Fungi and their potential for biofuels production (review)

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Abstract


World production of wheat, rice, sugar cane, vegetables and corn has reached approximately 62 billion tons per year. Over 3.48 billion tons per year agricultural wastes are accumulated. Improper disposal of agricultural waste is one of the causes of environmental pollution. Biodegradation of plant residues for biofuel production has become a modern alternative for the treatment of agricultural waste. Fungi producing lignocellulolytic enzymes include species of Ascomycetes, Basidiomycetes, and several anaerobic species that break down cellulose in the gastrointestinal tract of ruminants and some halophilic fungi. Anaerobic gut fungi have attracted huge interest as the most active cellulose degraders in nature. Some industrial production processes are carried out in the presence of high concentrations of NaCl. In such conditions, halophilic fungi with cellulase activity are already reported to be used in direct production of ethanol and butanol.

Keywords: agricultural waste; anaerobic digestion; bioenergy; biofuel; biogas; food waste; lignocellulosic biomass; methane; methanogens; halophilic fungi

Introduction

FAOSTAT (2012) reports that world production of wheat, rice, sugar cane, vegetables and corn has reached approximately 62 billion tons/year, from which over 3.48 billion tons/year agricultural wastes are accumulated (Pathak & Chaudhary, 2013). Agricultural wastes include organic and inorganic materials such as plant residues, animal excreta, compost, plastic etc. Most of agricultural waste residues are either burned in the field or utilized in an inefficient way (Sadik et al., 2010). For example, improper disposal of agricultural waste is one of the causes of environmental pollution (Kadarmidheen et al., 2012). Biodegradation for both economic and environmental reasons has become a popular alternative for the treatment of agricultural waste. The high calorific value of plant biomass allows plant waste to be converted into biofuel (Niedziółka et al., 2015).

Lignocellulose is the main component of plant biomass and is the most common renewable organic resource in the soil. Lignocellulose biomass, which represents one-third of the dry mass of the plant material, consists of cellulose (35–50%), hemicellulose (25-30%) and lignin (25–30%) (Sun et al., 2010) and can be a potential source of fuels (Wang et al., 2011). The biodegradation processes of agricultural wastes are mainly performed by microbial community which includes bacteria, fungi, actinomycetes, etc. For instance, bioconversion from cellulose to ethanol can be performed by various anaerobic thermophilic bacteria, such as Zymomonas (Matthew et al., 2005), Clostridium thermocellum (Ingram et al., 1987), engineered Escherichia coli (Millichip & Doelle, 1989), as well as by some filamentous fungi, such as Neurospora sp. (Yamauchi et al., 1989), Aspergillus sp. (Sugawara et al., 1994), Trichoderma viride (Ito et al., 1990), Neurospora crassa (Gong et al., 1981), Monilia sp. (Saddler & Chan, 1982), Paecilomyces sp. (Gervais & Sarrette, 1990) and Zygosaccharomyces rouxii (Pastore et al., 1994).
Anaerobic fungi are a potential source of biological H$_2$

Most known fungi are aerobic. Anaerobic fungi have been found in different sites like rumens of herbivores (Orpin, 1975; Khejornsart & Wanapat, 2010; Liggenstoffer et al., 2010), in herbivorous reptiles such as the green iguana (Liggenstoffer et al., 2010) and termites (Lee et al., 2015), deep-sea sediments (Nagano & Nagahama, 2012), freshwater lakes and landfill sites (McDonald et al., 2012). The discovery of obligate anaerobiosis in fungi was in 1975. Orpin (1975) reported the isolation of the species Neocallimastix frontalis from a sheep and its cultivation. As the cell wall of these organisms contains chitin, they were affiliated to Kingdom Fungi (Orpin, 1977).

Anaerobic gut fungi have attracted huge interest as the most active cellulose degraders in the biological world (Wood & Wilson, 1995). Anaerobic fungi can degrade these substrates more efficiently than commonly used anaerobic bacteria (Procházka et al., 2012) and they are key players in the degradation of lignocellulosic plant fiber (Khejornsart & Wanapat, 2010; Liggenstoffer et al., 2010; Gruninger et al., 2014). Fungi producing lignocellulolytic enzymes belong to the genera Ascomycetes, Basidiomycetes, including white rot fungi, brown rot fungi and several anaerobic species that break down cellulose in the gastrointestinal tract of ruminants (Ljungdahl, 2008; Yoon et al., 2007).

