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15 Genetic and Metabolic Aspects of Primary and Secondary Metabolism of the Zygomycetes

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I. Introduction

Zygomycetes constitute a remarkable group of microscopic fungi (formerly classified into the phylum *Zygomycota*) basal to *Ascomycota* and *Basidiomycota* (for review see Voigt 2012; Voigt and Kirk 2014). These fungi are mainly soil inhabitants living as saprobes and decomposers of organic matter and herbivorous feces (coprophiles). Some taxa are parasitic or predacious, in which case developing mycelium is immersed in the host tissue:

Traditionally, the Zygomycota, represent the most basal terrestrial phylum of the kingdom of Fungi. The Zygomycota are not accepted as a valid phylum (as "Phylum des Zygomycètes"; Whittaker 1969; Cavalier-Smith 1981 because of a lacking compliance to the International Code of Botanical Nomenclature/International Code of Nomenclature for algae, fungi and plants (Hawksworth 2011) and lacking resolution of the basal fungal clades (James et al. 2006). Molecular phylogenetic analyses based on informal phylogenetic trees where molecular phylogenies are substituted with traditional taxonomic information revealed dispersal into five subphyla containing one to four orders (Hibbett et al. 2007; Hoffmann et al. 2011, for review see: Benny et al. 2014). The phylogenetic relationships between these subphyla and their orders is still not well resolved. However, based on the potential of all five subphyla to produce zygospores during conjugation of two yoke-shaped gametangia it is referred to a phylogenetically coherent group named zygosporic fungi as a whole group, which share morphological features but consists of phylogenetically unrelated subphyla. Therefore, the

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phylum referred to as "Zygomycota" is employed to make clear the term is being used in a colloquial sense, for instance the inclusion of all basal lineages of terrestrial fungi with the potential to form zygospores or sharing any other of the plesiomorphic morphological characters of the former phylum.

Cavalier-Smith (1981, 1998) introduced the name as "cl. nov." and comments that it does not appear to have been validly published elsewhere. Likewise, this class *Zygomycetes* does not appear to be monophyletic (James et al. 2006, for review and comprehensive phylogeny see Voigt and de Hoog 2013). Here the term "zygomycetes" is printed in lower-case letters and used in a colloquial sense for ecological groupings sharing soil as their main habitat. Zygomycetes are common and cosmopolitan components of the mycoflora of dung, soil, and other substrates that support their growth and sporulation.

A. Zygomycetes: Evolution, Systematics, and Ecology

Zygomycetes are ubiquitously distributed. The occurrence of zygomycetes dates back to the Precambrian era, 800–1400 million years ago (Heckman et al. 2001; Mendoza et al. 2014). During the course of their long evolutions, which has its roots in the Precambrian, they have learned to interact with many other microorganisms in a wide variety of interplays, e.g., symbiosis with endo- and ectosymbionts; commensalism and parasitism during zygomycetes become successful pathogens of plants, animals, and human. Interaction pattern appears to be instrumental for fitness reasons as shown in aphid–bacterium–fungus alliances lowering the rate of transmission diseases (Scarborough et al. 2005).

Zygomycetes encompass nine orders: *Asellariales*, *Dimargaritales*, *Endogonales*, *Entomophthorales*, *Harpellales*, *Kickxellales*, *Mortierellales*, *Mucorales*, and *Zoopagales* (for review see Voigt and Kirk 2014). Members of the *Asellariales* (18 species) have filamentous, branched thalli and reproduce asexually by arthrospore-like cells that disarticulate from their corresponding thallus. They inhabit the digestive tract of terrestrial, aquatic, and marine isopods as well as springtails by attachment to the cuticle or digestive tract via a holdfast. They are not immersed in the host tissue (Moss 1975; Lichtwardt and Manier 1978). The *Dimargaritales* (18 species) is comprised by **obligate haustorial mycoparasites of the** *Mucorales* (rarely, species of *Chaetomium* [*Ascomycota*: *Sordariales*]) which are saprobic, or coprophilous, and share the same habitat.

The *Endogonales* (15 species) is an order of **mainly ectomycorrhizal fungi**, in addition to some saprobes. Endogonalean fungi are widely associated with the earliest branching land plants. During their evolution, they give way to arbuscular mycorrhizal glomeromycotan fungi in later lineages. It has been hypothesized that *Endogone*-like fungi rather than (as previously proposed, Simon et al. 1993; Parniske 2008) the *Glomeromycota* enabled the establishment and growth of early land colonists and thus facilitated terrestrialization (Bidartondo et al. 2011).

The *Entomophthorales* (250 species) consists of **mainly entomogenous/entomopathogenic fungi** producing one of the most spectacular insect-killing mechanisms. They are occasionally saprobic and found in soil, but mainly parasites of insects ("insect destroyers"), and other arthropods, rarely of nematodes and tardigrades as hosts. Most species are obligate parasites, and, therefore, these are so highly adapted to their hosts that their lifestyle obligately relies on the close relation to the host insect throughout the entire fungal ontogeny making a fungal cultivation in axenic cultures impossible. Few recipes of pure cultures have been reported which are highly complex media often containing natural products or biopolymers (Grundschober et al. 1998; Delalibera et al. 2003). Even under these conditions, it is unlikely that growth will be typical, and certainly sporulation will rarely be present. The exceptions to this general property are species of the genus *Conidiobolus* which are saprobes from the soil and are of widespread distribution. They are frequently isolated from the soil and are easy to grow in culture. *Conidiobolus coronatus* is found to be associated with medical and veterinary cases of mainly local, chronically lapsed, entomophthoromycotic infections (for review see Rothhardt et al.

2011; Mendoza et al. 2014). Zygospores, where known, are formed on differentiated hyphae.

The ecology of the *Harpellales* (252 species) is similar to that of the *Asellariales* by endocommensal association with the aquatic larvae of arthropods (incl. crustaceans and diplopods, rarely isopods), found attached to the gut lining of the aquatic larvae.

The *Kickxellales* (37 species) comprise mainly **saprobes from soil or coprophilous in dung**, rarely as mycoparasites. The *Kickxellales* are of widespread occurrence apparently favoring somewhat dry climates rather than the wet tropics but are relatively under-recorded, so their true distribution, like that for many of the fungi, is unclear.

The order *Mortierellales* (79 species) possesses an extremely high ecological and physiological diversity enabling them to be distributed worldwide (for comprehensive phylogeny, see Nagy et al. 2011; Wagner et al. 2013). Most species are polyunsaturated fatty acid-based lipid-accumulating organisms (e.g., *Mortierella alpina*, for overview see Münchberg et al. 2012, 2015). One thermotolerant species, *M. wolfii*, has clinical relevance and appears as a causative agent of bovine abortion (Papp et al. 2011). Zygospores are mostly thin walled, not ornamented, and nonpigmented.

The order *Mucorales* (237 species) is the most prominent and the most studied group among the zygomycetes (Voigt and Kirk 2014). Members of the *Mucorales* constitute a remarkable group which encompass a wide variety of morphological appearances, ecological niches, and lifestyles (saprobic, facultative parasitic, opportunistic pathogenic) facilitating extensive evolutionary studies (Voigt and Wöstemeyer 2001; Voigt et al. 2009, 2013; Hoffmann et al. 2009, 2013).

Mucoralean species are **predominantly saprotrophic**, **soil inhabitants**, rarely mycoparasites (biotrophic fusion parasites) on other mucoralean hosts. Due to airborne spores, high germination, and growth rates, mucoralean species belong to the primary colonizers of organic substrates. As typical indoor contaminants and post-harvest pathogens on fruits and food causing food spoilage, mucoralean fungi are the most successful and most abundant zygosporic fungi encountering permanent presence in the human environment. Some mucoralean species are able to develop life-threatening infections within immunocompromised patients (mucormycosis) (de Hoog et al. 2000, 2014; Mendoza et al. 2014; Ibrahim 2011; Chayakulkeeree et al. 2006; Greenberg et al. 2004; Bitar et al. 2009; Chakrabarti et al. 2008, 2009; Morace and Borghi 2012; Casadevall and Pirofski 2001). On the other hand, mucoralean species are used for fermentation of soy-based food in Asia since centuries and for the application of *Rhizopus* species in biotechnological production of enzymes for decades.

The *Zoopagales* (208 species) is, even though species rich, relatively unknown concerning its frequency and distribution. They appear to be cosmopolitan as obligate haustorial parasites of fungi and animals (nematodes, Amoeba, and other small terrestrial invertebrates).

