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Review

LysM proteins in mammalian fungal pathogens

José A. OGUIZA

Genetics, Genomics and Microbiology Research Group, Institute for Multidisciplinary Research in Applied Biology (IMAB), Universidad Pública de Navarra (UPNA), Campus de Arrosadia, 31006 Pamplona, Spain

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ABSTRACT

The LysM domain is a highly conserved carbohydrate-binding module that recognizes polysaccharides containing N-acetylglucosamine residues. LysM domains are found in a wide variety of extracellular proteins and receptors from viruses, bacteria, fungi, plants and animals. LysM proteins are also present in many species of mammalian fungal pathogens, although a limited number of studies have focused on the expression and determination of their putative roles in the infection process. This review summarizes the current knowledge and recent studies on LysM proteins in the main morphological groups of fungal pathogens that cause infections in humans and other mammals. Recent advances towards understanding the biological functions of LysM proteins in infections of mammalian hosts and their use as potential targets in antifungal strategies are also discussed.

1. Introduction

Mammals are highly resistant to most invasive infections by fungi due to the high body temperature and both the innate and adaptive immune systems (Casadevall, 2007). Only a reduced number of species in the fungal kingdom can cause infections in mammals and cover primary pathogens, commensals and opportunistic pathogens (Sexton and Howlett, 2006; Heitman, 2011; Mayer *et al.*, 2013; Köhler *et al.*, 2017; Gostinčar *et al.*, 2018; Seyedmousavi *et al.*, 2018). However, a limited proportion of the mammalian pathogenic fungi may be considered primary pathogens that can cause infection in immunocompetent hosts. Commensal fungi generally survive without tissue invasion and can emerge to cause occasional infections only when the association with the mammalian host is disrupted. On the other hand, most opportunistic fungi are environmental species with a saprophytic lifestyle that are not specialized for the mammalian host, and usually cause life-threatening systemic infections in immunocompromised hosts (Casadevall, 2007; Rappleye and Goldman, 2008; de Hoog *et al.*, 2014). The innate immune system of mammals recognize fungi by the specific detection of chitin, a polysaccharide of N-acetylglucosamine (GlcNAc) residues that is a major component of fungal cell walls (Kombrink *et al.*, 2011; Bueter *et al.*, 2013; Kombrink and Thomma, 2013; Gow *et al.*, 2017).

Polysaccharide recognition by carbohydrate-binding modules (CBMs) of proteins is essential for multiple biological processes in all living organisms (Boraston *et al.*, 2004; Guillén *et al.*, 2010; Mesnage *et al.*, 2014). The LysM domain is a highly conserved CBM that recognizes polysaccharides containing

E-mail addresses: jose.oguiza@unavarra.esjose.oguiza@unavarra.es
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GlcNAc residues. LysM domains are found in a wide variety of extracellular proteins and receptors from viruses, bacteria, fungi, plants and animals (Buist *et al.*, 2008; de Jonge and Thomma, 2009; Zhang *et al.*, 2009; Mesnage *et al.*, 2014). The large number and diversity of LysM proteins identified in fungal genomes suggests that the functional repertoire of these proteins in fungi has been only partially discovered (Akcapinar *et al.*, 2015). The expanding availability of fungal genomes, has also led to the identification of LysM proteins in many species of mammalian fungal pathogens that remain largely unexplored (Akcapinar *et al.*, 2015; Muraosa *et al.*, 2019). The aim of this review is to summarize the current knowledge and recent studies on LysM proteins in the main morphological groups of fungal pathogens that cause infections in mammals: obligate parasitic fungi, yeasts and yeast-like fungi, filamentous fungi, and thermally dimorphic fungi. The biological functions of fungal LysM proteins in mammalian pathogenesis and their use as potential targets in antifungal therapies will also be considered.

2. The LysM domain

The LysM domain is a small globular non-catalytic CBM approximately between 40 and 50 amino acids in length that specifically recognize polysaccharides containing GlcNAc residues (Pfam: PF01476; PROSITE: PS51782) (Fig. 1A). Different LysM domains have been reported to bind bacterial peptidoglycan, fungal chitin and chitin oligosaccharides such as chitin fragments, nodulation or mycorrhization factors (Buist *et al.*, 2008; de Jonge and Thomma, 2009; Mesnage *et al.*, 2014; Visweswaran *et al.*, 2014). Although the primary sequence of a typical LysM domain is not highly conserved, there is a particular high sequence conservation in the first 16 amino acids, and none or only one conserved cysteine residue (Fig. 1A). The three-dimensional structure of the LysM domain has a characteristic conserved βααβ fold, in which both α-helices are packed on the same side of the two antiparallel β-sheet (Buist *et al.*, 2008). Moreover, several LysM domains of eukaryotes contain additional conserved cysteine residues that are probably involved in disulphide bridge formation to provide stability to the domain (Buist *et al.*, 2008; de Jonge and Thomma, 2009).

