 3. All authors contributed to the writing of the manuscript.

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