

GALACTOMYCES GEOTRICHUM – MOULDS FROM DAIRY PRODUCTS WITH HIGH BIOTECHNOLOGICAL POTENTIAL*

Anna Grygier, Kamila Myszkka, Magdalena Rudzińska✉

Faculty of Food Science and Nutrition, Poznań University of Life Sciences
Wojska Polskiego 31, 60-624 Poznań, Poland

ABSTRACT

The article reviews the properties of the *Galactomyces geotrichum* species, the mould that is most important for the dairy industry. *G. geotrichum* mould has been isolated from milk, cheeses and alcoholic beverage. Its presence in food products makes it possible to obtain a characteristic aroma and taste, which corresponds to the needs and preferences of consumers. *G. geotrichum* plays an important role in ecology, where the mould is employed for the degradation of various hazardous substances and wastewater treatment. It has also been found to have potential for biofuel production. In addition to this, *G. geotrichum* can be applicable in two further major areas: agriculture and health protection.

Keywords: *Galactomyces geotrichum*, cheeses, DDT, angiotensin I converting enzyme, PUFA

INTRODUCTION

Based on the publications from the Web of Science database, it can be stated that *Galactomyces geotrichum* mould is widely used in biotechnology, although only about 100 publications about its identification, metabolism and occurrence in food products and environment are available in the literature. The first publication on *G. geotrichum* dates from 1977, when Redhead and Malloch classified this mould to *Endomycetaceae* genera (Redhead and Malloch, 1977). Next, *G. geotrichum* was isolated from various types of cheeses and response of the characteristic properties of the selected cheeses. Knowledge of the presence specific microorganisms in the cheese meant a starter culture could be prepared in order to obtain repeatability in cheese production. Most of the publications from the database were connected with research on the about application of this mould in food,

amounting to 46% of all publications. *G. geotrichum* was also able to biodegrade harmful compounds, which could be used to improve the quality of the environment. Moreover, many publications on biodegradation and bioremediation processes – 38%. Other papers present the possibility to use the *G. geotrichum* mould in medicine – 6%; production biofuels – 5% and in agriculture – 5%. All the possibilities of *G. geotrichum* mould are not known. Publications about the practical use of *G. geotrichum* are described in this review.

THE CLASSIFICATION OF GALACTOMYCES GEOTRICHUM

Lumbsch and Huhndorf (2007) classified the *Galactomyces geotrichum* mould as belonging to *Ascomycota*

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✉ magdar@up.poznan.pl, phone +48 61 848 7276

Phylum (the largest Phylum of Fungi), *Saccharomycotina* Subphylum, *Saccharomycetes* Class and *Dipodascaceae* Family. This mould is also referred to as *Endomyces geotrichum* and *Dipodascus geotrichum*. To determine the subdivisional affinities of *Galactomyces geotrichum*, a test with diazonium blue B can be used (Summerbell, 1985). According to Kurtzman and Fell (1998), *Galactomyces geotrichum* is in a teleomorphic state, that is, at a sexually reproductive stage. The opposite – anamorphic (asexual) – stage of this microorganism is *Geotrichum* sp., which has not been named (Skóra et al., 2009). However, since 2004, *Geotrichum candidum* has been reported in the literature as an anamorph, with a teleomorphic state in *Galactomyces candidus* (Sacristan et al., 2013). The International Code of Botanical Nomenclature (Vienna Code) from 2005 required different genera names to be given to anamorphic and teleomorphic forms. This regulation was replaced in 2012 by the International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) (Kurtzman, 2015). The new code allows for assignment of both an anamorphic and teleomorphic stage to the same genus. Thus, the anamorph of *Galactomyces geotrichum* is currently referred to as an unnamed *Geotrichum* species (De Hoog and Smith, 2011).

MORPHOLOGY, GROWTH AND BIOCHEMICAL PROPERTIES

G. geotrichum forms white, tomentose colonies with vegetative hyphae. The asci are produced on a wide hyphae and the ascospores are broadly ellipsoidal (Butler and Petersen, 1972; De Hoog et al., 1986). The time of the *G. geotrichum* lag phase is long (10 h), and so is the generation time (3.6 h). *G. geotrichum* has a high proteolysis activity and it grows at 25–30°C. The *G. geotrichum* mould is able to grow at 3 to 11 pH, but the optimal is 5–7 pH (Skóra et al., 2009).