One or multiple LysM domains can be found in a wide variety of extracellular proteins and receptors from all kingdoms of life, and LysM proteins have been identified in bacteriophages, bacteria, fungi, plants and animals (Buist *et al.*, 2008; Kombrink and Thomma, 2013; Akcapinar *et al.*, 2015). Mainly, LysM proteins are secreted proteins, but also comprise membrane proteins, cell wall proteins and lipoproteins. The LysM domain was first identified in bacteriophage lysin proteins that hydrolyze peptidoglycan of the bacterial cell wall. In plants, LysM proteins play important roles in defence responses against pathogens and in the establishment of symbiosis (Buist *et al.*, 2008; Gust *et al.*, 2012; Akcapinar *et al.*, 2015; Buendia *et al.*, 2018; Hu *et al.*, 2021). In bacteria and fungi, LysM domains are present in many different types of extracellular proteins involved in the degradation or modification of peptidoglycan and chitin including glycoside hydrolases (GH), transglycosylases, peptidases, amidases and chitinases (Buist *et al.*, 2008; Akcapinar *et al.*, 2015). Although bacterial and fungal LysM proteins can also be involved in many other biological functions such as esterases, reductases or nucleotidases.

Based on phylogenetic analysis and sequence profiles, fungal LysM domains have been classified into a fungal/bacterial group and a fungal specific group (Gruber *et al.*, 2011; Martinez *et al.*, 2012; Akcapinar *et al.*, 2015). Fungal specific LysM domains contain a characteristic 53 amino acid consensus pattern with several conserved residues that are not localized in the fungal/bacterial LysM domains. These include four highly conserved cysteines (positions 5, 17, 42 and 52) and an asparagine in the WNP motif (positions

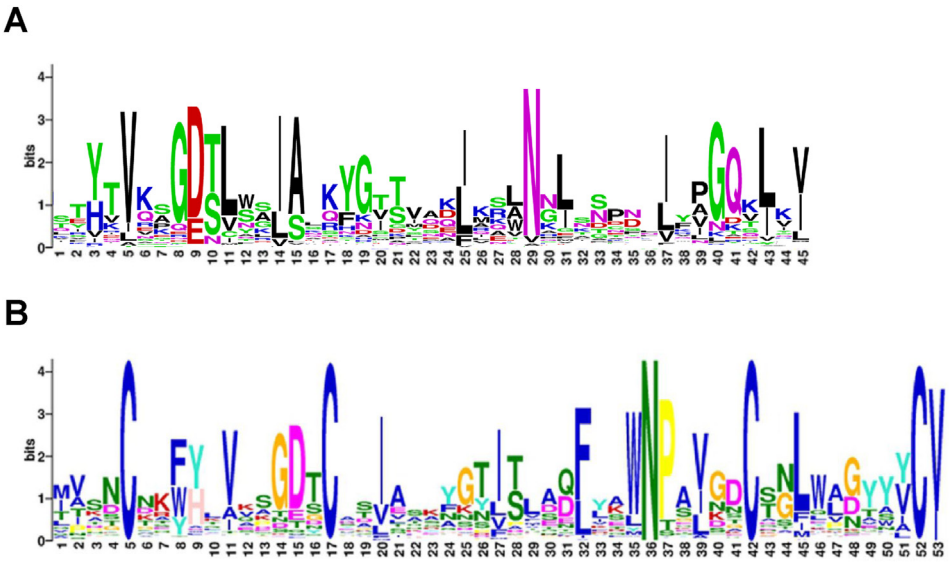

Fig. 1 – Hidden Markov model (HMM) sequence logos for (A) the fungal/bacterial LysM domain (PROSITE: PS51782) and (B) the fungal-specific LysM domain (Akcapinar et al., 2015). Height of each letter is related to frequency of amino acid at that position.

35–37) (Akcapinar *et al.*, 2015) (Fig. 1B). Presence of these conserved cysteines suggests that disulphide bridges might stabilize fungal specific LysM domains (Akcapinar *et al.*, 2015).

