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PRODUCTION OF BIOACTIVE COMPOUNDS BY *GEOTRICHUM CANDIDUM*

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Abstract–The focus of the present study was to evaluate the production of bioactive compounds from *G. candidum*. *G. candidum* is used as a starter culture in dairy industry for cheese ripening. *G. candidum* produces various beneficial enzymes like amylase, lipase, gelatinase, glutaminase, L-asparaginase and glutamate decarboxylase. Furthermore, strain showed prebiotic utilization, GABA and cholesterol lowering activity. *G. candidum* can be used to prepare cheese that will confer additional benefit to the consumers.

INTRODUCTION

Bioactive compounds are produced by a wide range of microorganisms (Shukla, 2015), but currently, just a few of these possibilities are known. *Galactomyces geotrichum*, often known as *G. geotrichum*, is a little-known mold that is employed as a starter or nonstarter culture in the manufacturing of numerous cheeses around the world (Chaves-López *et al.,* 2017). According to the literature, *G. geotrichum* can produce peptides that inhibit the angiotensin I converting enzyme (Grygier *et al.,* 2017).

GABA is a nonprotein amino acid that acts as a major inhibitory neurotransmitter in the mammalian central nervous system (Schousboe & Waagepetersen, 2007). Furthermore, GABA has hypotensive, calming, and diuretic properties and can help prevent diabetes (Li and Cao, 2010). L-asparaginase is a clinically acceptable anti-cancer agent that has been used in combination with other agents in the treatment of acute lymphoblastic leukaemia (mainly in children), reticle sarcoma, Hodgkin disease, acute myelocytic leukaemia, acute myelomonocytic leukaemia, chronic lymphocytic leukemia, lymphosarcoma and melanosarcoma chemotherapy (Abbas *et al.,* 2010). Lipases are used in a variety of industries, including food, degreasing formulation, dairy, medicine, detergents, and fine chemical synthesis (Gupta *et al.,* 2004). *G. candidum* lipase research is limited; however, it is of particular importance due to its vast industrial demand and use in the food sector (Kocabiyik and Ozel, 2007).

The focus of the present study was to evaluate the production of bioactive compounds from *G. candidum*. Various enzymes like amylase, lipase, gelatinase, glutaminase, asparaginase and glutamate decarboxylase, furthermore prebiotic utilization, antimicrobial activity and antibiotic susceptibility. The potential of *G. candidum* to biosynthesize GABA and cholesterol lowering activity was also studied.

MATERIALS AND METHODS

Yeast strain

G. candidum, proprietary strain Gc2 of Enformtech, New Zealand, was grown on glucose, peptone, and yeast extract (GPY) agar plates and incubated at 30°C for 24 h. Activated culture was inoculated in 100 mL GPY medium, incubated at 30°C for 24 h and used for biomass production.

Cholesterol removal assay

The method of Gilliland *et al.* (1985) was used to estimate cholesterol removal by *G. candidum.*

Amylase production

Production of amylase was performed by using the method of (Hols *et al.,* 1994).

Lipase production

G. candidum culture was streaked on tributyrin agar (tributyrin 1%, yeast extract 0.3%, peptone 0.5%, agar 2% w/v); plates were incubated at 30°C for 72 h. Lipase production is indicated by the formation of a clear zone around the colony.

Gelatinase production

G. candidum culture was streaked on Gelatine agar plate, incubated at 30°C for 24 h. Plates were flooded with Frazier's reagent. Formation of clear zone indicates gelatin hydrolysis.

Asparaginase production

Production of asparaginase was performed by the method of (Gulati *et al.,* 1997).

Glutaminase production

G. candidum culture was streaked on modified glutamine agar (MGA) peptone 1%, yeast extract 0.5%, meat extract 0.1%, K_3PO_4 0.2%, $MgSO_4$ 0.02%, $MnSO_4$ 0.05%, tween 80 0.1 %, ammonium citrate 2%, L-glutamine 2%. Phenol red was added to the agar as pH indicator. All plates were incubated at 30°C for 48-72 h. Microbial colonies exhibiting a pink or red zone on the plate consider as positive result.

Rapid glutamate decarboxylase assay

The method of Rice *et al.* (1993) was used for the glutamate decarboxylase assay.

GABA production

The method of Lee *et al.* (2010) was used to evaluate GABA production by *G. candidum.*

Prebiotic utilization test

Prebiotic utilization was performed using the method of (Kaplan and Hutkins, 2000).