Chen et al. (2010) examined the yeast and moulds obtained from raw milk. Selected *G. geotrichum* isolates were able to grow on glucose and galactose, but could not ferment glucose and assimilate nitrate. Moreover, these microorganisms did not utilize an urea as a carbon source. A test with cycloheximide showed that *G. geotrichum* was resistant to urea, but it was not resistant to cadaverine. Furthermore, no growth of *G.*

geotrichum was detected on cellobiose, maltose, mellobiose, raffinose, mannitol, erythritol and α -methyl glucoside (Chen et al., 2010).

CURRENT AND POTENTIAL USES OF GALACTOMYCES GEOTRICHUM

Dairy and alcohol industries

The *G. geotrichum* mould was isolated from various food products. The most frequently described sources of *G. geotrichum* isolation are summarized in Figure 1. The majority of publications concerning the *G. geotrichum* mould are related to its presence in the dairy industry, especially in cheeses (Binetti et al., 2013; Chebeňová-Turcovská et al., 2011; Flores-Magallón et al., 2011; Goerges et al., 2008; Majcher et al., 2014; Sadecka et al., 2016; Wyder and Puhán, 1999; Wyder et al., 1999). This mould belongs to secondary microflora and is able to survive in ripening cheese where the pH is low and where water activity and salt concentration are high (Binetti et al., 2013). Yeasts and mould affect the sensory properties of dairy products through the fermentation of sugar, assimilation of lactate, and also by means of lipolytic and proteolytic activities (Chaves-López et al., 2012).

The activity of *G. geotrichum* in cheese has also been explored by Wyder et al. (1999), who studied Raclette cheese. The sample used in the study was foilwrapped Raclette cheese. It is a semi-hard cheese made of cow's milk, which is often used for melting. Four types of yeast or mould were selected – *G. geotrichum*, *Pichia jadinii*, *Yarrowia lipolytica* and *Debaryomyces hansenii* B. – and were added for the production of this cheese at the same time as lactic cultures. The aim was to investigate lactic acid utilization, lipolysis, proteolysis and flavour development. The results demonstrated that only the addition of *G. geotrichum* increased the proportion of L(+) lactic acid after the maturation of the cheese, although what typically happens with yeast is utilization of lactic acid – de-acidification, associated with an increase in pH. The effect of lipolysis is free short-chain acids which are associated with flavour development. From all the samples examined, the Raclette cheese with *G. geotrichum* contained the smallest amount of free short-chain acids. This is most likely due to the formation of esters from free short-chain acids, and the



Fig. 1. On the map was presented the places where the *G. geotrichum* mould was isolated from groceries

former's inhibitory effect on microorganisms which liberate free short-chain acids. The advantage of using *G. geotrichum* in cheese production is a reduction of bitterness in the cheese due to the breakdown of bitter peptides (proteolysis). A sensory analysis confirmed that Raclette cheese produced with *G. geotrichum* was less bitter than cheeses produced with other moulds. Moreover, it was found that this mould had an inhibitory activity on the formation of biogenic amines (Wyder et al., 1999).

Another food that owes its characteristic organoleptic properties to microorganisms such as *G. geotrichum* is Slovakian bryndza cheese. In these products *G. geotrichum* are responsible for flavour formation and for proteolysis (Chebeňová-Turcovská et al., 2011). Gamero et al. (2016) checked the production of aroma by fungi. *G. geotrichum* was a species capable of biosynthesis of aroma compounds at high level, therefore it can be used for food application. A second important finding of this research was that the profile of aroma compounds produced by *G. geotrichum* did not differ in other species of *G. geotrichum* (Gamero et al., 2016). In 2016 Sadecka et al. became interested in another traditional cheese from Slovakia – barrelled ewe's cheese. From winter bryndza cheese production the intermediate product

is taken out and it is subjected to long-ripening. The mould *G. geotrichum* with lactococci and lactobacilli prevails in this product. In this particular case *G. geotrichum* was also responsible for aroma formation (Sadecka et al., 2016).

