Fungal aegerolysin-like proteins: distribution, activities, and applications

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Received: 29 September 2014 / Revised: 13 November 2014 / Accepted: 14 November 2014
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Abstract The aegerolysin protein family (from aegerolysin of the mushroom Agrocybe aegerita) comprises proteins of ~15–20 kDa from various eukaryotic and bacterial taxa. Aegerolysins are inconsistently distributed among fungal species, and variable numbers of homologs have been reported for species within the same genus. As such noncore proteins, without a member of a protein family in each of the sequenced fungi, they can give insight into different species-specific processes. Some aegerolysins have been reported to be hemolytically active against mammalian erythrocytes. However, some function as bi-component proteins that have membrane activity in concert with another protein that contains a membrane attack complex/perforin domain. The function of most of aegerolysins is unknown, although some have been suggested to have a role in development of the organism. Potential biotechnological applications of aegerolysins are already evident, despite the limited scientific knowledge available at present. Some mushroom aegerolysins, for example, can be used as markers to detect and label specific membrane lipids. Others can be used as biomarkers of fungal exposure, where their genes can serve as targets for detection of fungi and their progression during infectious diseases. Antibodies against aegerolysins can also be raised as immuno-diagnostic tools. Aegerolysins have been shown to serve as a species determination tool for fungal phytopathogen isolates in terms of some closely related species, where commonly used internal transcribed spacer barcoding has failed. Moreover, strong promoters that regulate aegerolysin genes can promote secretion of heterologous proteins from fungi and have been successfully applied in simultaneous multi-gene expression techniques.

Keywords Fungi · Aegerolysin · Hemolysin · Lipid markers · Fungal biomarkers · Heterologous expression

Introduction

The inventory of potential molecules available from fungi has been traditionally exploited in human and animal nutrition and medicine, and in biotechnological applications (Robson et al. 2007; Pointing and Hyde 2001; Erjavec et al. 2012; Nayak et al. 2013). On the other hand, fungi and many of their products might threaten the health of humans, animals, and plants, and the ecosystem (Fisher et al. 2012). Here, we have focused on the aegerolysins, a family of proteins that have molecular weights of 15 to 20 kDa. Since the 1990s, this protein family has been known as the aegerolysins, according to aegerolysin from the mushroom Agrocybe aegerita, which is coded by the Aa-PriA1 gene (Fernandez Espinar and Labarere 1997; Berne et al. 2002). The members of the aegerolysin protein family given in databases have increased tremendously more recently, most of which have been predicted from genomic data. However, the biological roles of the aegerolysins remain generally elusive. In this mini-review, we will present the current data on fungal aegerolysins and their relevance for various biomedical and biotechnological applications.

Distribution of aegerolysins in fungi

The aegerolysin family (Pfam 06355, InterPro IPR009413) currently contains over 300 protein entries in the UniProt
database. These proteins are from various bacterial and eukaryotic (e.g., animals, plants, and fungi) taxa, and also from a virus (Berne et al. 2009). So far, studies on the aegerolysins have been predominately focused on the fungal representatives, rather than the bacterial representatives. Aegerolysins are small proteins that have a low isoelectric point and two conserved cysteine residues. Only the crystal structure of the bacterial aegerolysin Cry34A (from *Bacillus thuringiensis*) has been determined to date (Schnepf 2003; Schnepf et al. 2005). This solved structure confirmed previous experimental (Berne et al. 2005; Nayak et al. 2011a; Shibata et al. 2010) and prediction data, all of which argued that the aegerolysins are β-structured proteins (Berne et al. 2009; Ota et al. 2014).