B. The Cooperative Nature of Zygomycetes: Bacterial–Fungal Alliances

The observation that progressive coupling of fungal host and bacterial endosymbiont metabolic and reproductive interests leads to an acceleration of studies reporting the cooperative nature of bacterial-fungal alliances in zygomycetes (Partida-Martinez and Hertweck 2005). The macrocyclic polyketide metabolite rhizoxin has been frequently isolated from cultures of *Rhizopus microsporus*, which is infamous for causing rice seedling blight (Tsuruo et al. 1986; White et al. 2002: *R. chinensis* synonym of *R. microsporus*; Dolatabadi et al. 2014a). Among other antimicrotubule agents, **rhizoxin** was proven to be particularly effective in small-cell lung cancer cell lines with a potential application in the salvage treatment of refractory or relapsed patients suffering small-cell lung cancer to overcome drug-resistance (Ikubo et al. 1999). **Rhizoxin is not biosynthesized by the fungus itself** but by an endosymbiotic, that is, intracellular living, bacterium of the genus *Burkholderia* (Partida-Martinez and Hertweck 2005). The remarkably complex symbiotic–pathogenic relationship that extends the fungus–plant interaction to a third, bacterial, key player unveils new perspectives for pest con-

trol. This finding appeared to be initially unexpected and unique, but the cases of endosymbiotic bacterial alliances with zygomycetes have increased during the following time (*Mortierella elongata*: Sato et al. 2010; Bonito et al. 2013, *Rhizopus chinensis*: White et al. 2002). All bacterial endosymbionts discovered so far in the zygomycetes belong to the family *Burkholderiaceae* (class *Betaproteobacteria*, Sato et al. 2010) and are closely related to *Glomeribacter gigasporarum*, which is an obligate endosymbiotic bacterium of the arbuscular mycorrhizal fungus *Gigaspora margarita* (Bianciotto et al. 2003). *Glomeribacter gigasporarum* reveals an interphylum network of nutritional interactions (Ghignone et al. 2012). On the other hand, the ~2.6 MB endosymbiont genome of *M. elongata* is larger than that of *Glomeribacter* but reduced compared to free-living *Burkholderia* (Bonito et al. 2013; Fujimura et al. 2014). Thus, intimate coevolution seems to be more recent than that of the alliance between *Glomeribacter gigasporarum* and *Gigaspora margarita*. Although many genes have been lost (e.g., genes encoding starch- or sucrose-degradation enzymes, phosphofructokinase leading to an incomplete glycolysis pathway, enzymes involved in the synthesis of the essential amino acids arginine, isoleucine, leucine, methionine, phenylalanine, tryptophan, histidine, and valine), some gene families have expanded including those involved in protein metabolism and electron transport (e.g., genes encoding amino acid transporters such as proteins involved in phosphate, zinc, and putrescine uptake; Ghignone et al. 2012). A gene cluster coding for a dipeptide/heme/δ-aminolevulinic acid transporters (*dpp* operon) contains the *dppA* gene, the product of which is responsible for the specificity of the imported oligopeptides and is present in at least 20 copies, suggesting that peptide uptake is crucial for bacterial cell function (Ghignone et al. 2012):

Rhizopus species appear to be trans-kingdom pathogens causing soil-, air and foodborne diseases in plants and humans (de Hoog et al. 2000; Dolatabadi et al. 2014b). The frequency of opportunistic mycoses in human began to rise since the mid 1990s (Ribes et al. 2000; Kauffman 2004; Chamilos et al. 2007). With regard to human pathogens, endosymbiotic toxin-producing bacteria in clinical *Rhizopus* isolates appear to be rather an exception then a general feature (Partida-Martinez et al. 2008). No evidence was found that bacterial endosymbionts and rhizoxin contribute to the pathogenesis of mucormycosis (Ibrahim et al. 2008). Consequently, it remains unclear if the paradigm of modulation of virulence of opportunistic fungi by widespread use of antibacterials can be applied (Chamilos et al. 2007).

II. Key Aspects in the Metabolism of Zygomycetes: Biotechnological Implications

Since centuries zygomycetes are traditionally used for the fermentative production of food in China and Southeast Asia, e.g., for tempeh or tofu (Wikandari et al. 2012; Hesseltine 1983). However, recent studies of the last few years have shown that mucoralean species can also produce a large amount of interesting and biotechnological relevant metabolites, including organic acid, e.g., lactic acid and fumaric acid, biofuels, e.g., ethanol and biodiesel, polyunsaturated fatty acids, carotenoids, chitosan, and various enzymes, e.g., amylases, cellulases, steroid 11α-hydroxylases, phytases, proteases, and lipases. Additionally, biomass can be used as animal and fish feed due to its high nutritional value (Ferreira et al. 2013; Karimi and Zamani 2013; Meussen et al. 2012).

Zygomycetous fungi show several characteristics which are advantageous in biotechnological applications: (1) one of the **highest fungal growth rates**, enabling fast biomass accumulation; (2) ability of growing at **higher temperature** for many of the species; (3) **dimorphism** of various genera, transition from filamentous growth to yeastlike growth under oxygen limitation or at high glucose concentrations (e.g., Orlowsky 1991); (4) simple demands on culture conditions; and (5) ability to produce a high diversity of enzymes enabling growth on diverse substrates, like starch or starch-containing residual materials, lignocellulosic substrates or whey, within wide temperature and pH ranges (Zhang et al. 2007; Dyal et al. 2005; Millati et al. 2005; Sautour et al. 2002; Nahas 1988; Sajbidor et al. 1988). Mucor-

alean species are amylase positive and are able to use pentoses; therefore, they can be directly applied to ethanol production from starch-containing or lignocellulosic substrates (SSF—simultaneous saccharification and fermentation) (Deng et al. 2012; Zhang et al. 2007; Jin et al. 2005). Nevertheless, the number of mucoralean species applied to established biotechnological processes is scarce:

> Currently, only few species have been fully characterized regarding their potential to produce metabolites and enzymes. Research is primarily focussed on three genera: *Rhizopus* species for the production of organic acids, *Mucor* species for the production of ethanol and single cell oil (Ferreira et al. 2013) and *Cunninghamella* species for single cell oil production (Fakas et al. 2009). Interestingly, the transition from filamentous growth to yeast-like growth in dimorphic species is triggered by similar conditions favourable for organic acid and ethanol production, particularly high glucose concentrations along with elevated CO_2 contents (Lennartsson et al. 2009; Sharifia et al. 2008; Wolff and Arnau 2002; Serrano et al. 2001; Orlowsky 1991; Bartnicki-Garcia 1968). Therefore, due to the highly beneficial characteristics and promising biotechnological potential of mucoralean species, research exploring metabolite and enzyme production is urgently needed.

Biotechnologically relevant metabolites produced by zygomycetes are ethanol, carotenoids, fatty acids, organic acids, and single cell oils (SCOs) rich in polyunsaturated fatty acids (PUFAs) which are also named storage lipids. SCOs are known for their bifunction as a supplier of functional oils, and feedstock for biodiesel production (Huang et al. 2013). Especially organic acids and single cell oils containing PUFAs have a high market value, but suitable production strains and economically efficient processes are not available. Therefore, research on these substances would imply a high impact on white biotechnological issues.

A. Carotene Biosynthesis and Degradation: Primary Meets Secondary Metabolism

Despite their negative impact on humans and agriculture, zygomycetes could also be used in a positive way, like fermentations of food and sterols or the production of additives for food, feed, or pharmaceuticals with major interest in biological production of carotenoids, e.g. zeaxanthin, lycopene, or carotene (Hesseltine 1991; Nout and Kiers 2005; Liu et al. 2012; Rodríquez-Sáiz et al. 2012; Voigt and Kirk 2014). Since animals are not able to produce carotenoids by themselves, they depend on external sources and producers like plants, microorganisms, or fungi. Carotenoids are important pigments in animals and plants serving light protection and other physiological functions, e.g., as antioxidants, chromophores in photosynthesis or photoprotection, membrane stabilizers, and precursors for vitamin A. Carotenoid biosynthesis is known to be light stimulated (Rodríguez-Ortiz et al. 2012). Within the fungi, precursors of the carotenoids originate from the mevalonate pathway and are processed via geranylgeranyl diphosphate, phytoene, lycopene to, e.g., β-carotene. Known enzymes involved in **β-carotene synthesis** (Fig. 15.1) comprise CarRA (CarRP in *Mucor circinelloides*, containing the two domains CarR (lycopene cyclase) and CarA (phytoene synthase) (Fig. 15.1; Torres-Martínez et al. 1980; Arrach et al. 2001), CarB (phytoene dehydrogenase) (Ruiz-Hidalgo et al. 1997), CarI (Roncero and Cerdá-Olmedo 1982), CarF (Mehta et al. 1997), CarC (Revuelta and Eslava 1983), and CarD (Salgado et al. 1989).