The *G. geotrichum* mould was also predominant in German Limburger cheese (Goerges et al., 2008). For the production of this cheese, pasteurized milk was used, as well as a thermophilic lactic acid starter culture. After 6 days of ripening, the percentage of *G. geotrichum* in the batch ranged from 5 to 83. However, two morphologically different types of this mould were found in the samples. One colony had a turquoise- to blue-coloured surface and was present in all batches, and the other had a light green and fluffy appearance and was present in half of the batches (Goerges et al., 2008).

In Cotija cheese, produced in Mexico, the dominant microflora are lactic acid bacteria (Flores-Magallón et al., 2011). Samples of this artisanal cheese were examined for the presence of other microorganisms, and four strains of *G. geotrichum* were found. A phenotypic characterization demonstrated that three out of four strains grew in 37°C, and one was able to grow on acetic acid, both of which is rare for these microorganisms (Flores-Magallón et al., 2011).

In a study by Wyder and Puhan (1999), several cheeses were prepared that contained various microorganisms. The preparation process was the same in each case. A sensory panel judged the aroma of the samples. The best scores were given to the cheese produced with *G. geotrichum*. The sample was a mouldy, mild, soft cheese such as Brie and Camembert (Wyder and Puhan, 1999).

An interesting study was conducted by Binetti et al. (2013), whose aim was to check cheese starter autochthonous microorganisms for their functional properties. In the experiment, which was conducted in Argentina, two strains of *G. geotrichum* were isolated from the starter culture. These strains constituted 2% of all isolated strains. In the study, the properties of the obtained strains were examined. *G. geotrichum* was the only strain that tolerated a 15% (w/v) concentration of NaCl and a 3% (v/v) concentration of lactic acid in the medium. These strains also had proteolytic and lipolytic activity ability. In simulated gastrointestinal digestion, the ability to survive was different for the two strains isolated from the cheese. After 150 min of digestion, the percentage count reduction for one strain was 80%, and for the other 0%. These two strains also had the highest auto-aggregation levels (Binetti et al., 2013).

An interesting property of *G. geotrichum* was observed by Majcher et al. (2014). The mould was isolated from a traditional cheese product – fried cottage cheese. The production of the cheese starts with the ripening of the quark cheese until it acquires a yellowish colour, viscous texture and a specific flavour. Subsequently, the cheese is melted at a low heating temperature – 90°C – with butter. In the experiment, odour active compounds of the cheese were identified, such as phenylacetaldehyde, 2-phenylethanol and phenylacetic acid, which all bring a rosy and honey-like flavour. The *G. geotrichum* mould isolated from the cheese has potential for the conversion of L-phenylalanine to 2-phenylethanol, a phenomenon described by Ehrlich (1907). This finding was confirmed using labelled [2H5]-L-phenylalanine in the culture medium of *G. geotrichum* (Majcher et al., 2014). The next step of the study was to optimise the culture medium, so as to obtain the most efficient results of L-phenylalanine conversion. After examining a series of cultures against various factors, such as medium components (various

types of carbon source, nitrogen source, amounts of mineral components and a single precursor, L-phenylalanine), culture conditions (pH value) and types (batch culture or fed-batch culture), the most effective combination of these factors was selected. The most efficient production was obtained when the medium was composed of sucrose (80 g/l), L-phenylalanine (21 g/l) and when the pH value in the batch culture was 5.0. In this medium, the mould was also able to produce phenylacetaldehyde, as well as phenylacetic and phenyllactic acid (Grygier et al., 2015).

In dairy industry moulds, *G. geotrichum* occurs not only in various types of cheeses but also in other milk products such as kumis, a traditional low alcoholic drink made of fermented cow's milk. Of all 93 strains of yeasts and moulds that were isolated from Colombian kumis coming from different geographic regions, *G. geotrichum* was the dominant species. It represented almost 23% of all isolated microorganisms (Chaves-López et al., 2012).

Chaves-López et al. (2012) studied the microorganisms in kumis and found that depending on the species forming the co-culture with *G. geotrichum*, the bitterness of the final product changes. For instance, bitterness was close to zero when the co-culture was created with *Lactobacillus plantarum* LAT03 and *Enterococcus faecalis*. Hence, these strains have a negative impact on the quality of kumis. In this respect, the presence of the *G. geotrichum* mould can also be considered undesirable, as it affects the growth in the number of *Lactobacillus plantarum* cells (Chaves-López et al., 2014).