Fungal aegerolysins are inconsistently distributed among species, and a variable number of homologous proteins belonging to this family can be found in different species within the same genera (Lakkireddy et al. 2011; Nayak et al. 2013). In April 2014, we performed a bioinformatics search for the taxonomic distribution of putative aegerolysin proteins in the fungal kingdom, with the collection of data from different databases. BLAST algorithms for the aegerolysins based on osteolysin A (OlyA) (from the mushroom *Pleurotus ostreatus*), Asp-hemolysin (from *Aspergillus fumigatus*; encoded by the *hlyA* or *AspHS* gene), and the Pfam domain PF06355 were used to search the databases of the National Center for Biotechnology Information (NCBI), the Joint Genome Institute (JGI) Mycocosm (Grigoriev et al. 2012), and the *Aspergillus* genome database (AspGD) (Arnaud et al. 2012), in combinations with hits from the Pfam database (version 27.0) (Finn et al. 2014). The taxonomic distributions were adopted according to JGI Mycocosm webpage (Grigoriev et al. 2012), to provide insight also about the number of sequenced fungal species without aegerolysins. Putative aegerolysins were recognized as noncore proteins (identified only in some, but not all, of the sequenced fungi), and they can be found in more than 20% of species across the fungal kingdom. Indeed, no obvious correlations to different lifestyles were observed. Putative aegerolysins are present in edible mushrooms, phytopathogens, and opportunistic human (animal) pathogens, as well as in saprotrophs (Kraševce, personal communication). The level of species that have putative aegerolysins now ranges from about one fifth of the species in *Agaricomycotina, Pezizomycetes, Dothideomycetes*, and *Sordariomycetes*, to about three quarters of the species in *Eurotiomycetes* (Table 1). For all of the other listed taxa, no putative aegerolysin sequences have been identified to date. In total, 160 putative aegerolysin sequences were identified in our searches, with about half of the representatives in *Eurotiomycetes*. This relates to the over 300 genomes with mostly biased sampling that have been sequenced to date (including mostly model fungi, biotechnological relevant species, and serious pathogens), out of the estimated 1.5 million fungal species, so the recorded observations still represent only a glimpse into the patchy distribution pattern of the aegerolysins.

Activities of the aegerolysin proteins

The first reported aegerolysin-like protein was Asp-hemolysin from the filamentous fungus *A. fumigatus*, which is the main causative agent of invasive aspergillosis in immunocompromised patients; Asp-hemolysin was isolated and characterized as a hemolytic protein (Sakaguchi et al. 1975). It was later shown to bind to lysophospholipids and to oxidized low-density lipoprotein (Kudo et al. 2001; Sakai et al. 1994), to be toxic for animals and different cells, such as macrophages, leukocytes, and vascular endothelial cells (Ebina et al. 1982; Kumagai et al. 1999; Kumagai et al. 2001), and to trigger immune responses in humans, by induction of the production of histamine and cytokines (e.g., tumor necrosis factor-α, and interleukins 6 and 8) (Iwata et al. 1962; Kumagai et al. 2001). Consequently, it was believed that Asp-hemolysin might be one of the important virulence factors of *A. fumigatus*, although recent findings have contradicted this belief (Wartenberg et al. 2011), as hemolysis and cytotoxicity were not altered in both single-deletion and double-deletion mutants lacking either one or both of the aegerolysin genes present in *A. fumigatus*.

Along with Asp-hemolysin, the native aegerolysin Aa-Pri1 from *Ag. aegerita* and recombinant terrelysin from *Aspergillus terreus* have been suggested to be hemolytic (Berne et al. 2002; Nayak et al. 2011a). On the contrary, OlyA, pleurotolsin A (PlyA), and erylsin A (EryA), which are aegerolysins from *Pleurotus* spp., are not hemolytically active per se, as each functions as part of the bi-component pore-forming proteins that include pleurotolsin B (PlyB) and erylsin B (EryB), respectively, which belong to the family of proteins with a membrane attack complex/perforin (MACPF) domain (Anderluth et al. 2014; Gilbert et al. 2013; Ota et al. 2013; Shibata et al. 2010; Tomita et al. 2004). As well as having hemolytic activity, OlyA (or an isoformic PlyA) and its partnering protein (PlyB, with the MACPF domain) can also form pores in lipid vesicles composed of either total erythrocyte lipids or sphingomyelin and cholesterol (Ota et al. 2013; Sepić et al. 2004; Tomita et al. 2004).