Antimicrobial activity

Schillinger and Lucke's (1989) method was used with minor modification to test the ability of *G. candidum* cells to inhibit the test pathogen was using a spot inoculation method. According to Landa *et al.,* (1997), the antifungal activity of *G. candidum* against food spoilage fungi was investigated.

Antibiotic susceptibility test

The method described by Charteris *et al.,* (1998 b) was used for antibiotic susceptibility test.

RESULTS

Cholesterol removal assay

G. candidum Gc2 showed 53 % cholesterol removal after 24 h incubation with 100 mg/L of cholesterol in GPY medium. % Cholesterol removal showed in (Fig. 1, Table 1).

Table 1 Antibacterial activity of *G. candidum* Gc2

Test Strains	Control	SGF	SGF-SIF
B. megaterium	24±0.2	17±0.4	16±0.7
B. subtilis	23±1.4	23±0.7	18±3.5
B. cereus	23±0.0	21±1.4	18±0.7

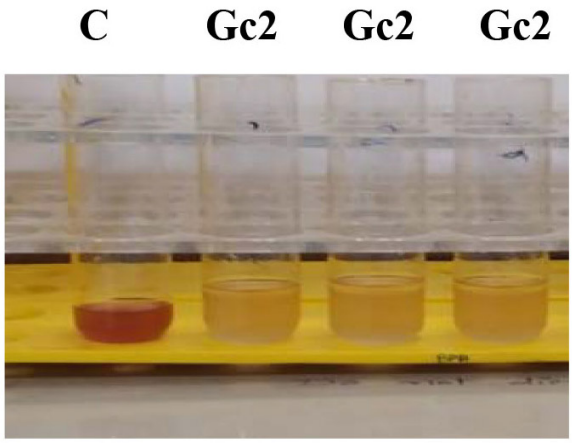

Fig. 1. Cholesterol removal by *G. candidum* Gc2

Amylase, Lipase, Gelatinase Activities

G. candidum exhibited zone of hydrolysis on starch, tween and gelatin agar plates. It indicates *G. candidum* Gc2 produce extracellular enzymes namely amylase, lipase and gelatinase (Fig. 2)

Beneficial enzymes

(i) Asparaginase production

G. candidum Gc2 showed a strong pink zone surrounding the colonies in medium containing L-asparagine with phenol red as an indicator, showing an increase in pH caused by ammonia formation in the medium. (Fig. 2).

(ii) Glutaminase production

Culture was screened using MGA agar containing

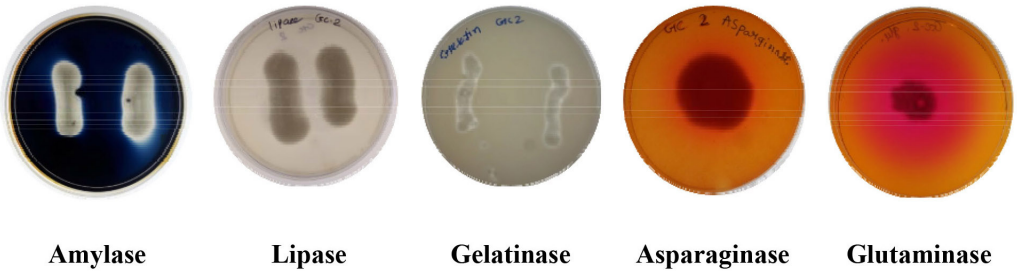

Fig. 2. Enzymes production by *G. candidum* Gc2

phenol red as pH indicator. *G. candidum* Gc2 demonstrated a pink zone around the colony in MGA agar medium with phenol red as pH indicator, phenol red turn to pink due to increased pH by ammonia production (Fig. 2).

(iii) Rapid glutamate decarboxylase assay

Glutamate decarboxylase production was evaluated of *G. candidum* Gc2. GAD is a pyridoxal enzyme which catalyzes in the removal of carboxyl group of L-glutamic acid that is adjacent to the amino group, producing the neurotransmitter, γ-aminobutyric acid (GABA). Glutamate decarboxylase production was observed in *G. candidum* Gc2 after 4 h of incubation showed distinct change from yellow to blue (Fig. 3) considered a positive result.