3. Classification and domain architecture of fungal LysM proteins

LysM proteins are widely distributed in fungi of diverse lifestyles, including symbionts, mycoparasites, saprophytes and pathogens of plants, insects and animals (Bolton *et al.*, 2008; de Jonge and Thomma, 2009; Kombrink and Thomma, 2013; Akcapinar *et al.*, 2015; Agrawal *et al.*, 2016; Cen *et al.*, 2017). Fungal LysM proteins show a high variability in amino acid sequence, protein length, number of LysM domains and domain organization, even between closely related species (de Jonge and Thomma, 2009; Martinez *et al.*, 2012; Akcapinar *et al.*, 2015; Cen *et al.*, 2017). The number of LysM domains in fungal proteins ranges from 1 to 12, and often LysM domains are present in tandem (Buist *et al.*, 2008; de Jonge and Thomma, 2009; Zhang *et al.*, 2009; Martinez *et al.*, 2012; Visweswaran *et al.*, 2014; Akcapinar *et al.*, 2015). Several LysM proteins of fungi are composed of catalytic domains linked to one or multiple non-catalytic LysM domains and, frequently, LysM domains are separated by a short peptide spacer that can form a flexible region (de Jonge and Thomma, 2009; Visweswaran *et al.*, 2014). Many fungal LysM proteins contain putative N-terminal signal peptides, indicating that they are secreted proteins (de Jonge and Thomma, 2009; Martinez *et al.*, 2012). Studies based on the domain architecture and/or phylogenetic analysis have shown that there are mainly two types of LysM proteins in fungi: LysM effectors and subgroup C chitinases (de Jonge and Thomma, 2009; Gruber *et al.*, 2011; Martinez *et al.*, 2012; Akcapinar *et al.*, 2015).

During colonization of their niches, fungi secrete a variety of effector proteins that facilitate the establishment of interactions with host organisms (Kombrink and Thomma, 2013). LysM effectors are secreted proteins with a variable number of non-catalytic LysM domains but without any catalytic domain. The modular architecture of fungal LysM effectors is relatively simple consisting of an N-terminal signal peptide for secretion and single or multiple LysM domains (Fig. 2A). Fungal LysM effectors have been linked to plant pathogenesis, mycoparasitism, insect pathogenesis and mammalian pathogenesis (Bolton *et al.*, 2008; de Jonge and Thomma, 2009; de Jonge *et al.*, 2010; Gruber *et al.*, 2011; Martinez *et al.*, 2012; Kombrink and Thomma, 2013; Cen *et al.*, 2017; Romero-Contreras *et al.*, 2019; Dubey *et al.*, 2020; Suarez-Fernandez *et al.*, 2021).

Subgroup C chitinases are large proteins containing single or multiple non-catalytic LysM domains at the 5′-end in combination with a catalytic GH18 domain (PF00704) (Fig. 2B) (Seidl, 2008; Gruber *et al.*, 2011; Goughenour *et al.*, 2021). All fungal chitinases belong exclusively to the GH18 family and catalyze the hydrolysis of glycosidic bonds in chitin and chitin oligosaccharides (Seidl, 2008; Chen *et al.*, 2020; Goughenour *et al.*, 2021). The domain architecture of subgroup C chitinases with one or multiple LysM domains and a GH18 domain is specifically found in the fungal kingdom (Zhang *et al.*, 2009; Gruber *et al.*, 2011; Akcapinar *et al.*, 2015). Subgroup C chitinases also can have an N-terminal signal peptide that serves to direct secretion and/or an additional chitin-binding type-1 domain (PF00187) (Fig. 2B) (Seidl, 2008; de Jonge and Thomma, 2009; Gruber *et al.*, 2011; Martinez *et al.*, 2012; Goughenour *et al.*, 2021). Potential functional roles of fungal chitinases include chitin metabolism, fungal cell wall modification and degradation, and competition and defence against other fungi, insects or nematodes (Seidl, 2008; de Jonge and Thomma, 2009; Chen *et al.*, 2020).

LysM effectors and subgroup C chitinases are the major types of LysM proteins found in fungi. Some LysM proteins of mammalian fungal pathogens can also include additional functional domains such as a polysaccharide deacetylase type-1 domain (PF01522) or an Hce2 domain (PF14856, homolog of Ecp2 effector protein from the tomato pathogen Cladosporium fulvum) (Fig. 2C) (de Jonge and Thomma, 2009; Martinez *et al.*, 2012; Akcapinar *et al.*, 2015; Persinoti *et al.*, 2018; Lopes *et al.*, 2020).