Haitjema et al. (2014) reported that the anaerobic fungi are part of the microbial community in biogas reactors. It is known that anaerobic fungi and methanogenic microbial communities can form methane-producing co-cultures when growing together (Gilmore, 2019). Some authors have shown that anaerobic fungi can stimulate the growth of methanogens and maintain their diversity (Yuqi et al., 2021). Anaerobic fungi secrete significant amounts of endocellulase and endoprotease, which gives them a competitive advantage over rumen bacteria in the degradation of plant structural material (Mountfort, 1987). Degradation of cellulosic biomass in nature is performed by cellulases. In the fungal kingdom, anaerobic fungi are the only ones that have cellulosomes (Dollhofer et al., 2015). Degradation of lignocellulose biomass results in the formation of long-chain polysaccharides, mainly cellulose and hemicellulose, and subsequent hydrolysis of these polysaccharides to their components of 5- and 6-carbon sugar chains. Hydrolysis of the β-1,4-glycosidic bonds in cellulose can be achieved by cellulases. Cellobiohydrolases hydrolyze β-1,4-glycosidic bonds, producing cellobiose. The hydrolysis of soluble cellobiose and cellodextrins to glucose is performed with the participation of β-glucosidases (Henrissat, 1991; Teter et al., 2014). Cellulases, hemicellulases and β-glucosidases belong to the group of glycosidic hydrolases.

Hydrogenosomes – “powerplants” in anaerobic fungal cells

In anaerobic eukaryotes, adaptation to an anaerobic environment has led to a reduction in mitochondria to inconspicuous cell compartments, a loss of the electron transport chain, and its energy-saving potential (Figure 1) (Yarlett & Hackstein, 2005). Instead of mitochondria, anaerobic fungal species have hydrogenosomes (Yarlett et al., 1986; Khejornsart & Wanapat, 2010; Gruninger et al., 2014). Fungal hydrogenosomes are membrane-bound organelles that contain hydrogenase and produce molecular hydrogen, carbon dioxide, acetate, and other compounds as metabolic waste products (Figure 2) (Yarlett et al., 1986; Khejornsart & Wanapat, 2010; Gruninger et al., 2014). They cannot use oxygen as an electron acceptor. All hydrogenosomes reduce protons to molecular hydrogen (Figure 2) (Müller, 1998; Müller, 1993; Martin & Müller, 1998; Embley et al., 1997).

Hydrogenosomes possess all of the enzymes necessary for hydrogen production. Hydrogenase, pyruvate:ferredoxin oxidoreductase, NADPH:ferredoxin oxidoreductase and “malic” enzyme were found in the hydrogenosomes obtained from Neocallimastix patriciarum (Yarlett et al., 1986). The hydrogenosomes in Neocallimastix L2 have been shown to have not only energy and H$_2$ generation functions, but also...
Hypothetical origin of hydrogenosomes

Many authors suggest that hydrogenosomes share a common ancestor with mitochondria (Sogin, 1997; Bui et al., 1996; Hackstein et al., 2001). Translocation studies have revealed compatibility in membrane protein import between mitochondria and hydrogenosomes using hydrogenosomal ADP/ATP carrier. This interchangeability shows that the targeting signals in the two proteins can be recognized by similar receptors/translocases (Dyall & Johnson, 2000). Two of theories, the Hydrogen hypothesis (Martin & Müller, 1998; Doolittle, 1998) and the Syntrophy hypothesis (Lopez-Garcia & Moreira, 1999; Moreira & Lopez-Garcia, 1998) suggest that the cause of symbiosis between mitochondria and hydrogenosomes was a requirement for hydrogen from an autotrophic methanogenic archaeal host cell. Hydrogenosomes also contain heat-shock proteins, Hsp70, Hsp60 and Hsp10, which are known to participate in protein translocation and folding in mitochondria (Dyall et al., 2000). Yarlett et al. (2005) reported that hydrogenosomes of the ciliate N. ovalis have typical mitochondrial cristae and contain cardiolipin.