β-carotene itself is processed differently in different fungal phyla, e.g., to neurosporaxanthin in *Ascomycota* or dihydroactinidiolide and β-ionone serving as flavor compounds in the *Basidiomycota* (Zorn et al. 2003). In the basal fungal lineages, however, carotene is cleaved to pheromones facilitating sexual recognition between mating partners—the sesquiterpene sirene in the *Chytridiomycota* (*Blastocladiomycetes*, *Neocallimastigomycetes*, *Monoblepharidomycetes*, and *Chytridiomycetes* for review see Voigt et al. 2013) and trisporoids in the zygomycetes (for review see Wöstemeyer et al. 2005). Zygomycetes recruit their recognition molecules from β-carotene biosynthesis (Fig. 15.1) and degradation (Fig. 15.1) pathways bridging primary and secondary metabolism, a feature comparable to abscisic acid in plants (Schwartz et al. 1997). Trisporoids are a rather

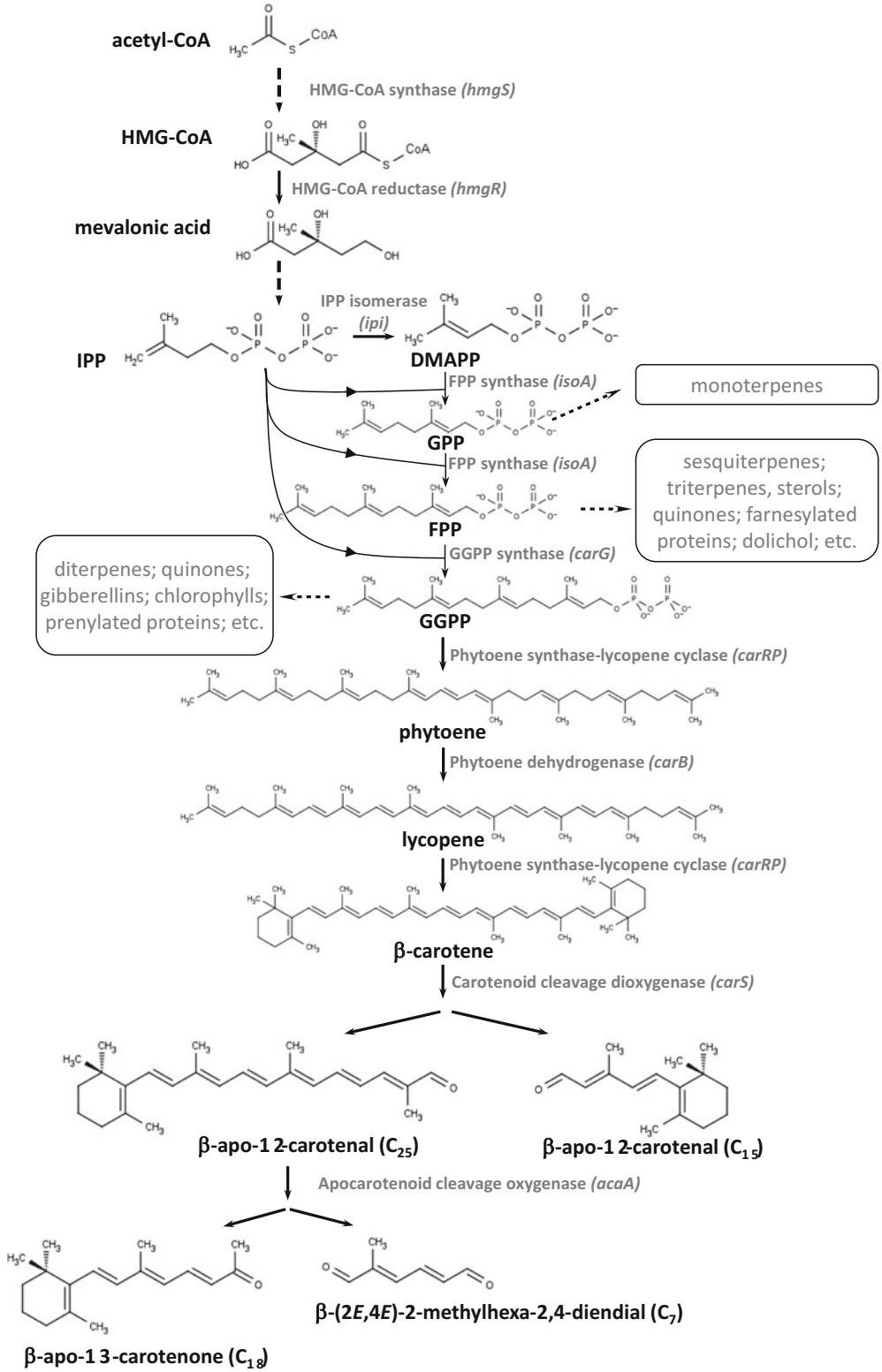

Fig. 15.1 Carotene biosynthesis and degradation pathways: Main steps of the acetate–mevalonate pathway, the specific β-carotene biosynthesis in *M. circinelloides* and presumable cleavage of β-carotene (C40)

unusual degradation product since it is not involved in cell-supporting or cell-protective functions but in pheromone action in sexual communication of those fungi if compared to higher fungi which rely on modified peptides (Gooday 1974; Jones and Bennett 2011). Since a multitude of structural diverse carotenoids is involved in a very broad and diverse spectrum of applications in all organisms, an even much more diversity of enzymes involved in carotenogenesis and processing should be reasonable. Enzymes cleaving specific double bonds are termed **carotenoid cleavage oxygenases** (CCOs) or more specified mono- (CMOs) or dioxygenases (CCDs):

Elucidation and clarification of enzymatic mechanisms started only several years ago with the first crystal structure of a CCO from *Synechocystis* sp. converting β-apo-carotenals as sole substrates (Kloer et al. 2005). Since then, only few amino acid residues are believed to be essential for enzyme activity, namely four histidine and three glutamate/aspartate residues (Poliakov et al. 2005; Takahashi et al. 2005). According to the first structurally described CCO, these amino acids correspond to sequence positions Glu150, His183, His238, His304, Glu370, Glu426 and His484 of *Synechocystis*.

All representative carotenoid cleavage enzymes (shown in Fig. 15.1) possess these conserved amino acids. In accordance with their first description by Medina et al. 2011, few sequences (clustering within the zygomycetous order *Mucorales* and termed "unknown") possess similar sequences but lack some of the conserved residues (essentially His183 and His238). The gene coding for the CCO from *Phycomyces blakesleeanus* (acaA) seems to have been duplicated recently. A functional characterization remains to be done for the duplicated *aca*A and the presumed genes of unknown function (Medina et al. 2011). The phylogeny of the CCOs (Fig. 15.2) shows that each clade has evolved its more or less specific carotenoid cleavage enzymes with similar cleaving sites, but with different natural substrates, which is presumably due to their wide variety within carotenogenesis and specific organismal requirements. The carotenoid-cleaving enzymes in the *Mucorales* are unique for this group of fungi with no similar cleaving enzymes in other fungal groups (Sahadevan et al. 2013):

Mucorales seem to possess also only one enzyme capable to cleave β-carotene, an enzyme crucial for all subsequent utilizations of β-carotene. This gene, called *carS*, is an 11′–12′ carotenoid cleavage dioxygenase (Fig. 15.1; Medina et al. 2011; Tagua et al. 2012; Rodríquez-Sáiz et al. 2012; Rodríguez-Ortiz et al. 2012), which cleaves β-carotene (C_{40}) into β-apo-12-carotenal (C_{25}) and β-apo-12-carotenal (C_{15}, Fig. 15.1, Sahadevan et al. 2013). CarS should not be misapplied as an orthologue of the *carS* in the ascomycete *Fusarium* sp., which codes for a regulatory protein, most likely corresponding to CrgA from *Mucor circinelloides* (Navarro et al. 2001). After cleavage by CarS, β-apo-12-carotenal (C_{25}) is further processed by the apocarotenoid cleavage oxygenase AcaA, presumably cleaved at its 13–14 position, resulting finally in two more fragments, namely β-apo-13-carotenone (C_{18}, also named d'orenone, Sahadevan et al. 2013), and probably (2*E*,4*E*)-2-methylhexa-2,4-diendial (C_7) (Fig. 15.1; Polaino et al. 2010; Medina et al. 2011).