In the genome of the filamentous fungus *Aspergillus niger*, we identified two genes that encode aegerolysin proteins, and two genes that encode proteins with the MACPF domain. Whether these aegerolysins have similar characteristics as the others remains to be determined, although hemolysis has been observed while growing *As. niger* on agar plates supplemented with sheep blood (Fig. 1). However, a very recent study revealed that rather than aegerolysins, the hemolysis seen with *As. niger* might be caused by increased production of free fatty acids (Novak et al. 2014). In addition, this hemolytic activity could also be caused by another neurotoxic
hemolysin found in this fungus, nigerlysin (α-helical protein with a molecular weight of 72 kDa) (Donohue et al. 2006), or extracellular proteases that have been found in hemolytically active fractions of As. terreus culture supernatant along with the native terrelysin (Nayak et al. 2011a, b).

Despite numerous studies, the functions and biological roles of fungal aegerolysins still remain matters of speculation. OlyA (from Pl. ostreatus), Aa-Pr1 (from Ag. aegerita), Pe. PlyA and Pe. ostreolysin (=EryA) (from Pleurotus eryngii), and two aegerolysins from the cocoa tree pathogen, Moniliophthora perniciosa, have all been shown to be present or highly expressed in primordia and fruiting bodies (Berne et al. 2002; Fernandez Espinar and Labarere 1997; Joh et al. 2007; Kurahashi et al. 2014; Lee et al. 2002; Vidic et al. 2005).

Time-course studies performed for the expression of Asp-hemolysin and terrelysin have also showed that their expression is highest during early fungal growth (Nayak et al. 2012; Wartenberg et al. 2011). All of this suggests that these aegerolysins have important roles in fungal development (Berne et al. 2009; Nayak et al. 2013; Ota et al. 2014; Table 1).

<table>
<thead>
<tr>
<th>Taxona</th>
<th>Number of species in taxon</th>
<th>Number of species with aegerolysins</th>
<th>Percent of species with aegerolysins</th>
<th>Number of aegerolysin sequences</th>
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\*Taxonomical distribution was adopted according to JGI Mycocosm web page (Grigoriev et al. 2012) as on April, 2014.

Fig. 1 Hemolysis induced by Aspergillus niger. Top (a) and bottom (b) views of Aspergillus niger N402 mycelia (4 days old) and the filtrate (c) from minimal medium liquid culture after 2 or 3 days on tryptic soy agar supplemented with 5 % defibrillated sheep blood. Partial hemolysis is observed as the greenish agar around and underneath the colony and around the hole in the agar.
Vesper and Vesper 2004). However, taking into account their inconsistent distributions among closely related fungi, their ability to form pores in vesicles of specific lipid compositions, and their hemolytic activities that are in some cases achieved only when combined with the B component, it is possible that their roles are pleiotropic, and thus, they might be involved in various other physiological processes related to the fungal lifestyle. Although these functions and biological roles of the fungal aegerolysins remain to be determined, they have potential biotechnological appeal due to certain of their characteristics (Fig. 2).

Are aegerolysins involved in mushroom fruiting?

The identification of genes and proteins involved in the fruiting of edible mushrooms, and the monitoring of the formation of the mushroom fruiting body following stimulation of fruiting by different stimuli, are of utmost importance for the control of mushroom fructification in biotechnological processes. Fruiting initiation in mushrooms can be triggered by a variety of environmental (e.g., light and temperature) and biochemical stimuli, which include substances of natural and synthetic origin. In particular, the fruiting in Pl. ostreatus has been shown to be induced by addition of veratryl alcohol (Suguimoto et al. 2001), β-adenosine (Domondon et al. 2004), phenol (Upadhyay and Hofrichter 1993), saponins (Magae 1999; Magae et al. 2009), associated bacteria (Cho et al. 2003), and some synthetic (Magae et al. 2005) and natural (Berne et al. 2008) surfactants.

Analyses of the spatial and temporal expression of some fungal and bacterial aegerolysins have suggested their involvement in the initiation of fructification and/or sporulation. For example, OlyA is preferentially located in the basidia and basidiospores of young fruiting bodies, and not in the vegetative mycelium of Pl. ostreatus, and its expression has been shown to be initiated at the stage of primordia, and to reach its maximum at the stage of young fruiting bodies (Berne et al. 2002; Vidic et al. 2005). Direct involvement of OlyA in sporulation is, however, unlikely, as it has been detected in large amounts also in a nonsporulating strain of Pl. ostreatus (Berne et al. 2007). The same expression pattern has been reported for another almost identical protein, aegerolysin from the black poplar mushroom Ag. aegerita (Fernandez Espinar and Labarere 1997), while some bacterial (Barloy et al. 1998) and ascomycetal (Bando et al. 2011) aegerolysins appear to be related to bacterial and fungal sporulation, respectively.

