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Characterization of lipids and fatty acids of the soil derived fungus *Cladosporium sp.*

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RESUMEN

Caracterización de lípidos y ácidos grasos de hongos derivados del suelo *Cladosporium sp.*

El hongo *Cladosporium sp.* fue estudiado por a su potencial para producir lípidos, ácidos grasos y proteínas. Los lípidos fueron caracterizados por la cuantificación de acilgliceroles y ácidos grasos. Alteraciones en la acumulación de proteínas y lípidos fue observada por cambios en el medio de crecimiento, el tipo y contenido de la fuente de carbono (glucosa o dextrosa), el período de crecimiento y por desarrollo de estrés salino a través de la incorporación de NaCl en el medio de formulación. El contenido lipídico vario desde 6.8% ± 0.3 hasta 27.3% ± 2.8 %, w/w. Los principales ácidos grasos acumulados fueron ácido palmítico, oleico y linoleico. La fracción lipídica obtenida con el medio de patata-dextrosa (conteniendo 2% dextrosa y 4% NaCl) acumulo un 73.7% ± 3.7 w/w de ácido oleico. Algunas fracciones lipídicas encontradas tuvieron una proporción saturado: monoinsaturado: poliinsaturado cercana a 1:1:1 w/w/w. Los triglicérido fueron los principales constituyentes de la fracción lipídica.

PALABRAS-CLAVE: Cladosporium sp. – *Contenido lipídico – Perfil de ácidos grasos.*

SUMMARY

Characterization of lipids and fatty acids of soil derived fungus *Cladosporium sp.*

The fungus *Cladosporium sp.* was explored for its potential ability to produce lipids, fatty acids and protein. The lipids were characterized by the quantification of acylglycerols and fatty acids. Alterations in lipid and protein accumulation were observed by changing the growth medium, carbon source type (glucose and dextrose) and content, growth period and by developing salt stress through the incorporation of NaCl in the formulation of the medium. Lipid content was found to vary from 6.8 ± 0.3 to 27.3 ± 2.8%, w/w. The major fatty acids accumulated were palmitic, oleic and linoleic. The lipid fraction obtained from a Potato- dextrose medium (containing 2% dextrose and 4% NaCl) accumulated 73.7 ± 3.7%, w/w, oleic acid. Some lipid fractions were found to have fatty acid ratios like saturated, monounsaturated, and polyunsaturated close to 1:1:1 w/w/w. Triacylglycerol was obtained as the major constituent of lipid fractions.

KEY-WORDS: Cladosporium sp. – *Fatty acid profile – Lipid content .*

1. INTRODUCTION

Fatty acids are the starting material for synthesis of the bulk of chemicals. They are used in industries like foods & pharmaceuticals; soaps, detergents & cosmetics; paints and varnishes, rubber, textile, polymer, etc (SBP Board of consultants & Engineers, 1996). Vegetable oils and animal fats are considered to be the main source of fatty acids but due to adverse climatic conditions and natural calamities, their production may be affected. Hence, it is essential to find some alternative natural sources for fatty acids. Under such circumstances, microorganisms are known to be the best choice.

Certain strains and species of both yeast and filamentous fungi are known to produce high amounts of oils and fats. Fungal lipids and their fatty acids have been well recognized from the late nineteenth century and considerable studies have been done to determine the potential application of fungi for the production of fat. The production of lipids and PUFA from microbial sources has been investigated and reported by a number of researchers (Bajpai *et al*. 1991; Shimizu *et al* 1988; Saxena *et al* 1998; Chaudhuri *et al* 1997; De and Kumar 2005). Bajpai *et al* (1991) and Shimizu *et al* (1988) have investigated the production and optimization of arachidonic acid and eicosapentaenoic acid in the fungus of the *Mortierella* genus. De and Kumar (2005) have optimized the production of lipids and PUFA especially in some fungi belonging to the genus *Aspergillus* and *Mucor*. They have altered growth conditions and exposed the fungi to UV radiation to determine their effect on PUFA and lipid production. The efficacy of producing lipids of the fungi *Aspergillus oxysporum* (76.60%), *A. solani* (45.80%), *A. ustus* (38.00%), and *A. semitectum* (37.80%) was reported by Naqvi *et al* (1997). The effect of three nitrogen sources on the chemical composition of seven fungi *Aspergillus niger, Penicillum crustosum, Altemaria tenuis, Rhizoctonia solani, Mucor sp.* and *Pythium irregulare* was investigated by Bhatia *et al* (1972) and they found that lipid contents varied from 3.2 to 41.5%, w/w. According to the American Heart Association (AHA) oils and fats having saturated-,

mono- and polyunsaturated fatty acids in the ratio of 1:1:1 is considered ideal due to their nutritional significance (www.telegraphindia.com/1090202/jsp/atleisure/story_10472623.jsp; and www.americanpalmoil.com/publications/PO%20on%20human%20health.pdf). It has been reported that oil high in oleic acid has a high demand in commercial food service applications due to its long shelf life and cholesterol reducing properties (Kaushik and Agnihotri 2000). Although extensive work has been done and is currently going on to meet the demand of fatty acids, no such detailed investigation on the chemical composition of the fungus *Cladosporium sp.* has yet been reported.