1. Regulation, Genetic Manipulation: What Have We Learned from the Major Model Organisms *Mucor circinelloides*, *Phycomyces blakesleeanus*, and *Blakeslea trispora*?

Members of the order *Mucorales* are known as β-carotene-producing fungi. Among them, *Blakeslea trispora*, *Mucor circinelloides*, and *Phycomyces blakesleeanus* are involved in the study of the carotenoid biosynthesis as model organisms. *B. trispora* is already an industrial

Fig. 15.1 (continued) at position C11–C12 by the carotenoid cleavage dioxygenase CarS, resulting in two fragments of β-apo-12-carotenal, (C25) and (C15) in *P. blakesleeanus*, the final cleavage of β-apo-12-carotenal (C25) to β-apo-13-carotenone (C18) and probably (2*E*, 4*E*)-2-methylhexa-2,4-diendial (C7) by the apocarotenoid cleavage oxygenase AcaA. The most important enzymes and the encoding genes are indicated with *gray*. *HMG* hydroxymethylglutaryl, *IPP* isopentenyl pyrophosphate, *DMAPP* dimethylallyl pyrophosphate, *GPP* geranyl pyrophosphate, *FPP* farnesyl pyrophosphate, *GGPP* geranylgeranyl pyrophosphate

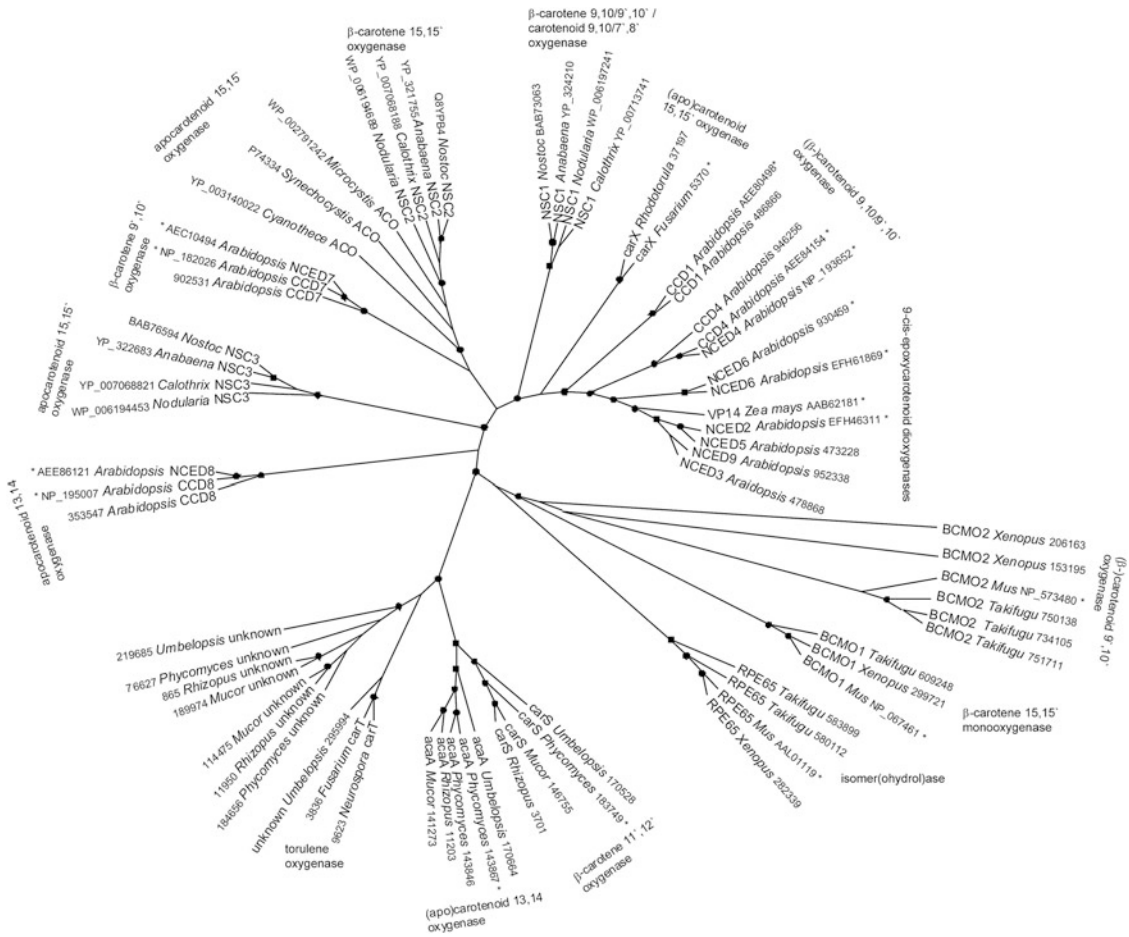

Fig. 15.2 Evolutionary relationships of representative genes involved in β-carotene degradation and their representative cleavage sites. The tree can be roughly divided into three sub-trees, each following species phylogeny. Carotenoid cleavage oxygenases from *Mucorales* comprise CarS and AcaA as well as several so far uncharacterized sequences. Bootstrap values greater or equal to 90 % are indicated by *black dots*. *Names on branches* indicate prominent cleavage sites

source of β-carotene, while the application of *M. circinelloides* and *P. blakesleeanus* is in a developmental phase (Dufossé 2006, 2008). However, improvement and study of the carotenogenesis in *B. trispora* and *P. blakesleeanus* are hampered by the lack of efficient methods for genetic manipulation; i.e., their genetic transformation has still been unsuccessful (Obraztsova et al. 2004; Sanz et al. 2011; Garre et al. 2015). *M. circinelloides* seems to be more amenable to molecular techniques as well-developed transformation systems including vectors, promoters, recipient strains, and methods (i.e., PEG-mediated protoplast transformation and electroporation) are available (van Heeswijck and Roncero 1984; Wolff and Arnau 2002; Appel et al. 2004; Papp et al. 2010; Gutiérrez et al. 2011). Moreover, this fungus has an ability to maintain and express exogenous genes from related fungi (e.g., *P. blakesleeanus*, *B. trispora*, or *Rhizomucor miehei*) and other organisms (e.g., *Xanthophyllomyces dendrorhous* or *Paracoccus* sp. N81106) (Iturriaga et al. 1992; Ruiz-Hidalgo et al. 1999; Quiles-Rosillo et al. 2003; Rodríguez-Sáiz et al. 2004; Lukács et al. 2009; Papp et al. 2006, 2013; Csernetics et al. 2015).

Carotenoids are terpenoid compounds, and their biosynthesis can be regarded as a side route of the general acetate–mevalonate (AMV) pathway, in which precursors of the different terpene derivatives are synthesized from acetyl CoA. Several genes encoding the enzymes, which catalyze the main steps of the AMV pathway and carotenoid biosynthesis, have been isolated and characterized in *M. circinelloides* (Velayos et al. 2000a, b, 2003; Csernetics et al. 2011; Nagy et al. 2014). Carotenogenic genes of *B. trispora* and *P. blakesleeanus* were also identified, and their functions were analyzed by expressing them in *M. circinelloides* (Rodríguez-Sáiz et al. 2004; Sanz et al. 2011).

One of the key enzymes of the general AMV pathway is 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which catalyzes the formation of HMG-CoA from mevalonic acid. As HMG-CoA is a common intermediate of numerous different terpenoid compounds, such as carotenoids, ergosterol, prenyl groups of certain proteins, and ubiquinone, its formation is considered to be rate limiting for the carotenoid synthesis (Wang and Keasling 2002). *M. circinelloides* has three HMG-CoA reductase genes (*hmgR*), which respond differently to temperature and the oxygen level of the environment (Nagy et al. 2014). Among them, *hmgR2* and *hmgR3* seem to be especially involved in the carotenoid biosynthesis. Overexpression of these genes by changing their own promoter to that of the endogenous glyceraldehyde-3-phosphate dehydrogenase 1 gene (*gpd1*) and elevating their copy numbers increased the whole carotenoid content of the fungus 1.5–1.7-fold (Nagy et al. 2014).