Despite their common origin, both mitochondria and hydrogenosomes are very diverse in the function, protein composition, and size of their proteomes. (Gabaldón & Huynen, 2004). Hydrogenosomes lack many mitochondrial features like mitochondrial genome and proton-pumping electron transport chain. These organelles do not contain their own DNA, although DNA has already been detected in one anaerobic ciliate (Benchimol, 2009). For this reason, the discussion of the origin of hydrogenosomes remains open.

Biotechnological applications of halophilic fungi

Halophilic microorganisms are known for various biotechnological applications such as production of pigments, production of polyhydroxyalkanoate, decomposition of hydrocarbons, production of exopolysaccharide, production of bioemulsifiers, production of halocin and production of halotolerant enzymes (Oren, 2010). Ali et al. (2014) reported that low protease and lipase presence were observed in halophilic fungi Aspergillus gracilis and Aspergillus restrictus isolated from Ban Laem district, Phetchaburi province, Thailand. Other possible uses of halophilic microorganisms such as treatment of saline and hypersaline wastewaters and biofuel are being investigated (Oren, 2010). The main reason for studying halophilic fungi is related to the biotechnological application of their metabolites capable of activity under extreme conditions. In times in which the world is searching for alternative sources of energy, biofuel is a good alternative. There are several reports of halophilic microorganisms and fungi showing cellulytic activity (Gunny et al., 2015). Several industrial production processes are carried out in the presence of high concentrations of NaCl. In high salt concentration conditions, halophiles with cellulase activity will be more useful. There are some reports about direct production of ethanol and butanol by halophiles (Amiri et al., 2016).

A suitable source of halophilic fungi in Bulgaria are the salt lakes in Burgas. In addition, several halophilic fungal strains were isolated from our collective, and their study and characterization is in progress (Table 1). These halophilic and halotolerant fungi have been isolated from Burgas salty lake mud. Some of these fungal strains had shown growth in both 0% and 3.8% NaCl (Data not shown). Up to now, there are no data about halophilic fungi isolated from the Burgas salty lakes mud.

Methanogenesis in high-salinity ecosystems in the salt pool in the Gulf of Mexico has been reported for the first time in 1979 (Brooks, 1979). Many microbiological researchers have focused on the aerobic, halophilic microflora, mainly isolated from the Dead Sea. Furthermore, few authors have reported anaerobic halophiles and their potential for biodegradation of organic substances under anaerobic conditions. Mathrani et al. (1987) reported cellulase activity in a hypersaline African lake. Halophilic and halotolerant anaerobic
microorganisms have been isolated from hypersaline media (Ollivier et al., 1994), but there are no data about isolated halophilic anaerobic fungi.

Enzymes from halophilic and halotolerant fungi

Fungi are actively involved in the production of biofuel. One environmental problem is the great amount of water required for the production of biofuels. During the process of biofuel synthesis, the pH and salt concentration increase. As a result, environment conditions become similar to alkaline and saline environments. For this reason, halophilic fungi can be used successfully in this process.

Lignin degrading halophilic enzymes have many potential industrial applications including delignification of pulp, textile dye decolorization, effluent detoxification etc. The use of halophilic enzymes to break down cellulosic biomass can help reduce the need for high temperatures and pH neutralization of the pretreated biomass before fermentation (Begemann et al., 2011). Thus, it is important to improve the efficacy of halophilic cellulase production by newly isolated strains. There is great potential for the development of biotechnologies with halophilic/halotholerant filamentous fungi for the production of biodiesel as well as halophilic lignocellulosic enzymes for biomass treatment.

However, in the recent years the research has diversified to topics like extremozymes, followed by bioremediation studies. Extremozymes are very resistant in extreme conditions and they propose new opportunities for biocatalysis and biotransformations, also for the development of the economy. Recent trends are aimed at utilising the many novel and unique molecules found in halophiles for molecular, biomedical and biotechnological applications (Oren, 2010). Fungal biotechnology or “mycotechnology“ has made significant progress over the past five decades. Halophilic fungi can be used as expression hosts as well as a source of new genes. With modern molecular genetic tools, fungi can be used as “cell factories” for enzyme production.

Conclusion

The world economy is highly dependent on fossil energy sources such as oil, coal, natural gas, etc. Fuels from agricultural and lignocellulosic wastes are considered as one of the most important strategies in the world to reduce climate change, increase energy security, and rural economic development. Conversion of agricultural waste into fuel using fungal strains can increase productivity of agricultural products and reduce environmental pollution.

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References


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