Another dairy product that was examined was nunu, an African product made of spontaneously fermented raw unpasteurized cow's milk. *Saccharomyces cerevisiae* and *Pichia kudriavzevii* are dominant in that product, but *G. geotrichum* is also present in some samples (Akabanda et al., 2013).

Yet another popular type of fermented milk is shubat, a Kazakhstani drink made from unpasteurized fresh camel's milk, which is also known in Mongolia, Uzbekistan, Turkmenistan and some regions of Russia. It is consumed both as a nutritional drink and as a medicinal remedy. The presence of *G. geotrichum* has been demonstrated in this drink, but in scant amounts (Akhmetsadykova et al., 2014). In nunu and shubat the presence of *G. geotrichum* affects the flavor

development and synthesis aminopeptidases, which reduce bitterness (Akabanda et al., 2013; Akhmet-sadykova et al., 2014).

G. geotrichum was also identified in a beverage made from a product other than milk. Boza, a low alcohol drink from the Balkans, is made of cereals. In a study by Osimani et al. (2015), boza was examined by means of PCR-DGGE (polymerase chain reaction denaturing gradient gel electrophoresis) analysis. Here, the overwhelming number of strains were lactic acid bacteria; other microorganisms included some yeast and mould. Peptides inhibiting the angiotensin I-converting enzyme have also been documented in boza (Osimani et al., 2015).

Another alcoholic drink was examined by Wu et al. (2012), who aimed to identify the microorganisms which affect the flavour of Maotai-favour liquor, a traditional Chinese alcohol produced from fermented sorghum. The *G. geotrichum* mould was isolated from a sample of this alcohol. Here, a new morphology of *G. geotrichum* colony was discovered: it was light green in colour, and had a white rim. The colony also had an interesting topography: it was large and had a thorny surface and a circular margin. All the microorganisms isolated from the Maotai liquor were treated with stress factors such as temperature; the study was the first to demonstrate that *G. geotrichum* was able to survive a temperature of 46°C. Compared with the volatile compounds produced by other isolated microorganisms, *G. geotrichum* produced 3-methylbutanol and phenylethyl alcohol, but the amount was 235 times lower than that produced by *Saccharomyces cerevisiae*. Of esters, ethyl acetate, ethyl hexanoate, and phenylethyl propionate were produced but the amount was also insignificant (only 4–10 µg/l). This could have been due to the lack of precursors suitable for the synthesis of aromatic compounds. Of all the isolated strains, *G. geotrichum* presented the smallest levels of volatiles (Wu et al., 2012).

In another type of Chinese liquor – daqu – microorganisms were identified for the preparation of culture starters for manufacture. Depending on the maximum temperature during the production process, there are three types of this product: qing cha, hou huo, hong xin. In the study, there were 5 samples of these types from two different factories. In 3 samples, the *G. geotrichum* mould was the dominant

fungal species. The authors postulate that this species can affect the flavour of the liquor by degrading proteins and lipids into precursors of volatile compounds (Zhang et al., 2014). Another traditional Chinese product where *G. geotrichum* mould is present is Chinese gruel, which is fermented from proso millet and millet. To prepare starters for industrial production of that product, microorganisms from the gruel were isolated. The microorganisms most frequently isolated from the product were *Lactobacillus*, *Acetobacter*, *Pichia*, and *G. geotrichum*. This means that the product for microbiological testing is free of pathogens. In the samples *G. geotrichum* mould coexisted with the *Pichia* species (Qin et al., 2016).