Another important section of this pathway is the synthesis of the prenyl-chain intermediate compounds, which serve as precursors in the different specific side routes. The most important steps of this process are the isomerization of dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) catalyzed by the IPP isomerase, the condensation of IPP and DMAPP to form geranyl pyrophosphate (GPP), and the extension of the prenyl chain by the addition of further IPP units to the carbon chain forming farnesyl and geranylgeranyl pyrophosphate (FPP and GGPP, respectively). Synthesis of GPP and FPP is catalyzed by the FPP synthase, while formation of GGPP is managed by the GGPP synthase. GGPP is the direct precursor of carotenoids as their specific biosynthesis starts with the condensation of two 20-carbon GGPP units leading to the synthesis of carotenoid phytoene (Iturriaga et al. 2000). In *M. circinelloides*, the IPP isomerase and the FPP and GGPP synthases are encoded by the *ipi*, *isoA*, and *carG* genes (Velayos et al. 2003, 2004; Csernetics et al. 2011). Overexpression of these genes significantly enhanced the carotenoid biosynthesis. In this study, the step determined by the *carG* gene proved to be the first bottleneck for carotenoid production, placing it under the control of the *Mucor gpd1* promoter resulted in a fourfold increase in the carotenoid content (Csernetics et al. 2011). Total carotenoid content of these strains was more than 2 mg/g (dry weight). Similarly, the expression of the *ipi* and the *carG* genes of *B. trispora* in an engineered, carotenoid-producing *E. coli* strain led to a twofold increase in the carotenoid production of the bacterium (Sun et al. 2012). These studies indicated that *ipi* and *carG* genes can be applied to improve the carotenoid production of mucoralean fungi. *M. circinelloides* requires light for carotenoid biosynthesis and transcription of *carG*, and the carotenoid-specific genes (i.e., *carB*-encoding phytoene dehydrogenase and *carRP*-encoding phytoene synthase–lycopene cyclase) are induced by blue light (Velayos et al. 2000a, b, 2003). White collar-1-like proteins, Mcwc-1b, and Mcwc-1c were found to be involved in the activation of the carotenogenic genes of *M. circinelloides* (Silva et al. 2006, 2008), while the protein CrgA proved to be a repressor of the carotenoid biosynthesis in *Mucor* (Navarro et al. 2001). Deletion of the *crgA* gene resulted in enhanced accumulation of carotenoids under both dark and light conditions (Navarro et al. 2001). Moreover, deletion of *crgA* could be used to increase the lycopene production of a *mutant M. circinelloides* strain achieving a lycopene content of 54 g/L (Nicolás-Molina et al. 2008). Recently,

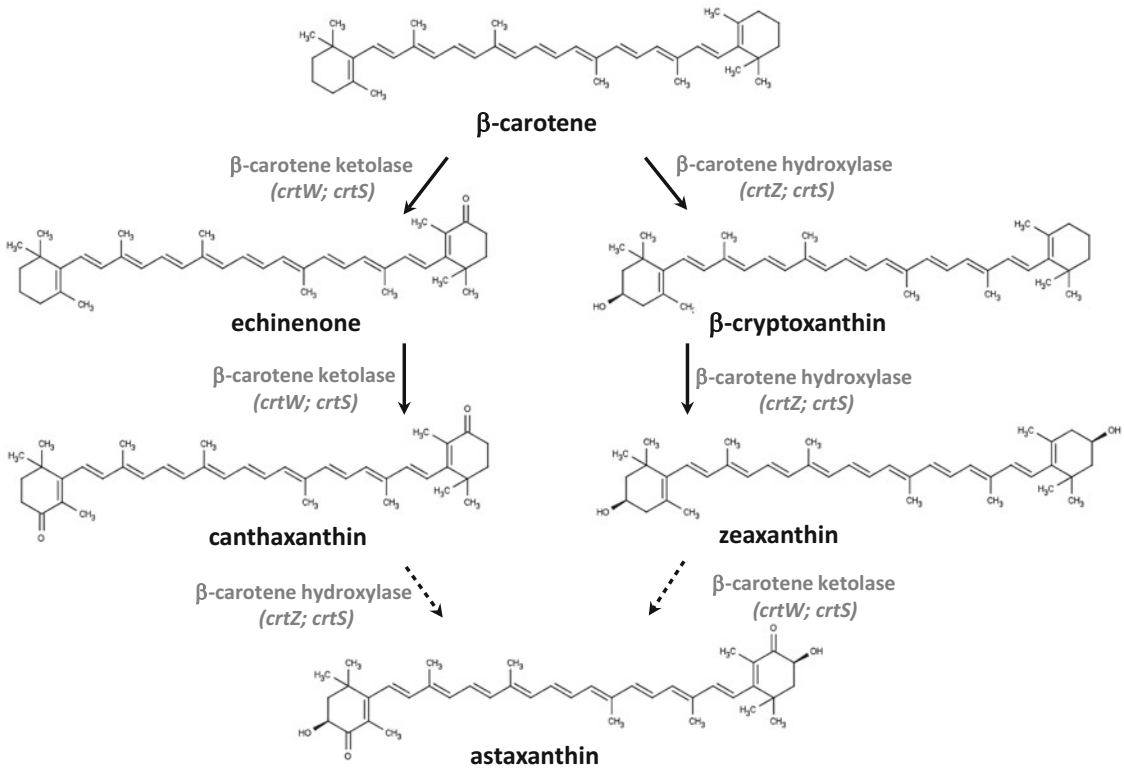

Fig. 15.3 Conversion of β-carotene to its oxygenated derivatives, the carotenoids (xanthophylls)

it has been supposed that CrgA may be an ubiquitin ligase, and one of its functions may include preventing Mcwc-1b to activate the transcription of the carotenoid biosynthesis genes (Silva et al. 2008; Navarro et al. 2013):

By expressing exogenous carotenoid biosynthesis genes, production of new carotenoid compounds, such as oxygenated derivatives of β-carotene, can be achieved. *Paracoccus* sp. N81106 is a marine, astaxanthin producing bacterium, in which the conversion of β-carotene to astaxanthin is catalyzed by the enzymes β-carotene ketolase (CrtW) and hydroxylase (CrtZ). Production of xanthophylls (i.e. β-cryptoxanthin, zeaxanthin, canthaxanthin and astaxanthin, Fig. 15.3) could be carried out by transforming *M. circinelloides* with autonomously replicating vectors, which harboured the *crtW* and the *crtZ* genes fused with the regulatory sequences of *Mucor gpd1* (Papp et al. 2006). Multiple integration of the *crtW* gene into the *Mucor* genome resulted in strains accumulating canthaxanthin as the main carotenoid instead of β-carotene (Papp et al. 2013). The astaxanthin biosynthesis gene (*crtS*) of *Xanthophyllomyces dendrorhous* also could be used to obtain xanthophyll-producing *M. circinelloides* strains (Álvarez et al. 2006; Csernetics et al. 2015; Rodríquez-Sáiz et al. 2012). In these experiments, the *crtS* gene was driven by the promoter of the *Blakeslea carRA* or the *Mucor gpd1*.

2. Carotene Degradation Is Linked to Sexual Interactions

All zygomycetes are coherently united by the potential to form the chemotactic pheromone **trisporic acid** (Fig. 15.4; for review see Wöstemeyer et al. 2002, 2005). This compound is morphogenic by its ability to induce the genesis of zygophores subsequently followed by zygospores during conjugation of two yoke-shaped gametangia (**gametangiogamy**) in compatible mating interactions. Trisporic acid is the universal gamone, which is cooperatively formed between both mating partners (Schimek et al. 2003; Schachtschabel et al. 2008). Trisporoid acid has a multitude of derivatives (trisporoids), which possess deviating biological activ-