When native ostreolysin (Oly=OlyA contaminated with minor amounts of PlyB; Ota et al. 2013) was applied externally at 1 to 250 ng/mm² to solid nutrient media inoculated with mycelia of Pl. ostreatus, slight and transient inhibition of mycelial growth was observed, in comparison with control plates. However, Oly strongly induced the formation of primordia, which appeared 10 days earlier than in the control plates. Moreover, Oly stimulated the subsequent development of primordia into fruiting bodies (Berne et al. 2007). These findings make OlyA and the other aegerolysin-like proteins and their engineered recombinant forms interesting from the commercial point of view. Indeed, knowledge of the cellular processes involved in fructification is lacking in several edible mushrooms, including Pl. ostreatus, so all of the findings related to the identification of genes and proteins involved in their fruiting might contribute to the control of fruiting body initiation, the pivotal step in the production of mushrooms.

Aegerolysin proteins as membrane lipid markers

The binding and lytic activity of native Oly has been shown to be greatest on membranes that are highly enriched in

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![Fig. 2](image-url)  
**Fig. 2** Scheme summarizing the putative functions of aegerolysins in fungi (a), and the possible applications for diagnostic or biotechnological purposes (b). Putative model of aegerolysin-like protein from *Aspergillus niger* showing actinoporin-like fold is inferred by Phyre2 server (Kelley and Sternberg 2009) on the OlyA6 model (Ota et al. 2013). MACP, membrane attack complex/perforin domain
combined cholesterol and sphingomyelin (Sepčić et al. 2004; Rebolj et al. 2006). Immuno-detection studies have shown that native Oly binds to different cell lines and that this binding is inhibited if the cells are pre-treated with a cholesterol scavenger, methyl-β-cyclodextrin (Chowdhury et al. 2008; Resnik et al. 2011). Immuno-staining showed no co-localization of Oly with some established raft-binding proteins, like the cholera toxin B-subunit (Chowdhury et al. 2008). Native OlyA and recombinant OlyA were further shown to be responsible for binding to sphingomyelin/cholesterol-enriched membrane domains (Ota et al. 2013). As OlyA is non-toxic in itself, the idea to use this protein as a probe for cholesterol-rich domains in natural and artificial membranes has emerged (Ota et al. 2013).

OlyA fused with the mCherry fluorescent protein (OlyA-mCherry) was produced for direct labeling of fixed and living cells (Fig. 3) (Skočaj et al. 2014). Similarly, another almost identical aegerolysin, pleurotolysin A2 (PlyA2), which has also been shown to bind to sphingomyelin- and cholesterol-rich membranes, was fused with enhanced green fluorescent protein (EGFP) and used to label fixed HeLa cells (Bhat et al. 2013). Pre-incubation of the cells with methyl-β-cyclodextrin or sphingomyelinase totally abolished the binding of both OlyA-mCherry and PlyA2-EGFP to the cells, which demonstrated that both cholesterol and sphingomyelin are prerequisites for the membrane binding of these aegerolysins. The binding specificity of the PlyA2-EGFP fusion protein to different sterols was characterized by incubation of PlyA2-EGFP with equimolar mixtures of sphingomyelin and various sterols. High binding affinity was observed for cholesterol, β-sitosterol, and 7-dehydrocholesterol, and only weak binding affinity was seen for ergosterol. In contrast, no binding of PlyA2-EGFP was observed for lanosterol, 6-ketocholestanol, and epicholesterol. This indicated the importance of the stereochemical configuration of the 3-hydroxyl group on the sterol. Modification of the sterol tail had little influence, while bulky groups on the A-ring and B-ring of the sterol backbone completely abolished PlyA2-EGFP binding (Bhat et al. 2013). Membrane binding of Oly was also shown to be dependent on the intact sterol 3β-OH group, and to decrease following minor modifications to the steroid skeleton (Rebolj et al. 2006).