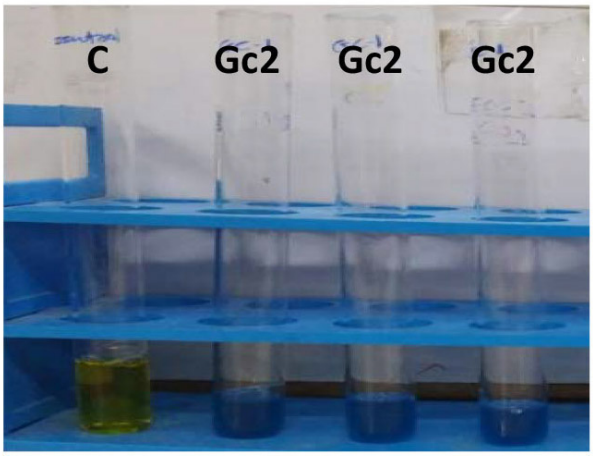

Fig. 3. Rapid glutamate decarboxylase production by *G. candidum* Gc2

(iv)GABA production

Cell free supernatant of *G. candidum* Gc2 showed production of GABA (Fig. 4, Lane 3) *G. candidum* Gc2 exhibited GABA spot corresponding to the standard. Culture showing conversion of glutamate to GABA must have glutamate decarboxylase enzyme which is the prime requirement for the conversion in GABA. The TLC system described here gave good results with only 10 µL samples.

Prebiotic utilization test

Prebiotic utilization plate assay was carried out using three prebiotics Inulin, FOS and Lactulose. All three prebiotics was utilized by *G. candidum* Gc2 as indicated by yellow zone against purple background on GPY agar plates indicating acid production (Fig. 5).

Table 2. Antifungal activity (%) of *G. candidum* Gc2 against food spoiling fungi

Test Strains	Gc2
A. niger	18±0.0
A. flavus	4±0.1

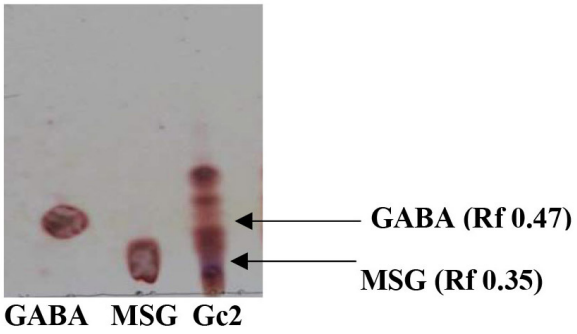

Fig. 4 Thin-layer chromatography (TLC) analysis of GABA by *G. candidum* Gc2.

Table 3. Antibiotic susceptibility test of *G. candidum* Gc2

Antibiotics	Gc2
Amphotericin B (100 µg)	11
Fluconazole (25 µg)	26
Nystatin (100 µg)	12
Voriconazole (1 µg)	27
Itraconazole (30 µg)	10

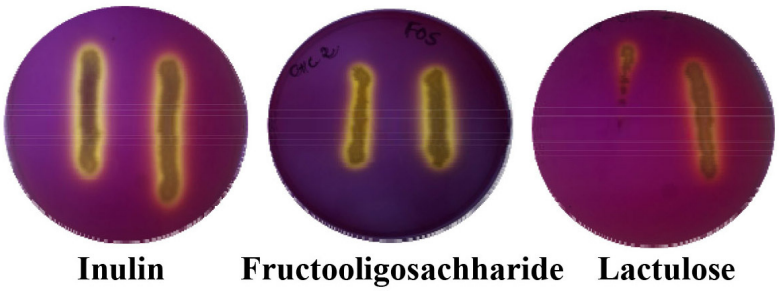

Fig. 5 Prebiotic utilization by *G. candidum* Gc2

Antibacterial activity

G. candidum Gc2 exhibited antibacterial activity against Gram positive *Bacillus* spp., while no activity detected against Gram negative gastrointestinal pathogens. (Table 1). The extent of antibacterial action of Gc2 varied with Gram-positive and Gram-negative bacteria. Gc2 demonstrated higher antibacterial activity against *Bacillus* spp. tested.

Antifungal activity

G. candidum Gc2 showed inhibition of *A. niger* and *A. flavus* food spoiling and aflatoxin producing fungi respectively (Table 2).

Antibiotic susceptibility test

G. candidum Gc2 exhibited susceptibility to tested antifungal antibiotics (Table 3).

DISCUSSION

Bioactive compounds are gaining attention in the food and pharmaceutical sectors due to their health benefits including antibacterial activity. Microbes are known to be producer of diverse beneficial metabolites but not explored much, however some of them have been recognized. *Geotrichum geotricum* or *Galactomyces geotrichum* is use as a starter or nonstarter culture in the manufacturing of numerous cheese across the world (Chaves-López *et al.*, 2017). Therefore, it is noteworthy to explore the potential of *Geotrichum* culture for production of bioactive compounds.