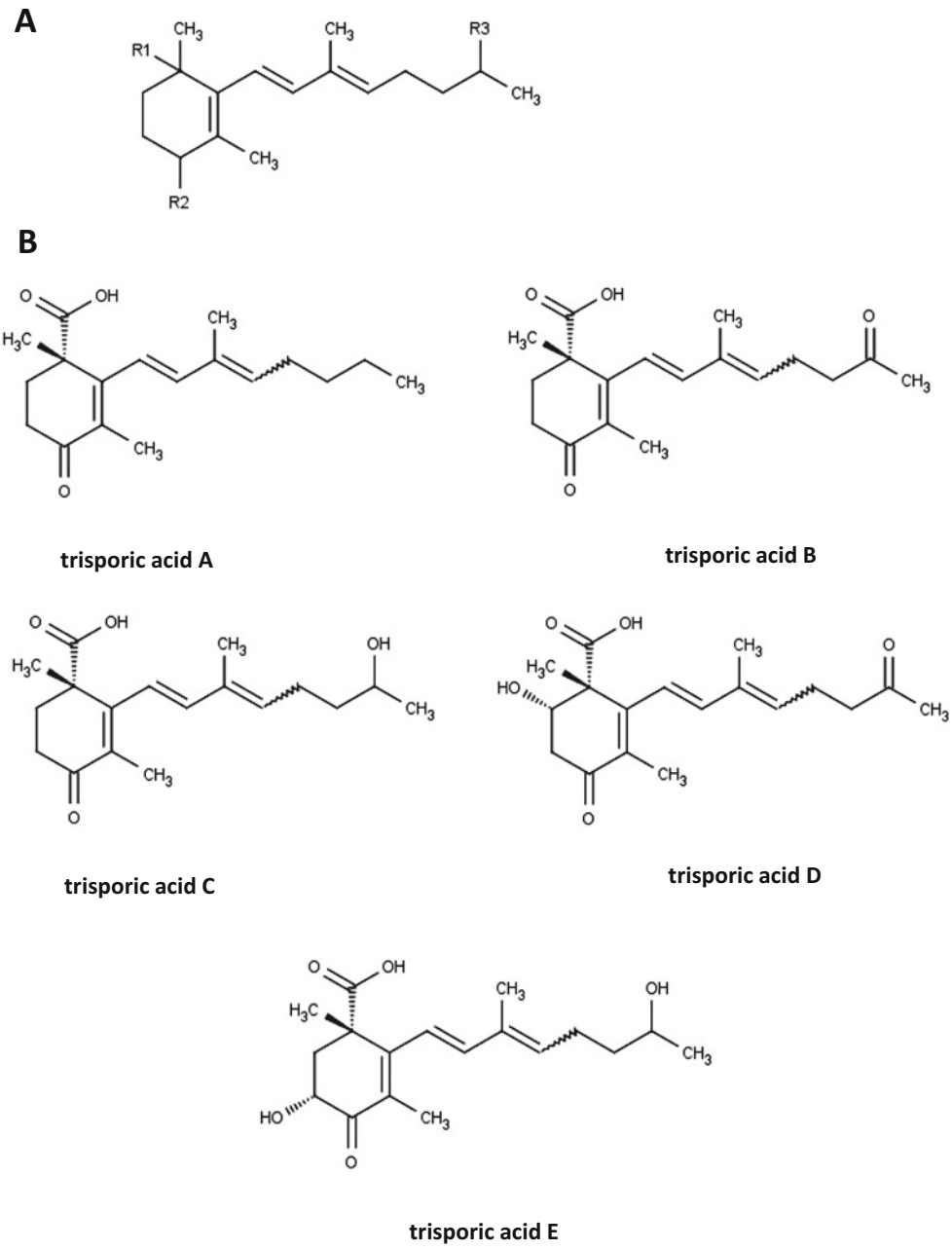

Fig. 15.4 Chemical structures of trisporic acid, the universal sexual pheromone of the zygomycetes. (**a**) Basic chemical structure. (**b**) Structures of trisporic acids A–D. Trisporic acid D was postulated but was never experimentally proven

ity (Schachtschabel et al. 2005). The biosynthesis of trisporoids starts with the cleavage of β-carotene which is mediated by a trisporic acid-regulated β-carotene oxygenases *tsp3* and *tsp4* aiming a C_{18} compound (Burmester et al. 2007). The sexual pheromones of the *Mucorales* are processed from the C_{18} compound resulting finally in trisporic acids. Yet many of the enzymatic steps remain unknown. The only enzymes known so far in the processing of the

C_{18} compound are two enzymes belonging to two different families of oxidoreductases: *tsp2*, a short-chain dehydrogenase involved in the processing of 4-dihydrotrisporin, and *tsp1*, an aldo-keto reductase involved in the processing of 4-dihydromethyltrisporate (Czempinski et al. 1996; Wetzel et al. 2009).

B. Fatty Acids

Fungi of the order *Mortierellales* and *Mucorales* have attracted considerable interest as **industrial lipid producers**. They are easily cultivated in solid or liquid culture and have been shown to grow on various different carbon sources (Dyal and Narine 2005; Gao et al. 2013; Zeng et al. 2013), on numerous different agricultural waste products (Chaudhuri et al. 1998; Jang et al. 2000; Zeng et al. 2013), and on glycerol, a by-product of biodiesel production (Hou 2008; Dedyukhina et al. 2011; Chatzifragkou et al. 2011). Hence, industrial and agricultural waste products can be converted as low-cost substrate into valuable products, providing an excellent biotechnological application for the zygomycetes. For example, lignocellulosic biomass, which is the most available and renewable source in nature, might be an ideal raw material for single cell oils (see Sect. D) production (Huang et al. 2013). Especially *Mortierella* spp. can accumulate large amounts of unusual lipids containing polyunsaturated fatty acids depending on species, strain, and growth conditions (Münchberg et al. 2012, 2015). The characterization of the genomes from oleaginous fungi like *Mortierella alpina* (Wang et al. 2011) and *M. elongata* (Bonito et al. 2013) provides insights into the genomic basis of fatty acid production. First insights into the *M. elongata* genome reveal preliminary enrichments of genes related to lipid metabolism (e.g., sphingolipids, ether lipids, and glycerophopholipids), tryptophan metabolism, siderophore group nonribosomal peptides, and glucan 1,4-alpha glucosidases compared to genome sequences of other basal fungi (Bonito et al. 2013).

C. Organic Acids

The production of relevant organic acids, namely, **L-lactic acid** and **fumaric acid**, is based on pyruvate, the end product of the glycolysis. Whereas L-lactic acid is formed directly from pyruvate by lactate dehydrogenase (Skory 2000; Pritchard 1971, 1973), fumaric acid is formed via the oxidative branch of the TCA cycle located in the cytoplasm (Fig. 15.5; Goldberg et al. 2006).

Both organic acids can be diversely applied in food industry, textile sector, cosmetic industry, and chemical and pharmaceutical industry. Lactic acid is the most abundantly produced organic acid in nature. Therefore, lactic acid production by *Rhizopus* species is a subject of intensive research and has the potential to replace the established lactic acid production processes using chemical methods or lactobacilli fermentation. When producing lactic acid by *Rhizopus* species, low-cost substrates (e.g., agricultural waste products containing any kind of plant fibers) and a wide variety of carbon sources ranging from monosaccharides to polysaccharides can be used (Guo et al. 2010; Vially et al. 2010; Yen and Lee 2010; Bulut et al. 2009; Bai et al. 2008), resulting in very high yields ranging near the theoretical maximum (Ferreira et al. 2013; Meussen et al. 2012).

Fumaric acid, a C_4-dicarboxylic acid, was identified by the US Department of Energy as one of 12 promising platform chemicals from biomass with high added value (Werpy and Petersen 2004). Presently, fumaric acid is chemically produced from crude oil and is applied in food industry as acidulant, food preservative, and flavor enhancer. Due to its bifunctionality and the double-bond fumaric acid, it is also suitable to act as polymerization starter unit for plastics or resins (Anonymus 2007; Willke and Vorlop 2004). As for lactic acid, high yields near the theoretical maximum can be achieved by microbial fermentation when using glucose as carbon source (Meussen et al. 2012; Roa Engel et al. 2008; Cao et al. 1996). Noteworthily, for each molecule of formed fumaric acid, one molecule CO_2 is fixated (Osmani and Scrutton 1985; Overman and Romano 1969).

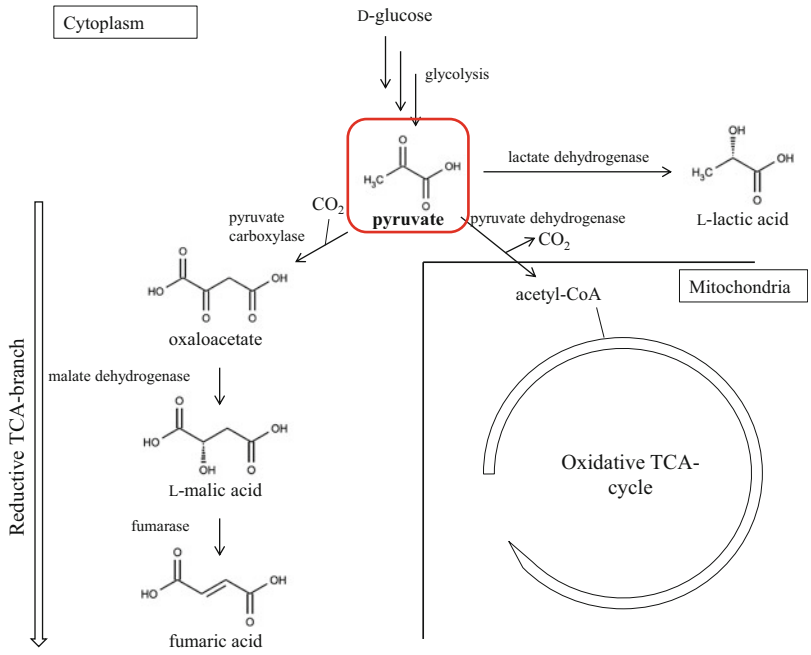

Fig. 15.5 Formation of lactic and fumaric acid is based on pyruvate, the end product of the glycolysis. Whereas L-lactic acid is formed directly from pyruvate by lactate dehydrogenase (Skory 2000; Pritchard 1971, 1973), fumaric acid is formed via the oxidative branch of the TCA cycle located in the cytoplasm

Whether fumaric or lactic acid is produced from pyruvate depends on activity and substrate affinity of the respective enzymes and seemed to be strain dependent. However, Saito et al. (2004) proved that *Rhizopus oryzae* strains with two genes for lactate dehydrogenase produce mainly lactic acid, whereas strains with only one gene produce mainly fumaric acid.