The expression of both OlyA-mCherry and PlyA2-EGFP inside the cells revealed the absence of sphingomyelin/cholesterol-rich domains in the cytosol-exposed leaflets of the intracellular membranes. PlyA2 co-localized with the raft marker CD59 and with a late endosomal marker, but not with the transferrin receptor or with markers of early endosomes or the Golgi apparatus. Similarly, the mode of OlyA-mCherry binding to the plasma membrane was compared to the binding patterns of natural toxin analogs that specifically target raft-associated lipids (Skočaj et al. 2013, 2014). These data clearly showed that OlyA-mCherry labels different pools of lipids compared to other toxins that bind to components of membrane rafts. OlyA-mCherry was also endocytosed when incubated with living Madin-Darby canine kidney cells, and the internalization pathway was followed (Skočaj et al. 2014). The protein entered the cell mainly by the raft-dependent caveolin-1 pathway. After internalization, OlyA-mCherry was found in early endosomes (after 5 min), in late caveolar compartments (after 30 min), and finally in the juxta-nuclear region of the cell near to the Golgi apparatus (after 90 min). OlyA-mCherry and PlyA2-EGFP have demonstrated lipid rafts in cells, and therefore these nontoxic proteins show good potential to be used as versatile tools to track membrane rafts in living cells, as well as providing tools to investigate their overall biological roles in healthy and abnormal tissues or cells. Moreover, OlyA was suggested to be used as a vehicle to direct other proteins of choice into the endosomal recycling route upon fusion with OlyA (Skočaj et al. 2014).

**Biomarkers of fungal exposure**

For the diagnosis of personal exposure to fungi in general, and more importantly to causative agents of fungal infection (in plants, animals, humans), suitable biomarkers are needed. Some aegerolysin-like proteins from various species of *Aspergillus* genus have been reported to be released into the

![Fig. 3](image_url)  
**Fig. 3** Labeling of living Madin-Darby canine kidney cells with OlyA-mCherry. Representative fluorescent images of living Madin-Darby canine kidney cells grown at 37 °C after 5-min (a) and 30-min (b) incubations with 1 μM OlyA-mCherry at 37 °C. Internalization can be seen after 30 min of incubation. Blue, DAPI; red, OlyA-mCherry. Scale bars, 20 μm.
Invasive aspergillosis is a serious nosocomial infection that is caused by *Aspergillus* spp., and difficulties in its early diagnosis have increased the already high mortality rates. The existing methods have limited diagnostic value, and even rapid methods, such as those based on galactomannan detection, are not completely reliable for the identification of invasive aspergillosis. Within the *Aspergillus* genus, *A. fumigatus* is the main species that causes invasive aspergillosis; however, *Aspergillus flavus*, *As. niger*, and *As. terreus* have also been reported as causative for aspergillosis. The galactomannan test cannot identify the specific species that causes this infection, and it also shows variable sensitivity. Most of the more recent studies on the clinical application of quantitative real-time PCR for invasive aspergillosis detection have used multiplex gene targets, such as 18S or 28S ribosomal DNA (rDNA) sequences. However, the rDNA sequences are highly conserved in nature as well, so they are more suitable for fungal detection in general, or for *Aspergillus* genus detection, and they cannot distinguish between different species. The product of the *aspHS* gene is an aegerolysin that is overexpressed in vivo during infection, and it has been proposed as a novel target for specific detection of *A. fumigatus* by quantitative real-time PCR (Abad-Diaz-de-Cerio et al. 2013). In combination with the DNA extraction method, this method can detect *A. fumigatus* with high specificity. Furthermore, this method can distinguish between nongerminating conidia, where the aegerolysin is not detected, and germinating conidia, where the aegerolysin is detected, which can thus indicate the initiation of infection. Abad-Diaz-de-Cerio et al. (2013) suggested that these techniques can be sufficiently sensitive and rapid to help clinicians in earlier diagnosis, although the presence of PCR inhibitors in clinical samples like human bronchoalveolar lavage fluids needs to be addressed.