The present study revealed that *Geotrichum* is one of the potential sources of bioactive compounds. There is growing interest in healthy food products that lower cholesterol as that will decrease risk of coronary heart disease, and probiotics are very well known for reducing cholesterol (Tran and Nagano, 2002). Over the last few years, *Saccharomyces boulardii*, *Pichia kudriavzevii* and *Saccharomyces cerevisiae* have been studied as potential probiotics for cholesterol absorption (Psomas *et al.*, 2003). *Galactomyces* sp. BY1 and *Galactomyces* sp. BY2 reported to assimilate 7-21% of cholesterol after 24 h (Chen *et al.*, 2010). While *G. candidum* strain showed 53% cholesterol assimilation within 24 h.

Preliminary studies exhibited that *G. candidum* produces beneficial enzymes associated with utilization of raw material namely amylase, lipase and gelatinase. Some of the previous studies reported *G. candidum* UCMA 91(ATCC 204307) produces metallolipase-like lipase (Muhammad *et al.*, 2017). Lipase, Gelatinase and Amylase producer organisms gained attention as it can be novel enzyme source. *G. candidum* lipase possesses a number of interesting and advantageous properties for industrial biotechnological applications. *G. candidum* Gc2 showed lipase production showing clear zone around the colony. This finding suggests that *G. candidum* Gc2 and its enzymes could be beneficial to the dairy, pharmaceutical, and biofuel industries.

Gelatinase has recently received a lot of attention as a drug development target due to their potential role in connective tissue degradation associated with tumor metastasis (Pacheco, 1998). Because of the potential applications and high demand for gelatinase, there is a need for the discovery of new strains of yeast that produce enzymes with novel properties, as well as the development of low-cost industrial medium and extraction formulations. In our findings *G. candidum* Gc2 showed gelatinase production.

L-glutaminase is applied in food fermentations to produce glutamic acid, which is used as a taste enhancer in a variety of food products. L-glutaminase is employed as a therapeutic enzyme in pharmaceutical research because of its antitumor and anticancer capabilities (Bazaraa *et al.*, 2016).

Bacillus and *Pseudomonas* spp. are reported for production of L-Glutaminases but very little information about yeast sources such as *Zygosaccharomyces rouxii* (Binod *et al.,* 2017). *G. candidum* Gc2 exhibited glutaminase production after 48 h of incubation although optimization studies are in progress. Acute myelocytic leukaemia, chronic lymphocytic leukaemia, and lymphoblastic leukaemia are few of the malignancies that can be treated with L-asparaginase (Verma *et al.,* 2007). It can be utilized in the food industry to reduce 90% of acrylamide content in starchy fried meals without affecting taste or texture (Hendriksen *et al.,* 2007). In our findings *G. candidum* Gc2 showed production of L-asparaginase.

The enzyme catalysis γ-decarboxylation of glutamic acid to produce γ-aminobutyric acid and carbon dioxide. For the enzyme to be liberated, some kind of lytic agent is required (Jilly *et al.,* 1984). Glutamate decarboxylase productions was observed by *G. candidum* in our study. GABA helps to regulate cardiovascular processes including blood pressure and heart rate. As a result, GABA can be employed as a neuroactive compound in the food and fermentation industries. Yeasts strains *Pichia kudriavzevii 1–21, Kluyveromyces marxianus B13–5, Saccharomyces cerevisiae* DL6–20, and *Kluyveromyces lactis* DY1–10 were showed GABA production (Li *et al.,* 2021). Similar finding was observed in our study *G. candidum* Gc2 showed GABA production. *Saccharomyces boulardii* strains utilized galactose and palatinose while in our findings *G. candidum* Gc2 utilized all three prebiotics inulin, Fructooligosaccharide, and lactulose.

Additionally, it was discovered that *G. candidum* inhibited both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Providencia stuartii*, and *Klebsiella oxytoca* (Dieuleveux *et al.,* 1998). While in our finding *G. candidum* Gc2 showed antibacterial activity against *Bacillus* spp. The safety of novel probiotic strains can be determined by assessing strain-specific features like as antibiotic resistance activity. The strain of *G. candidum* employed in this study are resistant to a broad range of antibiotics, making them suitable for use as probiotics.

CONCLUSION

G. candidum Gc2 is potential producer of bioactive metabolites. *G. candidum* produce various enzymes like amylase, lipase, gelatinase, glutaminase, asparaginase and glutamate decarboxylase. Furthermore, Strain showed prebiotic utilization, antimicrobial activity and antibiotic susceptibility. The production of bioactive compounds by *G. candidum* can be used to prepare cheese enriched with bioactive compounds due to the presence of *G. candidum*.

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