Biodegradation and bioremediation properties of *Galactomyces geotrichum*

Degradation of harmful substances and wastewater treatment. One of the reasons why *G. geotrichum* can be used for environment protection is its ability to degrade a number of harmful substances. One of these is DDT (1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane), an organochlorine pollutant which contaminates the environment and has harmful effects on humans and animals. Despite the ban on the use of this compound for pest control and in the agricultural and forestry industry, high concentrations of organochlorine compounds still exist in soil, sediment, water and air, as it has been used since World War II (Tian et al., 2015). Purnomo et al. (2010) examined the potential of cattle manure compost to degrade DDT to DDD (1,1-dichloro-2,2-bis (4-chlorophenyl) ethane) and DDE (1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene), which are less toxic than DDT. There are three stages of the composting: mesophilic stage (a few days), thermophilic stage (from a few days up to several months) and maturation stage (several months). Each stage involves the activity of different types of microorganisms; the stages also differ with regard to temperature in the compost. These processes were examined by Purnomo et al. (2010). In the study, the highest temperature was at 9 h, and then it decreased slowly. In the thermophilic stage, the maximum temperature was 70°C and it is there that most of the DDT was degraded (as much as 48% of the toxic compound). In the other stages, the DDT degraded more slowly (only 10% for each stage). One of the goals of

Purnomo's et al., (2010) study was to check how different composting temperatures affected the degradation of DDT. It turned out that the degradation of DDT was better when the temperature was higher (60°C), and this finding was consistent for all stages. Moreover, the best results were obtained in the sample with manure from the thermophilic stage: here, as much as 88% of the DDT was degraded. Apart from the above, Purnomo et al. (2010) also identified the microorganisms that were involved in the degradation. There was twice as much fungi biomass as there was prokaryote biomass. In the fungi biomass, *G. geotrichum* and *Mucor circinelloides* were identified. In the mesophilic stage, there were both fungal species in the compost; subsequently, in the thermophilic stage, there was only *G. geotrichum*, while in the maturation stage, only *Mucor circinelloides*. Interestingly, it turned out that the strain of *G. geotrichum* isolated from the thermophilic stage was able to degrade as much as 93% of the DDT in a PBD (Potato Dextrose Broth) medium (Purnomo et al., 2010).

Another group of substances that can become biodegradable with the use of *G. geotrichum* is azo dyes. The first research carried out was connected with decolorization of Methyl red azo dye. The *G. geotrichum* strain MTCC 1360 after 1 h in deionized water at 30°C in shaking conditions showed a 100% decolorization of the toxic dye. The process speed increased when molasses (1 g/l) was added to the deionized water. Then the process lasted 10 minutes (Jadhav et al., 2008c). In Govindwar's et al. (2014) study, a static culture of this mould kept in a malt extract medium was treated with an azo dye; after 30 hours of such a treatment, 86% of the dye was decolorized. Less satisfying results were obtained when the culture was shaken. The optimum temperature and pH were 30°C and 7.0, respectively (Govindwar et al., 2014). The same results were obtained with decolorization of Rubine GFL by *G. geotrichum* (Waghmode et al., 2012a). Govindwar et al.'s (2014) research shows that the enzymes that are most important in the decolorization of azo dyes are laccase, azo reductase and intracellular tyrosinase (Govindwar et al., 2014). Similar findings were obtained with a mixture of azo dyes (Remazol red, Golden yellow HER, Rubine GFL, Scarlet RR, Methyl red, Brown 3 REL, and Brilliant blue; Waghmode et al., 2011).

Various microorganisms' decolorizing ability was also examined by Kurade et al. (2012). The authors looked at the decolorization of Scarlet RR dye by individual microorganisms – *G. geotrichum* MTCC 1360 and *Brevibacillus laterosporus* MTCC 2298, as well as by a consortium of these microorganisms. For all experimental conditions, the longer the duration of the treatment, the better the results of the decolorization. The best results were obtained when a consortium of *G. geotrichum* MTCC 1360 and *Brevibacillus laterosporus* MTCC 2298 was used for the decolorization. This was possible thanks to the cooperation of two different enzyme systems. The greatest activity was observed for the intracellular tyrosinase enzyme (Kurade et al., 2012). A similar experiment using a consortium of *G. geotrichum* and *Brevibacillus laterosporus* (GG-BL) was conducted by Waghmode et al. (2012a) with Rubine GFL dye. In their study, the activity of laccase increased from 188% to 325% during the decolorization process. The best results (100% decolorization) were obtained when the consortium was in aerobic/microaerophilic conditions (Waghmode et al., 2012b). The components of the culture medium that are most suitable for decolorization differ depending on the dye and microorganisms used. For decolorization of azo dye RY-84A with the use of *G. geotrichum*, the best carbon and nitrogen sources were glucose and ammonium chloride. For decolorization of Rubine GFL using the GG-BL consortium, in turn, full (100%) decolorization was obtained when the carbon source was glucose and the nitrogen source was a yeast extract. For Remazol Red, the best decolorization was obtained when the carbon source was pepton (Govindwar et al., 2014; Waghmode et al., 2012b; Waghmode et al., 2012c). For all the above cases, the decolorized dye is always less toxic than the dye on the plant seed. This is because the dye decreases germination and reduces the shoot- and root length of germinating plants such as *P. mungo* and *S. vulgare*. Also, a consortium of *G. geotrichum* and *Brevibacillus laterosporus* are the excellent catalyst for decolorization of sulphated Reactive Red 198 azo dye. A common culture allows the discoloration of dye to a degree of 92%. For comparison, separate cultures of microorganisms give only half of this result (Kurade et al., 2015). That consortium was also used to degrade the sulphur-containing