Phylogenetic studies using further independent DNA markers by Abe et al. (2007) revealed that fumaric acid and lactic acid producers can be separated into two sibling species, *R. oryzae* sensu stricto (also known as *R. arrhizus*) and *R. delemar* correlating with the lactic acid and fumaric–malic acid producers, respectively. Reclassification of strains in the fumaric–malic acid group as *R. delemar* and therefore reclassification of the genome strain *R. oryzae* 99-880 into *R. delemar* were proposed (Gryganskyi et al. 2010), which was later converted into *Rhizopus arrhizus* var. *delemar* (Dolatabadi et al. 2014a).

D. Storage Lipids and Single Cell Oils

All living organisms have to synthesize a minimum amount of lipids to build up membranes. However, only few organisms are able to accumulate more than 20 % of their dry biomass in form of storage lipids. The term “oleaginous” refers to microorganisms, including yeasts, fungi, and microalgae, which meet this criterion and store lipids in form of triacylglycerols (Ratledge and Wynn 2002). Storage lipids, which are also known as “single cell oils” (SCPs), are rich in polyunsaturated fatty acids and are of special interest due to their bifunction as a supplier of functional oils and feedstock for biodiesel production (Huang et al. 2013). Especially **γ-linoleic acid** (GLA, C18:3n−6) is biotechnologically relevant (Fig. 15.6). It is commercially applied in pharmaceutical industry and is currently obtained by extraction of selected plant oils. However, higher amounts of GLA are also produced by some mucoralean genera, like *Cunninghamella*, *Mucor* (including *Zygorhynchus*), *Rhizopus*

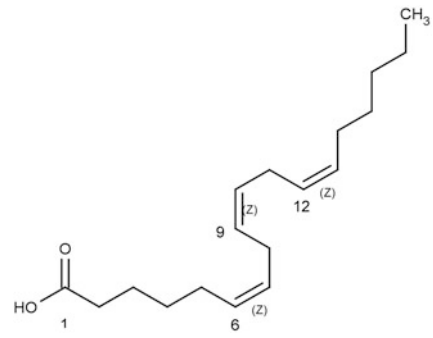

Fig. 15.6 Chemical structure of γ-linoleic acid (GLA, C18:3n−6)

(Kavadia et al. 2001; van der Westhuizen et al. 1994), *Choanephora*, *Phycomyces* (van der Westhuizen et al. 1994), and *Thamnidium* (Stredansky et al. 2000). Oleaginous microorganisms start the accumulation of single cell oil when grown in a medium with excess of carbon source but with a limitation of another nutrient. Oleagincity is characterized by the ability to produce a continuous supply of both acetyl CoA and NADPH as necessary precursors and reduction equivalent in fatty acid biosynthesis and is realized by the key enzymes ATP citrate lyase and AMP deaminase (Ratledge 2004). *Mortierellales* have great biotechnological importance as industrial producers of polyunsaturated fatty acids, such as arachidonic acid or eicosapentaenoic acid. Both the content of fatty acids and their rate of saturation are known to be dependent on the temperature during production and also vary due to utilization of different nutrients in the cultivation media (Münchberg et al. 2012, 2015).

E. Enzymes

Zygomycetes are known to produce a vast variety of enzymes, e.g., amylases, cellulases, xylanases, steroid 11α-hydroxylases, phytases, proteases, and lipases which have a multitude of applications in industrial and pharmaceutical applications (for review see Krisch et al. 2010; Voigt and Kirk 2014).

Amylases are one of the main enzymes used in industry. They hydrolyze starch molecules into polymers composed of glucose units or oligosaccharides. Amylases have potential application in industrial processes such as food, textile, paper, and detergent industries as well as fermentation and pharmaceutical industries. As starch is an important constituent of the human diet and is a major storage product of many economically important crops such as wheat, rice, maize, tapioca, and potato, starch-converting enzymes are used in the production of maltodextrin, modified starches, or glucose and fructose syrups. For the production α-amylases using submerged and solid-state fermentation systems, distribution, structural–functional aspects, physical and chemical parameters, and the use of these enzymes in industrial applications, see the review by Monteiro de Souza and de Oliveira Magalhães (2010).

Cellulases catalyze cellulolysis, the decomposition of cellulose, which is the most abundant organic source of feed/food, fuel, and chemicals (Spano et al. 1976). Cellulase breaks down the cellulose molecule into mono- and oligosaccharides by hydrolysis of the 1,4-beta-D-glycosidic linkages in cellulose in its derivative hemicellulose, lichenin, and cereal beta-D-glucans. Cellulases represent a naturally occurring mixture of various enzymes acting serially or synergistically to decompose cellulosic material. Zygomycetes (e.g., *Mucor circinelloides*) were frequently found as straw-colonizing fungi producing total cellulases, endo-beta-1,4 glucanase, and endo-beta-1,4 xylanase in solid-state fermentation (Lee et al. 2011).

Lipases are water-soluble enzymes that act on insoluble substrates and catalyze the hydrolysis of long-chain triglycerides. They play a vital role in the food, detergent, chemical, and pharmaceutical industries and have gained significant attention in the industries due to their substrate specificity and stability under varied chemical and physical conditions (for review see Gopinath et al. 2013).

Phytases are myo-inositol hexakisphosphate phosphohydrolases and represent any type of phosphatase enzyme that catalyzes the hydrolysis of phytic acid (myo-inositol hexakisphosphate)—an indigestible, organic form of phosphorus that is found in grains and oil seeds—and releases a usable form of inorganic

phosphorus (Mullaney et al. 2000). Phytases have been most commonly detected and characterized from fungi (Mullaney and Ullah 2003), specifically in the zygomycete *Rhizopus oligosporus* (DSMZ 1964), which is commonly used for tempeh production (Azeke et al. 2011). The phytases from *R. oligosporus* exhibit a broad affinity for various phosphorylated compounds. Practical interest in phytases has been stimulated by the fact that phytase supplements increase the availability of phosphorus in pig and poultry feed and thereby reduce environmental pollution due to excess phosphate excretion in areas where there is intensive livestock production.

Proteases produced by zygomycetes are rennin-like proteases secreted by several mucoralean species that are used in cheese production. In particular, mucoralean fungi (*Rhizopus oryzae*, *Circinella muscae*, *Mucor subtilissimus*, *Mucor hiemalis* f. *hiemalis*, *Syncephalastrum racemosum*, *Rhizopus microsporus* var. *chinensis*, and *Absidia cylindrospora*) were frequently isolated from maize flour, corn meal, and cooked cornflakes using surface and depth plate methods with subsequent measurement of significant proteolytic activities (de Azevedo Santiago and de Souza Motta 2008).

Steroid 11α-hydroxylases are encoded by genes of the cytochrome P450 superfamily of enzymes containing a heme cofactor (hemoproteins, Sigel et al. 2007). The cytochrome P450 proteins are monooxygenases that catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids, and other lipids. They are, in general, the terminal oxidase enzymes in electron transfer chains, broadly categorized as P450-containing systems. The term *P450* is derived from the spectrophotometric peak at the wavelength of the absorption maximum of the enzyme (450 nm) when it is in the reduced state and complexed with CO (Sigel et al. 2007). To overcome the chemically laborious stereo- and regioselective hydroxylation steps in the pharmaceutical production of corticosteroids and progestogens, zygomycetes, e.g. *Rhizopus* spp., are employed to perform the 11α-hydroxylation of the steroid skeleton, thereby significantly simplifying steroid drug production (Petrič et al. 2010).

Xylanases degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicellulose, one of the major components of plant cell walls (Beg et al. 2001). Zygomycetes (e.g. *Mucor circinelloides*) are straw-colonizing fungi producing xylanolytic enzymes such as endo-beta-1,4 xylanase in solid-state fermentation (Lee et al. 2011).