4. LysM proteins among mammalian fungal pathogens

Several studies have described wide variations in the repertoire of LysM proteins among diverse fungal species that cause infection in humans and other mammals (de Jonge and Thomma, 2009; Gruber *et al.*, 2011; Martinez *et al.*, 2012; Kombrink and Thomma, 2013; White *et al.*, 2014; Whiston and Taylor, 2016; Cen *et al.*, 2017; Looi *et al.*, 2017; Teixeira *et al.*, 2017; Persinoti *et al.*, 2018). On the one hand, LysM proteins are absent in obligate parasitic fungi, some species of yeasts and yeast-like fungi, and in the dematiaceous fungi *Rhinocladiella mackenziei* (de Jonge and Thomma, 2009; Kombrink and Thomma, 2013; Teixeira *et al.*, 2017); but on the other hand, the dermatophyte *Microsporum canis* contains 31 LysM proteins (Martinez *et al.*, 2012; Persinoti *et al.*, 2018). Moreover, significant differences in the number, primary sequence, length and domain architecture of LysM proteins have also been observed, even between species or species groups of mammalian pathogenic fungi closely related or sharing the same mammalian host.

4.1. *Obligate parasitic fungi, and yeasts and yeast-like fungi*

Microsporidia and atypical fungi are obligate parasites of humans and animals that depend on the presence of eukaryotic cells to complete their life cycle. The absence of LysM proteins has been described in the microsporidian *Encephalitozoon cuniculi* (de Jonge and Thomma, 2009) and the atypical fungus *Pneumocystis jirovecii* (Kombrink and Thomma, 2013). Likewise, some yeasts and yeast-like fungi such as the ascomycetes *Candida albicans* WO1, *Candida lusitaniae*, *Candida parapsilosis*, *Lodderomyces elongisporus* and the basidiomycete *Malassezia globosa* lack LysM proteins (de Jonge and Thomma, 2009; Kombrink and Thomma, 2013). Furthermore, other yeast and yeast-like mammalian pathogens contain a limited number of LysM proteins: a single protein in *Candida albicans* SC5314 and *Candida guilliermondii*, two proteins in *Candida tropicalis* and three proteins in *Cryptococcus neoformans* (de Jonge and

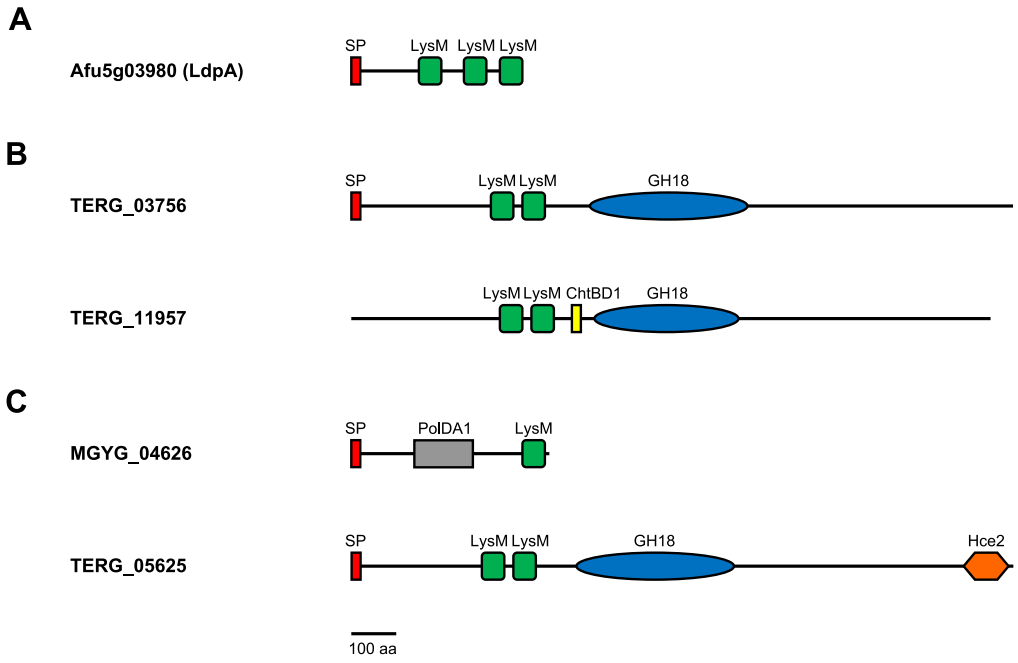

Fig. 2 – Domain architecture of LysM proteins in mammalian pathogenic fungi: (A) LysM effectors, (B) subgroup C chitinases and (C) LysM proteins with additional functional domains. Representative LysM proteins corresponding to each domain architecture are schematically shown. Signal peptide (SP), LysM domain (PF01476), glycoside hydrolase family 18 domain (GH18, Glyco_hydro_18: PF00704), chitin-binding type-1 domain (ChtB D1, Chitin_bind_1: PF00187), polysaccharide deacetylase type-1 domain (PolDA1, Polysacc_deac_1: PF01522), Hce2 domain (PF14856).