**Aegerolysins as markers in diagnosis of fungal diseases**

The detection of the fungal aegerolysins Asp-hemolysin and terrelysin produced by *A. fumigatus* and *As. terreus*, respectively, in patient sera and environmental samples has been suggested as a biomarker of fungal exposure (Nayak et al. 2012). *As. terreus* is of particular interest, as it causes rapidly disseminating infections with high mortality rates, which mostly affect immuno-compromised patients, due to its natural resistance to antifungal drugs and its production of vegetative aleuropocnidia during infection. As current diagnostic strategies are limited for *Aspergilli*, there is a critical need for techniques to enable early and specific identification of these causative agents. Nayak and co-workers (2012) described monoclonal antibodies developed against a recombinant form of terrelysin. These monoclonal antibodies have been used to detect the terrelysin expressed during conidial germination and early growth of *As. terreus* hyphae. As terrelysin was in the culture supernatant, it might be possible to detect it in the serum of infected patients. However, this aspect has yet to be confirmed, as the early expression of terrelysin in the *As. terreus* growth cycle might limit its usefulness for detection of an infection (Nayak et al. 2012). Heesemann (2010) generated monoclonal antibodies against the secreted aegerolysin-like hemolysin (Hly) from *A. fumigatus*, for which the hemolysin had to be first produced recombinantly. These monoclonal antibodies accurately recognized Asp-hemolysin secreted from fungal cultures; however, detection of Asp-hemolysin in patient sera was not successful. This might have been due to digestion of the Asp-hemolysin by serum proteases, or to too low a concentration of Asp-hemolysin in the sera. The sensitivity of this test might be further improved, e.g., in a sandwich ELISA test (Heesemann 2010).

**Aegerolysins as a species determination tool**

The accuracy and effectiveness of phytosanitary activities are dependent upon the correct identification of the pest of interest from the outset. The cosmopolitan genus *Alternaria* Nees contains many species of economic importance, including saprophytes, phytopathogens, and zoopathogens, which are known to produce a variety of mycotoxins and allergens. A recent class-wide phylogenetic analysis placed the genus *Alternaria* in the Pleosporales, a group that is heavily represented by phytopathogens in the Dothideomycetes (Schoch et al. 2009). Internal transcribed spacers represent pieces of non-functional RNA that are located between structural ribosomal RNAs, and these have been described and selected for fungal barcoding. However, it has been acknowledged that internal transcribed spacers do not work for some clearly recognizable species (Schoch et al. 2012), which indicates the need for additional barcode genes for these fungi. The relatively invariable internal transcribed spacer sequence for *Alternaria* species did not resolve isolates of *Alternaria*.
alternata, Alternaria gaisen, Alternaria yaliinificiens, Alternaria arborescens, Alternaria tenissima, Alternaria mali, and Alternaria brassicicola.

When an analysis was performed using the partial genomic sequences of putative aegerolysin L152, the lineages were resolved and they reflected the diversity previously hypothesized by morphological evaluation of sporulation patterns (Roberts et al. 2011). The presence of putative aegerolysin homologs in both phytopathogenic and nonphytopathogenic species argues against their involvement in pathogenesis. Also, the putative aegerolysin gene does not reside on one of the conditionally disposable chromosomes, and it is therefore likely to be present in all small-spored Alternaria spp., and not just the toxin producers (Roberts et al. 2011). Although the internal transcribed spacer rDNA sequence region is generally accepted as the most likely candidate for fungal barcoding, the analysis of aegerolysin L152 sequences might resolve closely related species or species groups where other loci cannot, including internal transcribed spacers (Roberts et al. 2011). The sequences of putative aegerolysin homologs are variable and parsimony informative, and they warrant additional analyses with a broader isolate set that includes related genera and species. These might also be suitable for the complex of pathogenic Fusarium species.