III. The Dogma of the Unability of Zygomycetes to Produce Natural Products

It has been a widespread dogma that zygomycetes are not capable to produce own secondary metabolites, despite of those produced by endosymbiotic bacteria (see Sect. I.B.) (for examples, see Jennessen et al. 2005). However, it has been shown that zygomycetes react on other fungal secondary metabolites by morphogenic changes as shown by the sesterterpene-type phytotoxin ophiobolin produced by fungi belonging mainly to the ascomycetous genus *Bipolaris* (Krizsán et al. 2010). Ophiobolin A caused morphological changes in *Mucor circinelloides*; the fungus formed degenerated, thick or swollen cells with septa and cytoplasm effusions from the damaged cells. Here we explore the potential of zygomycetes to produce secondary metabolites together with other microorganisms in a cooperative manner.

To estimate the genomic potential of the zygomycetes to produce secondary metabolites, all final, publicly available drafts of zygomycete genomes were scanned for the presence of genes encoding **polyketide synthases** (PKSs), **nonribosomal peptide synthetases** (NRPSs), and L-**tryptophan dimethylallyl transferases** (DMATs). For more information on regulation of secondary metabolism, see also Chap. 2. A total of eight genomes were screened and analyzed: one entomophthoralean (*Conidiobolus coronatus*), one kickxellalean (*Coemansia reversa*), two mortierellalean, and four mucoralean genomes (Table 15.1). All species possess the genomic prerequisite for the production of natural products. On average, each species encodes two DMATs and 1–2 NRPSs, with a

Table 15.1 The presence of gene families encoding polyketide synthases (PKSs), nonribosomal peptide synthetases (NRPSs), dimethylallyl pyrophosphate: L-tryptophan dimethylallyl transferase (DMAT synthase) which were predicted in the genomes of 15 species comprising five subphyla of the *Zygomycota*, as of 6th of July, 2015; genome resources (if not stated elsewhere): Joint Genome Institute Broad Institute of Harvard and MIT, Origins of Multicellularity Sequencing Project *Mortierella verticillata*

		Genome resource	(PKS)/FAS[a]	NRPS	DMAT
Mucorales	*Lichtheimia hyalospora*	JGI, unpublished	1	1	2
	Mucor circinelloides	Lee et al. (2014)	2	3	2
	Rhizopus microsporus var. chinensis	Wang et al. (2013)	1(+1)	2	6
	Rhizopus arrhizus var. *delemar* (syn. *R. oryzae*)[b]	Ma et al. (2009)	1	1	3
Kickxellales	*Coemansia reversa*	Chang et al. (2015)	4	1	2
Entomophthorales	*Conidiobolus coronatus*	Chang et al. (2015)	″(1)	3	2
Mortierellales	*Mortierella verticillata*	Bonito et al. (2013)	0	1	0
	Mortierella alpina	Wang et al. (2011)[c]	1	21	0

For an overview of genome projects on basal fungi incl. *Zygomycota*, see Shelest and Voigt (2014)

[a]The genes predicted as PKS-like have the typical structure of the FAS alpha subunit

[b]Nomenclature: Gryganskyi et al. (2010) and Dolatabadi et al. (2014a)

[c]Proteome of *M. alpina* available from phylomeDB: ftp://phylomedb.org/phylomedb/proteomes/ ftp://phylomedb.org/phylomedb/proteomes/685557.1.fa.gz

noteworthy exception detecting 21 NRPSs in the genome of *Mortierella alpina*. The genes encoding typical PKS/fatty acid synthase (FAS) ketosynthase domains are most likely FAS alpha subunits: they reveal a characteristic domain order. BLAST searches using the tools at the specific home pages of the genomes confirm their annotation as FASs. Most of the discovered NRPS genes encode monomodular enzymes, except for the one in *Mortierella verticillata*, where we find a five-module protein encoded. Many NRPS-like genes do not possess the minimal set of domains necessary for the full enzymatic activity. These genes are therefore characterized as NRPS-like. Three genes which putatively encode for NRPSs were found in *Mucor circinelloides* f. *circinelloides* (Table 15.1) and can also be considered NRPS-like (Lee et al. 2014). Within the genus *Lichtheimia*, genes encoding PKSs, NRPSs, and DMATs are present in *L. hyalospora*, but absent in *L. corymbifera* (Schwartze et al. 2014). In some cases it can be supposed that the genes predicted actually represent the full enzymes but cannot be correctly annotated due to erroneous gene prediction. Another problem connected with genome assembly is the high AT content, which renders the bioinformation content low and prevents motif-based cluster prediction. Transcription regulators of secondary metabolism have not been yet systematically characterized, as reliable data is scarce. At this stage, we are mostly aware of pathway-specific regulators of clusters, but it is premature to draw general picture yet. In fact, known clusters in the *Ascomycetes* build the main basis of such genome-mining analyses, whereas information on proven clusters in other fungal phyla is lacking. In the *Ascomycetes*, about 60 % of PKS- and NRPS-encoding gene clusters include an embedded transcription regulator gene, which encodes in majority of cases a **zinc cluster transcription factor** (TF) (Brakhage 2013). The neighboring sequence regions of the genes encoding PKSs, NRPSs, and DMATs in zygomycetes were analyzed in order to confirm this preference for Zn cluster TFs. For this, we predicted that all TFs genome-wide extracted the TF annotations in regions of ±10 genes around those encoding secondary metabolite enzymes. We assigned these TFs to families of DNA-binding domains based on InterProScan predictions as described by Shelest (2008). Interestingly, Zn clusters are very modestly represented among these SM-accompanying TFs, leaving the first place to C_2H_2 Zn finger TFs and TFs of the homeodomain-like class. This observation becomes less surprising, however, if we think

about the overall predominance of C_2H_2 Zn fingers and especially of homeodomain-like TFs observed in zygomycetes. Our analysis suggests that every second, NRPS has a TF in the vicinity of 10 genes (8 of 15 NRPSs). For DMATs this number is higher (20 of 34, ~60 %). This corresponds to the number of the TFs in known ascomycete clusters (60 % for PKSs and NRPSs). The number for zygomycetes can be lower because we consider only the vicinity of 10 genes, whereas the cluster can be longer; on the other hand, considering longer region can give more false-positive predictions. The most frequent TFs in the vicinity of NRPSs and DMATs are homeodomain-like DNA-binding domain family TFs and C_2H_2 Zn finger TFs, comprising in sum nearly half of the total number of TFs that can potentially be the regulators of secondary metabolism in zygomycetes. This is not very surprising since these two families are the most abundant in at least *Mucorales* and *Entomophthorales* genomes (Schwartze et al. 2014). It is interesting to notice, however, that the most numerous families take over the regulation of the secondary metabolite clusters in fungi: in *Ascomycetes*, where Zn cluster is the dominating TF family, Zn cluster TFs are most frequently embedded in SM clusters, and in a similar picture we observe now for C_2H_2 and homeobox TFs in zygomycetes.

One promising strategy to explore and to broaden the biotechnological potential of the zygomycetes could be the investigation of zygomycetes in co-cultures with other microorganisms sharing the same ecological niche. This procedure has been shown successful in *Aspergillus* spp. for activation of silent gene clusters (Schroeckh et al. 2009; Nützmann et al. 2011, 2012) and is consistent with the cooperative nature of the zygomycetes as shown at the cellular and molecular level (Schachtschabel et al. 2008; Schimek and Wöstemeyer 2009; Krizsán et al. 2010; Voigt and Kirk 2014). Cocultivation of zygomycetes with other microorganisms sharing the same habitat under nature-close cultivation conditions has a high potential to increase the metabolic activity of zygomycetes, which are commonly known to be low producers of secondary compounds.

To sum it up, we show that all considered zygomycetes have a pronounced potential to produce natural products.

IV. Conclusions

- Zygomycetes have an integral role in the development of microbial ecosystems, a property which has the potential to be converted for biotechnological and industrial applications ranging from food technology to drug development.
- Understanding the biology, ecology, and biotrophic interactions of the zygomycetes with other microorganisms can help to explore novel secondary metabolites which are central to ecological functions and are useful for effective and innovative biotechnological utilizations,
- The genomic architecture and transcription factor repertoire of the zygomycetes largely differs from that of more recent fungal lineages. C_2H_2 transcription factors are predominant transcription factors.
- The degradation of β-carotene to pheromones has been extensively studied. The impact on biotechnological importance and applications has been neglected so far. Systematic–phylogenetic approaches may help with the screening for suitable production strains for biotechnological applications.

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