Thomma, 2009). With the exception of the basidiomycete yeast *C. neoformans* that is an environmental saprophyte, all these mammalian pathogenic fungi without or with a reduced number of LysM proteins have an obligate parasitic or commensal lifestyle. Therefore, it has been suggested that this group of mammalian fungal pathogens could be deficient in the LysM proteins necessary for a free-living lifestyle (Kombrink and Thomma, 2013).

4.2. *Filamentous fungi*

The filamentous fungi infecting humans and animals are a broad group of species that can be classified into different morphological subgroups: hyaline fungi, dematiaceous fungi, zygomycete fungi, and dermatophyte fungi (Sciortino, 2017; Wanger *et al.*, 2017). In general, there is a high variability in the number of LysM proteins between filamentous fungal pathogens of mammals (de Jonge and Thomma, 2009; Gruber *et al.*, 2011; Martinez *et al.*, 2012; White *et al.*, 2014; Whiston and Taylor, 2016; Cen *et al.*, 2017; Looi *et al.*, 2017; Teixeira *et al.*, 2017; Persinoti *et al.*, 2018). Nevertheless, studies that deal with transcriptional or functional analysis of LysM proteins in this fungal group of mammalian pathogens have been performed only within a very limited number of species (Table 1).

Hyaline fungi are characterized by narrow, septate and colorless hyphae including mainly ascomycetes that are opportunistic pathogens such as *Aspergillus* and *Fusarium* species. *Aspergillus* species are prominent fungal pathogens that can cause systemic infections in immunocompromised mammalian hosts. *Fusarium* species, such as *Fusarium oxysporum* and *Fusarium verticilloides*, can also infect immunocompromised mammals. The number of LysM proteins ranges from 8 in *Aspergillus fumigatus* to 18 in *Aspergillus nidulans* and *F. oxysporum* (Whiston and Taylor, 2016; Cen *et al.*, 2017; Muraosa *et al.*, 2019). Transcriptional analysis of four A. nidulans genes encoding subgroup C chitinases (AN7613.2, AN0509.2, CBF71201, CBF74964) has revealed that their expression was highly induced during interspecific interactions with other fungi. Furthermore, deletion of any of these subgroup C chitinase genes affected the growth of A. nidulans (Tzelepis *et al.*, 2014). Functional analysis of A. fumigatus LdpA (Afu5g03980) and LdpB (Afu1g15420) has shown that these secreted LysM effectors are localized in the fungal cell wall and extracellular matrix, but without being essential for fungal morphology or pathogenicity. However, it has been suggested that LdpA and LdpB could be involved in host interactions (Muraosa *et al.*, 2019).

Dematiaceous fungi are mainly saprophytic ascomycetes found in soil and plant material that contain melanin in their cell wall, which produce the black or dark brown pigmentation of the septate hyphae (Revankar and Sutton, 2010). Significant variations in the number of LysM proteins have been observed between species of dematiaceous fungi implicated in opportunistic infections of mammals such as *R. mackenziei* (no LysM proteins); *Exophiala dermatitidis* (2 proteins); *Cladophialophora bantiana*, *Exophiala aquamarina* and *Phialophora europaea* (3 proteins); *Cladophialophora carrionii* and *Exophiala xenobiotica* (4 proteins); *Exophiala oligosperma*, *Exophiala spinifera* and *Fonsecaea multimorphosa* (5 proteins); *Verruconis gallopava* and *Corynespora cassiicola* (6 proteins); and *Fonsecaea pedrosoi* (7 proteins) (Chen *et al.*, 2014; Looi *et al.*, 2017; Teixeira *et al.*, 2017; Moreno *et al.*, 2018). It is particularly

Table 1 – Best-studied LysM proteins of mammalian fungal pathogens.

LysM protein	Species	Type of LysM protein	Putative function	References
FILAMENTOUS FUNGI				
AN7613.2	*Aspergillus nidulans*	Subgroup C chitinase	Fungal-fungal interactions	Tzelepis et al. (2014)
AN0509.2	*Aspergillus nidulans*	Subgroup C chitinase	Fungal-fungal interactions	Tzelepis et al. (2014)
CBF71201	*Aspergillus nidulans*	Subgroup C chitinase	Fungal-fungal interactions	Tzelepis et al. (2014)
CBF74964	*Aspergillus nidulans*	Subgroup C chitinase	Fungal-fungal interactions	Tzelepis et al. (2014)
LdpA (Afu5g03980)	*Aspergillus fumigatus*	LysM effector	Fungal-host interactions	Muraosa et al. (2019)
LdpB (Afu1g15420)	*Aspergillus fumigatus*	LysM effector	Fungal-host interactions	Muraosa et al. (2019)
LysM1 (TERG_05627)	*Trichophyton rubrum*	LysM effector	Binding chitin and glycoproteins	Kar et al. (2019); Martins et al. (2019)
LysM2 (TERG_01873)	*Trichophyton rubrum*	LysM effector	Binding chitin and glycoproteins	Kar et al. (2019); Martins et al. (2019)
TERG_03756	*Trichophyton rubrum*	Subgroup C chitinase	Keratin degradation	Lopes et al. (2020)
TERG_05625	*Trichophyton rubrum*	Subgroup C chitinase	Keratin degradation	Lopes et al. (2020)
TERG_01015	*Trichophyton rubrum*	LysM effector	Unknown function	Lopes et al. (2020)
TERG_05623	*Trichophyton rubrum*	LysM effector	Unknown function	Lopes et al. (2020)
THERMALLY DIMORPHIC FUNGI				
Cts3	*Histoplasma capsulatum*	Subgroup C chitinase	Endochitinase/chitobiosidase	Goughenour et al. (2021)