dye Brilliant Blue G (Jadhav et al., 2008b). Consortium *G. geotrichum* and *Bacillus* sp. VUS, in turn, is able to achieve 100% decolorization of Brown 3REL dye (Jadhav et al., 2008a).

An environmental problem where a solution is still being sought is harmful dairy effluents – organic contaminants requiring large amounts of oxygen to decompose. Djelal and Amrane (2013) examined the potential of a consortium of microorganisms such as *Aspergillus niger*, *Mucor hiemalis* and *G. geotrichum* to biodegrade the effluent by means of bioaugmentation. The study checked the relationship between the degradation of a compound of dairy effluents – lactose – and its concentration in the medium – reconstructed sweet whey. The best results were obtained when the concentration of lactose was 7.5 g/l in the medium. Another experiment conducted by Djelal and Amrane (2013) showed that the initial lactose amount does not have any impact on the removal of chemical oxygen demand (COD). Moreover, it was shown that the use of accelerated microorganisms improved COD removal more than the use of non-accelerated microorganisms. In the study, a detergent was added to the culture, as happens in the case of dairy effluents, but its presence did not have a negative effect on biodegradation; it was to the contrary, it had a positive effect on the final concentration of biomass, thanks to its containing phosphorous. Importantly, bioaugmentation using consortia of microorganisms such as the above also gives positive results on an industrial scale (Djelal and Amrane, 2013).

Another danger for the environment is the post-production of dregs from factories which produce medicines, especially antibiotics. An example here is the production of lincomycin, carried out by biotechnological methods such as the fermentation of *Streptomyces lincolnensis*. Residual lincomycin is present in waste fermentation dregs in approximately 2000 mg per kg of fermentation dregs, and this component is a hard-biodegradable pollutant. Zhang et al. (2015) isolated yeast and moulds from the soil and water from various waste fermentation residue storage locations. The degradation efficiency of the best strain was 37%; after identification, it was found to be *G. geotrichum*. In this case, the pH optimal for the degradation was 4.0, and the temperature – 28°C (Zhang et al., 2015).

Another biotechnological manipulation in wastewater treatment is flocculation, which is used in order to accelerate the separation of pollution from water. Zhu et al. (2014) isolated microorganisms from 21 samples of soil, sludge and wastewater to find new biofloculants. Their publication was the first to demonstrate the *G. geotrichum* mould's flocculation ability (Zhu et al., 2014).

In another study, *G. geotrichum* was observed in wastewater treatment plants. It was isolated from the sludge. The aim of the research was to identify such culture conditions that promote the degradation process. The study examined the influence of different organic loading rates on the growth of fungal filaments. More filamentous fungus were present in a bioreactor with the highest organic loading rate (Matos et al., 2012).

The potential of *G. geotrichum* for the production of biofuels. Another area where *G. geotrichum* has potential is in the production of biofuels. In a study by Marjakangas et al. (2015), *G. geotrichum* was isolated from palm oil mill effluent, which, when treated with microorganisms, can be used for the production of lipids for biodiesel and renewable diesel. In the culture, the quantity of triacylglycerols increased and the quantity of diacylglycerols decreased in two out of the four eukaryotic microorganisms that were isolated; one of these was *G. geotrichum* (Marjakangas et al., 2015).