The present work was started with the objective of isolating oleaginous fungi from the soil and *Cladosporium sp.* was obtained as one of the isolated species. It was therefore aimed at characterizing the isolated fungus chemically to explore its potential ability as a source of lipids and fatty acids by varying growth conditions.

2. MATERIALS AND METHODS

2.1. Isolation and Characterization of Fungus

The fungus *Cladosporium sp.* was isolated from the soil (collected from Kutch, Gujarat) using the standard procedure of the Pour Plate Method (Cappuccino and Sherman 1999) used for the isolation of soil fungi. Morphologically distinct colonies on the agar plate were selected for transfer on agar slants. The colonies were subsequently purified and stored on Potato Dextrose Agar slants at 4°C. The fungal strain was identified at the Agharkar Research Institute (Pune, Maharastra).

2.2. Culture Medium and Conditions

The fungus was cultivated in three different media-A, B & C having the following composition:

Medium-A: The ingredients were glucose (1, 2, 5 & 10%, w/v), peptone (5 g/L) and yeast extract (3 g/L). All the ingredients were dissolved in distilled water and the pH was maintained at 7.0. The liquid broth (Ca 100 mL) was placed in Erlenmeyer flasks (Cap. 250 mL) and sterilized (in laboratory autoclave) for 15 min. at 121°C under 15 psig steam pressure.

Medium-B: Potato-Dextrose Medium (Potato: 200 g/L; Dextrose: 1, 2 & 5%, w/v). To synthesize this, 200g of potato were scraped and boiled for 15 min in 750 mL of distilled water. The boiled potato was removed by filtration through a filter cloth and the aqueous extract was collected in a beaker. A required amount of dextrose was dissolved in it, the volume was made up to 1000mL and the pH was maintained at 7.0. Erlenmeyer flasks were then filled with this broth and sterilized as above.

Medium-C: Whey Medium, which is synthesized in the same way as Amul Butter Milk (A branded milk product of the Kaira District Co-operative Milk Producers Union Ltd, Anand, which is obtained through the curdling of milk and partial removal of fat and protein) was purchased from the local market and filtered through a filter paper (Whatman No. 1). To remove the fat phase, the filtrate was then slowly heated to a temperature of 60°C and maintained for 30 min. The fat layer separated onto the surface and the protein portion settled beneath. After that, the whey was filtered through Whatman filter paper (No. 1) under heated conditions and the filtrate obtained was allowed to settle in a separating funnel. The upper fat layer was discarded and the aqueous portion was used as a growth medium for fungus. This Medium was divided into two parts, one part was used as such (Medium-C1) without adding any other nutrient and the other part was used after adding 2%, w/w, glucose (Medium-C2). Finally, the medium was transferred into conical flasks (Cap. 250 mL) each containing 100 mL of medium and sterilized in autoclave under standard condition (15 psig steam pressure and 121°C temperature). After sterilization a slight precipitation of whey protein was observed which settled down with time.

The fungus was inoculated in conical flasks containing 100 mL of Medium A, B or C; incubated at 35°C (under diffused sunlight in the laboratory). The flasks were shaken manually and intermittently for 2 minutes at an interval of 15 minutes and harvested after 7, 10 & 13 days. The biomass was obtained as a mat on the surface of the medium. However in MediumC, the biomass was collected from the surface of the medium by using forceps without disturbing the pre-settled protein at the bottom. The biomass was then washed with distilled water, dried under vacuum and quantified gravimetrically.

2.3. Extraction of Lipids and Analysis

The fungal mass obtained from the different media (A, B and C) was dried in an oven at 60°C and quantified gravimetrically. The lipids were extracted for 5 h in a Soxhlet apparatus using methanol-chloroform (2:1, v/v). The solvent was removed by vacuum (730 mmHg pressure) distillation in a water bath maintained at 60°C and the total lipid content was determined. Fatty acid composition was analyzed by the GLC of fatty acid methyl esters (FAME) (refer to Metacalfe and Schmitz 1961) using a Perkin Elmer Auto-system XL Gas chromatograph equipped with a flame ionization detector and PE-Wax capillary column (length: 30 m and inner diameter: 0.25 mm). The oven temperature was programmed as follows: initial temperature: 100ºC, final temperature: 250°C, rise: 5 °C/min. The injector & detector temperatures were maintained at 250 and 280°C respectively. Hydrogen was used as carrier gas. The fatty acids were identified using standard FAME.