noteworthy the lack of LysM proteins in *R. mackenziei*, a dematiaceous fungus that causes primary central nervous system infection in humans and its environmental niche remains unknown.

Zygomycete fungi are a paraphyletic group of saprophytes in soil and plant debris that usually form aseptate hyphae. Zygomycetes of the order Mucorales, mainly species belonging to the genera *Rhizopus*, *Mucor*, *Lichtheimia* and *Apophysomyces*, cause life-threatening infections in immunocompromised mammalian hosts. *Rhizopus oryzae* contains 11 LysM proteins (de Jonge and Thomma, 2009), and in *Aphophysomyces* species similar numbers have been reported: 11 proteins in *Aphophysomyces variabilis*, 12 proteins in *Aphophysomyces trapeziformis* and 13 proteins in *Aphophysomyces elegans* (Prakash *et al.*, 2017).

Dermatophyte fungi are ascomycetes with septate hyphae belonging to the order Onygenales that are closely related phylogenetically to the thermally dimorphic fungi. Dermatophytes are obligate mammalian pathogens that exclusively invade keratinized tissues such as the skin, hairs and nails. Species within the genera *Trichophyton*, *Microsporum*, *Epidermophyton*, *Nannizzia* and *Arthroderma* commonly cause chronic infections in immunocompetent hosts (White *et al.*, 2014; de Hoog *et al.*, 2017; Persinoti *et al.*, 2018; Burstein *et al.*, 2020; Kumar *et al.*, 2021). LysM proteins vary in number and domain architecture across dermatophyte species (Martinez *et al.*, 2012; Cen *et al.*, 2017; Persinoti *et al.*, 2018; Kumar *et al.*, 2021). The number of LysM proteins ranges from 7 in *Epidermophyton floccosum* and *Trichophyton mentagrophytes* to 31 in *M. canis*, and the predominant domain architectures are LysM effectors and subgroup C chitinases (Martinez *et al.*, 2012; Cen *et al.*, 2017; Persinoti *et al.*, 2018; Liu *et al.*, 2021). Nevertheless, a polysaccharide deacetylase type-1 domain (PF01522) involved in chitin metabolism has only been identified in some LysM proteins of dermatophytes, and an Hce2 domain (PF14856) in the TERG_05625 subgroup C chitinase of *Trichophyton rubrum* (Fig. 2C) (Martinez *et al.*, 2012; Persinoti *et al.*, 2018; Lopes *et al.*, 2020). In comparison with other fungi, the LysM protein family of dermatophytes appears to have undergone a gene expansion (Martinez *et al.*, 2012). Additionally, variations in domain architecture can reflect the dynamic evolution of the LysM protein family in the dermatophyte fungi (Persinoti *et al.*, 2018). Analysis of *T. rubrum* LysM1 (TERG_05627) and LysM2 (TERG_01873) effectors has showed that these proteins can bind to the fungal cell wall chitin and to the N-linked oligosaccharides associated with human skin glycoproteins. Further characterization of *T. rubrum* LysM1 effector also revealed that each functional LysM domain can individually bind to chitin and to N-linked oligosaccharides in human skin and fungal glycoproteins (Kar *et al.*, 2019). A global transcriptional analysis of cell wall biosynthesis-related genes in *T. rubrum* highlighted that the TERG_05625 gene encoding a subgroup C chitinase was induced when the fungus was grown in presence of keratin and repressed by acriflavine and undecanoic acid (Martins *et al.*, 2019). Another expression analysis of the 14 *T. rubrum* LysM genes grown on different substrates showed marked changes in transcription levels of certain genes encoding subgroup C chitinases (TERG_03756 and TERG_05625) and LysM effectors (TERG_01015 and TERG_05623) when the dermatophyte was grown on keratinized sources. Consequently, it has been proposed that TERG_03756 and TERG_05625 subgroup C chitinases may be involved in keratin degradation (Lopes *et al.*, 2020).