Strong promoter for secretion of heterologous proteins from fungi

One way to improve protein expression in a host organism is to place a protein of interest downstream of transcription regulatory sequences that will promote the expression of the protein under given fermentation conditions. Significant glucose repression was detected in solid-state cultures based on steamed rice and wheat. Bando et al. (2011) reported on a novel promoter from a hemolysin-like gene (hlyA) that coded for an aegerolysin-like protein that was not repressed by glucose, and could be efficiently over-expressed in Aspergillus oryzae. Thus, for As. oryzae grown in wheat bran solid-state culture, expression of the homologous endoglucanase and two heterologous endoglucanases from Trichoderma reesei was shown to be stronger under the control of the hlyA promoter than under the control of the usually used amyA promoter, which suggests that the hlyA promoter might be useful for overproduction of other proteins as well. High-level transcriptional activity was also observed in liquid cultures where T. reesei does not sporulate. This suggests that the hlyA promoter is activated by conditions or factors that are not restricted to sporulation. Indeed, Bando et al. (2011) identified four putative CCAAT sequences, six putative Saccharomyces cerevisiae heat-shock transcription factor binding sequences (AGAAN), and two putative BrlA-binding sequences (MRAGGGR) that affected transcription. The CCAAT sequence is one of the most common cis elements in the promoter regions of numerous eukaryotic genes. The heat-shock transcription factor positively regulates the stress response gene family, and the brlA gene is a regulator of conidiation in As. oryzae.

Hatzmann (2011) reported on a new, strong, small, constitutive promoter that was derived from the As. niger hemolysin gene, or a truncated variant thereof, which had promoter activity in fungal host cells of As. oryzae, Penicillium chrysogenum, As. terreus, Aspergillus nidulans, As. niger, Aspergillus sojae, As. oryzae, Chrysosporium lucknowense, and T. reesei. A plasmid that contained the hlyA promoter and the glaB terminator was recently used as a target gene expression cassette (Hisada et al. 2013). Four highly secreted proteins, Taka-amylase A, glucoamylase, and two endoglucanases, were used as reader proteins and were fused to the heavy-chain antibody fragment to achieve high levels of production and secretion in As. oryzae. Hisada et al. (2013) also demonstrated high-level production of a heavy-chain antibody fragment fusion protein that approached industrial levels. Another example of the use of the aegerolysin promoter is the production of kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrene), one of the major secondary metabolites in As. oryzae that is widely used in food, pharmaceuticals, and cosmetics. Currently, kojic acid is produced from glucose, although Yamada and co-workers (2014) reported cost-effective production of kojic acid directly from inexpensive cellulose material using genetically engineered As. oryzae with a simultaneous multi-gene expression technique. In this case, a kojic acid transcription factor gene over-expressing strain was constructed to increase the transcription of three genes related to kojic acid production. Furthermore, this strain was additionally transformed by a three-cassette plasmid with the different promoters for the three different cellulase genes, one of which was controlled by the hlyA promoter and terminator.

Conclusions

Aegerolysins are widely and inconsistently distributed among fungal species in certain taxa, with no obvious correlation to various fungal lifestyles. Therefore, pleiotropic roles of aegerolysins are suggested in various physiological processes where the fungi communicate with their environment. When these data are taken together with the data from several studies over the past years, these reveal that not all aegerolysins have hemolytic activity, so still referring to them as hemolysins should be reconsidered. Even more importantly, reinvestigation of the hemolytic activity of Asp-hemolysin was suggested, as a non-cylolytic recombinant Asp-hemolysin has been reported (Ebina et al. 1994), and also, reinvestigation of A. fumigatus extracts has failed to demonstrate their
hemolytic activity (K. Sepčić, personal observation). At least some of the aegerolysins are successfully secreted into liquid fungal media without any classical secretion signal, so studying their secretory pathways might be of interest for further biotechnological applications for the production of heterologous proteins, and perhaps in combination with the already successfully applied promoters of these genes. Due to the different selectivities for lipids and combinations of lipids in lipid membranes, aegerolysins can be considered as key value biomarker tool for membrane studies. Nevertheless, the main challenge in aegerolysin research must be to discover their physiological roles and their relevance for the biology of the organism.

Acknowledgments The authors would like to thank to Sabina Belc for genome mining, Saža Rečonja and Nina Sluga for the hemolytic activity tests, Dr. Christopher Berrie for critical reading and English language editing, and the Slovenian Research Agency for financial support.

Conflicts of interest The authors declare that they have no conflicts of interest.

References


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