An earlier study, conducted by Sitepu et al. (2014), was also related to biofuels. In the experiment, lignocellulosic plants' biomass was used for the production of second generation biofuel. This type of biomass is difficult for microorganisms to digest. One solution to this problem is pretreatment of the biomass using ionic liquid. The conventional fermentative yeast, *Saccharomyces cerevisiae*, cannot be used for this process, as it is too sensitive for ionic liquid. To find a viable alternative, Sitepu et al. (2014) tested 98 yeasts and moulds and screened them for resistance to ionic liquid. Of all the strains that have been found to be tolerant to ionic liquid in concentrations of up to 5%, one was *G. geotrichum* (Sitepu et al., 2014).

***G. geotrichum* in agriculture**

An interesting area where *G. geotrichum* can be applied is agriculture. This stems from the mould's potential to solubilize phosphorus from rock phosphates. Phosphorus may be used for soil fertilization, so that optimal quantities of crop plants are delivered. In some areas, the phosphorus is in the soil, but it is not available for the plants. Yingben et al. (2012) isolated *G. geotrichum* from rhizosphere soil samples from the agricultural fields around phosphate mines in China. The highest efficiency of phosphorus solubilization was obtained at 40 h of culture growth at 30°C with an initial pH of 7.0. The best effects were obtained when the concentration of soluble phosphorus from rock phosphates was 5 g/l, and the shaking speed was 160 rpm. The maximum result of the solubilization of phosphorus was 1252 mg/l (Yingben et al., 2012).

A relevant study conducted by Waqas et al. (2014) found that *G. geotrichum* was able to produce a plant phytohormone. In this study, *G. geotrichum* was isolated from the *Trapa japonica* plant from an abandoned Zn mine. The strain is able to produce IAA (indole acetic acid), a phytohormone which is responsible for the enhancement of plant growth when they are exposed to biotic or abiotic stress. The second activity of the phytohormone is the reduction in crop plants' uptake of heavy metals; thus, the *G. geotrichum* mould can protect plants from the stress connected with the presence of heavy metals in soil. It was found that the application of *G. geotrichum* to soybeans improved the growth of the plant. This was demonstrated by the production of ROS (Reactive oxygen species), which are involved in the growth of various organs, for instance, in root elongation and leaf expansion. The best results were obtained when the application of *G. geotrichum* was performed with a hardwood-derived biochar. *G. geotrichum* was able to decrease the uptake of Zn to soybean roots and shoots (Waqas et al., 2014).

G. geotrichum has also been found to show resistance to three heavy metals – Pb, Zn and Ag, the best being its resistance to zinc. It needs to be remembered, though, that there are microorganisms more resistant to the above metals: for instance, the yeast *Trichosporon* sp., which is more suitable for such purposes (Munoz et al., 2012).

Health-related uses of *G. geotrichum*

G. geotrichum has also been found to have several potential health-related uses. For instance, the mould has been isolated from alpeorajo, a solid residue obtained in the production of olive oil. From this mould, Fernandez et al. (2008) isolated lipase I, which has a hydrolytic activity to triglycerides and which can be used for enriching fish oils with PUFAs, especially with eicosapentaenoic acid. A mutation of a gene of this enzyme is better for this enrichment than the enzyme of a wild type (Fernandez et al., 2008).

Grygier et al. (in press) found that *G. geotrichum* has the ability to produce polyunsaturated fatty acids. The authors isolated 39 strains from fried cottage cheese (Majcher et al., 2014). The strains were cultured in a Bajpai medium (2% glucose, 1% yeast extract) for 5 days, after which the culture medium was verified for the presence of fatty acids. A number of these were identified: α -linolenic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid. These acids belong to the n-3 polyunsaturated fatty acid group, which is important for the prevention of cardiovascular diseases (Adarme-Vega et al., 2012).