4.3. *Thermally dimorphic fungi*

The thermally dimorphic fungi are a unique group of ascomycetes with an environmental saprophytic phase and a mammalian pathogenic phase. These fungi are characterized by a reversible temperature-dependent morphological shift between filamentous mycelium with septate hyphae in the environment and yeast cells into the mammalian host. The thermally dimorphic fungi are considered primary pathogens capable of causing infection in healthy mammals, and include ascomycete species from the genera *Coccidioides*, *Histoplasma*, *Blastomyces*, *Paracoccidioides*, *Sporothrix*, *Emmonsia* and *Emergomyces* (Teixeira *et al.*, 2014; Gauthier, 2015, 2017; Seyedmousavi *et al.*, 2018; Van Dyke *et al.*, 2019). All thermally dimorphic fungi are members of the order Onygenales, with the exception of *Sporothrix* species that belong to the phylogenetically distant order Ophiostomatales. In comparison with

the closely related dermatophytes, the thermally dimorphic fungi in the order Onygenales harbour a reduced number of LysM proteins: only one in *Histoplasma capsulatum*; two in *Coccidioides immitis*, *Coccidioides posadasii* and *Paracoccidioides lutzii* (formerly *Paracoccidioides brasiliensis*); and 4 in *Blastomyces dermatitidis* (de Jonge and Thomma, 2009; Martinez *et al.*, 2012; Whiston and Taylor, 2016; Cen *et al.*, 2017; Goughenour *et al.*, 2021). A comparative phylogenomic analysis of pathogenic and nonpathogenic Onygenales species revealed a gene contraction of the LysM protein family in the thermally dimorphic fungi within the order Onygenales, instead of the previously proposed gene expansion in the dermatophytes (Whiston and Taylor, 2016). However, significantly higher numbers have been identified in thermally dimorphic fungi belonging to the order Ophiostomatales such as *Sporothrix brasiliensis* and *Sporothrix schenkii* that contain 13 and 14 LysM proteins, respectively (Teixeira *et al.*, 2014). It has been proposed that evolutionary adaptations from plant-associated to mammalian pathogenic lifestyle has led to a gene expansion of the LysM protein family in the *Sporothrix* lineage (Teixeira *et al.*, 2014; Huang *et al.*, 2020). In *H. capsulatum*, the *CTS3* gene encoding a subgroup C chitinase was the only chitinase gene with an increased expression in the mycelial phase compared with yeast phase, suggesting a functional role of the Cts3 chitinase in the mycelium of this fungus. Additionally, enzymatic activity assays demonstrated that Cts3 has endochitinase activity and chitobiosidase function consistent with hydrolysis of internal glycosidic bonds in cell wall polysaccharides (Goughenour *et al.*, 2021).

5. What are the biological functions of LysM proteins in mammalian fungal pathogens?

Considerable research has been carried out on LysM proteins of plant pathogenic fungi revealing a diverse and complex array of roles in plant-fungus interactions. Nevertheless, studies about the functional characterization and transcriptional regulation of LysM proteins in mammalian pathogenic fungi are still limited (Bueter *et al.*, 2013; Kombrink and Thomma, 2013; Akcapinar *et al.*, 2015; Muraosa *et al.*, 2019).

Fungi are characterized by the presence of chitin, a polysaccharide of GlcNAc units that is a major structural component of cell walls. The innate immune system of non-chitin-containing organisms like mammals and plants recognize fungal cells by the specific detection of chitin and produce chitinases to eliminate fungal pathogens (Lenardon *et al.*, 2010; Kombrink *et al.*, 2011; Bueter *et al.*, 2013; Kombrink and Thomma, 2013; Cen *et al.*, 2017; Gow *et al.*, 2017; Chen *et al.*, 2020). In plants, the hydrolytic activity of chitinases inhibit fungal growth and release chitin oligosaccharides from fungal cell walls that are recognized by host immune receptors to activate further immune responses. In many plant pathogenic fungi, secreted LysM proteins act as effectors that suppress chitin-induced immune response through scavenging of chitin oligosaccharides. Furthermore, LysM effectors can function as a barrier to protect fungal cell walls from the degradation by plant chitinases or can bind to plant chitinases inhibiting their activity (Bolton *et al.*, 2008; de Jonge and Thomma, 2009; Kombrink and Thomma, 2013; Romero-Contreras *et al.*, 2019; Sánchez-Vallet *et al.*, 2020). Likewise, some LysM proteins of entomopathogenic and saprophytic fungi may be involved in avoiding recognition of chitin and protecting fungal cells from chitinase hydrolysis (de Jonge and Thomma, 2009; Kombrink and Thomma, 2013; Cen *et al.*, 2017; Romero-Contreras *et al.*, 2019; Dubey *et al.*, 2020). In mammals, the mechanism of chitin recognition by the immune system still remains poorly understood. However, recently it has been reported that LYSMD3, a human protein containing a LysM domain, acts as a pattern recognition receptor for chitin in epithelial cells (He *et al.*, 2021).

Due to the absence of host specificity and the reduced host adaptation of many mammalian pathogenic fungi, it has been hypothesized that effector proteins are not necessary for the infection by mammalian fungal pathogens (Lowe and Howlett, 2012). However, the presence of LysM proteins in mammalian pathogenic fungi suggests that these proteins play important functional roles in this fungal lifestyle. It has been proposed that LysM proteins may bind and mask chitin to evade the immune system of mammalian hosts in a similar way as plant fungal pathogens (de Jonge and Thomma, 2009; Kombrink *et al.*, 2011; Martinez *et al.*, 2012; Kombrink and Thomma, 2013). Besides, it appears that the number of LysM proteins correlates with the cell wall chitin content in the morphological groups of mammalian fungal pathogens. The absence of chitin on the cell wall has only been described in *Pneumocystis* species (Ma *et al.*, 2016). Microsporidia lack chitin on the cell wall during the vegetative stage, but this polysaccharide is present in their spore walls (James and Berbee, 2012; Han *et al.*, 2020). Cell walls of yeasts and yeast-like fungi have a relatively low chitin content (0,5-5%), while filamentous fungi contain up to 20% or more of chitin in their cell walls (Hartl *et al.*, 2012; Akcapinar *et al.*, 2015; Garcia-Rubio *et al.*, 2020; Goughenour *et al.*, 2021). In addition to promoting host colonization, LysM proteins may also be necessary for survival in the environment of opportunistic fungal pathogens with a saprophytic lifestyle that are not specialized for the mammalian host (Kombrink and Thomma, 2013). This could also be an explanation for the absence or limited number of LysM proteins in some species of mammalian pathogenic fungi without a free-living lifestyle, such as obligate parasitic fungi, yeasts and yeast-like fungi and the dematiaceous fungus *R. mackenziei* (de Jonge and Thomma, 2009; Kombrink and Thomma, 2013; Teixeira *et al.*, 2017).

In dermatophyte fungi, LysM proteins may be required for cell wall modification, chitin metabolism, defence from other fungi or growth in a wide variety of niches such as the soil and keratinized tissues of mammals (Martinez *et al.*, 2012; Persinoti *et al.*, 2018; Lopes *et al.*, 2020). In this regard, a recent study has demonstrated that LysM1 (TERG_05627) and LysM2 (TERG_01873) effectors of *T. rubrum* are able to bind both fungal cell wall chitin and human skin glycoproteins. Consequently, it has been hypothesized that LysM proteins could also function as adhesion proteins during the infection process (Kar *et al.*, 2019). In contrast, based on the gene contraction of the LysM protein family in the thermally dimorphic fungi within the order Onygenales, it has been proposed that LysM proteins may be immunoreactive and could represent a disadvantage for mammalian fungal pathogens causing systemic infections

(Whiston and Taylor, 2016). As LysM domains specifically bind to fungal chitin or bacterial peptidoglycan, this gene contraction could also reflect the occurrence of reduced fungal-fungal or bacterial-fungal interactions during the life cycle of the thermally dimorphic fungi within the order Onygenales (Whiston and Taylor, 2016).

6. Conclusions

The high variability in repertoire, primary sequence, length and domain architecture of LysM proteins among mammalian pathogenic fungi, even between species or species groups closely related or sharing the same mammalian host, suggests that the LysM protein family plays important biological roles in this fungal lifestyle. However, studies dealing with the functional characterization of LysM proteins have been only performed within a few species of mammalian pathogenic fungi (Table 1) and many questions remain to be elucidated. Despite the availability of several host-infecting transcriptomes of mammalian fungal pathogens (Cairns *et al.*, 2010), further specific studies should be conducted on the transcriptional regulation of LysM genes in mammalian fungal pathogens to analyze their individual and global expression profiles during the infection process. Finally, as chitin is absent in mammals and is a major constituent of fungal cell wall, an interesting area of research is the development of new antifungal therapies based on LysM proteins of mammalian pathogenic fungi. For instance, the observed repressive effect of undecanoic acid against the TERG_05625 gene encoding a subgroup C chitinase of the dermatophyte *T. rubrum* highlights that LysM proteins can be used as potential targets in antifungal strategies (Martins *et al.*, 2019).

Declaration of competing interest

The author has declared that no competing interests exist.

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