Chaves-López et al. (2012) demonstrated that four strains of *G. geotrichum* isolated from kumis were able to produce peptides that could inhibit angiotensin I-converting enzyme and could therefore be used as functional compounds of food. This enzyme is responsible for the regulation of blood pressure in humans. Inhibition of this enzyme was recorded in patients who suffered from hypertension and congestive heart failure (Binkley et al., 1993). Of all the examined strains, six strains of *G. geotrichum* had the greatest inhibitory activity towards angiotensin I-converting enzyme. However, differences were found in the post-digestive activity of the peptides produced by the different species: the activity of peptides produced by *G. geotrichum* remained the same, while that of peptides produced by other species was increased after simulated gastrointestinal digestion (Chaves-López et al., 2012). The fermentation of kumis with a co-culture demonstrated that the co-culture with *G. geotrichum*, *Enterococcus faecalis* and *Lactobacillus plantarum* had the highest inhibitory activity for the angiotensin I-converting enzyme.

Above all, however, *G. geotrichum* could be used in the production of drugs. Wei et al. (2014) employed

the species for the production of taxol, a substance that can be used in cancer therapy (Wei et al., 2014). Taxol is produced by means of chemical synthesis. It is, however, also possible to biosynthesize taxol using microorganisms. Wei et al. (2014) isolated various strains of microorganisms from soil. These were, in turn, checked for the possibility of resolving racemic ethyl 3-phenylglycidate (rac-EPG) to (2R,3S)-ethyl-3-phenylglycidate, which is a key intermediate in the synthesis of taxol. Out of 200 strains examined, 30 demonstrated the activity of the epoxide hydrolase enzyme, which is important for the transformation of rac-EPG to (2R,3S)-EPG. The newly isolated *G. geotrichum* ZJUTZQ200 was found to have the greatest capacity for this transformation. After the conditions of the culture were optimised, the amount of the biomass with epoxide hydrolase increased from 8 g/l to 34 g/l.

Other

Interestingly, *G. geotrichum* was also isolated from olive paste and olive pomace, though it is not a dominant species there (Baffi et al., 2012). In a study by Baffi et al. (2012), the activity of some enzymes produced by this strain was examined. It was found that all *G. geotrichum* strains had a moderate β -glucosidase activity, 80% of them also had a moderate carboxymethylcellulase activity, and 40% had a moderate polygalacturonase activity. Furthermore, 40% had a weak polygalacturonase activity, and 40% had a strong lipase activity. Other microorganisms did not exhibit such activity (Baffi et al., 2012). This property of *G. geotrichum* adds to the potential of this species for further industrial applications.

Another interesting study aimed at verifying the presence of yeast and moulds in the gastrointestinal tract of piglets. To check this, microorganisms were isolated. Two sample types were compared: those of piglets from a commercial farm, and from an experimental one. In the samples from the commercial farm, the dominant cultures were *G. geotrichum*, *Kasachstania slooffiae* and *Candida catenulate*. In the samples from the experimental farm, in contrast, the dominant cultures were *Kasachstania slooffiae* and *Candida glabrata*. Some of the piglets were weaned 11 days before the samples were taken, while the other piglets remained with their mothers. *G. geotrichum* mould was

found more often in the samples from the unweaned piglets. This could be considered an advantage; if *G. geotrichum* has antimicrobial activity to other yeast in cheese, then it should also have beneficial effects in the case of suckling pigs (Urubschurov et al., 2008).

Like *G. candidum*, *G. geotrichum* is capable of producing a lipase. Yan et al. (2007) made cloning and purification of a lipase from *G. geotrichum* to know the enzyme potential for biodiesel production, selective hydrolysis, esterification for enrichment in PUFAs and for oil-contaminated biodegradation. A lipase isolated from *G. geotrichum* Y05 strain is thermostable and tolerant to organic solvents, therefore, it can be used as an excellent industrial catalyst (Yan et al., 2007).

CONCLUSIONS

G. geotrichum mould is widespread in the environment and has a wide range of current and potential uses. Its most popular living environment is dairy products, where it is possible to use the mould to produce differentiated products that correspond to consumers' needs and preferences. Another environment for this mould is alcohols. Furthermore, the species is also widely used in the removal of various hazardous compounds, as well as in wastewater treatment, thus reducing environmental pollution. Apart from this, *G. geotrichum* has been found to have properties which could lead to its use in agriculture. Finally, *G. geotrichum* has the potential for various health-related uses, including the enrichment of fish oils and the production of drugs. Nonetheless, more research is needed to find ways to employ the mould yet more effectively, both in the areas mentioned above, as well as in new ones.

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