After the extraction of lipids the biomass was characterized by the determination of percentage protein content using the Kjeldahl method and

total carbohydrate (TC) content using the Anthrone method (Sadasivam and Manickam 2004). The contents of triglyceride (TG), diglyceride (DG), monoglyceride (MG) and free fatty acids (FFA) were determined by column chromatography (Kates 1972) and the Phospholipid content (PL) of the lipid fraction was determined following the standard method of Chen *et al* (1956) and residual sugar was determined by Fehling's method (Furniss *et al* 2004).

All the experiments have been carried out in triplicate and results shown are the mean with their standard deviations.

3. RESULTS AND DICUSSION

The study was initiated with the objective of isolating some oleaginous fungi of soil origin. As a result of these screening 5 different fungal strains were obtained, one among them being *Cladosporium sp.* The literature reviewed so far shows that although numerous reports have been published on the isolation of soil fungi, the optimization and characterization of lipids or fatty acids of particular interest or protein in the cell mass, including a scientific study on the lipids and protein of *Cladosporium sp.* is lacking. A comprehensive study on the lipids and protein from *Cladosporium sp.* was therefore designed. In the entire study the fungus was grown under different growth condition, biomass was harvested, and the lipids were characterized after extraction.

The chemical composition of *Cladosporium sp.* grown under different conditions in Glucose-Peptone-Yeast medium (Medium A), Potato-Dextrose medium (Medium B) and Whey medium (Medium C) and lipid profiles of the lipids isolated are shown in Tables 1, 3 and 5. The fatty acid compositions of different lipid fractions have also been assayed (refer to Table 2, 4 and 6).

It has been reported (Fujii *et al* 2001, and Yuan *et al* 1989) that the presence of a higher amount of salt in a microbial medium enhances the accumulation of LC-PUFA in cellular lipids. The same result is claimed in marine microorganisms (Floreto *et al* 1994) as well. Hence the effect of salt (NaCl) content on the growth, lipids and fatty acid profile of the fungus *Cladosporium sp.* was investigated. To find out the different effects, the fungus *Cladosporium sp.* was cultured (5 times and each time for 7 days) through serial transfer in a solid medium containing 1, 2 and 4%, w/v, salt. Since the average salt content of sea water is 3.5 - 3.8%, w/v (http://en.wikipedia.org/wiki/seawater), salt addition in the medium formulation was limited to a maximum of 4%, w/v. These three pure cultures of *Cladosporium sp.* were then grown in a Potato Dextrose Medium containing 2%, w/v dextrose and 1, 2 and 4%, w/v salt (NaCl).

3.1. Dry Biomass Response

The fungal biomass was observed to increase with increasing glucose content in Medium A (refer to Table 1). Mycelium growth was found to be very weak at the minimum glucose concentration (1%, w/v glucose) studied and also reduced to 02.75 $\pm$ 0.2 (on day 13) from 04.32 $\pm$ 0.3 (on day 7). A closer observation reveals that 1%, w/v glucose is not sufficient for the organism to grow. The addition of 2%, w/v, glucose did not have much effect on mycelium growth and it was found to remain almost the same with increasing time after 7 days of incubation possibly due to the complete consumption of glucose. At concentrations of 5 and 10%, w/v, glucose, biomass increased with time indicating 2%, w/v glucose to be insufficient. However, the presence of residual glucose in the medium indicates an overaddition. Variation in dextrose content (1, 2 and 5%, w/v) did not bring about much change (6.31 $\pm$ 0.2, 6.89 $\pm$ 0.3 and 6.35 $\pm$ 0.2 g/L) in growth but the addition of 2%, w/v salt (NaCl) increased the growth to 7.41 $\pm$ 0.2 g/L. However, a reduction in growth was observed in the medium containing 4%, w/v, illustrating the adverse effect of excess salt on the growth of the fungus. No appreciable difference in mycelium growth was observed in Medium C2 containing 2%, w/v, glucose (10.25 $\pm$ 0.3 g/L) as compared to Medium C1 (without any nutrients) 09.10 $\pm$ 0.3 g/L giving the idea that the Whey medium itself is enough for fungus to grow.

3.2. Lipid Content

Increasing the percentage of glucose (Table 1) and dextrose (Table 3) in the medium resulted in enhanced lipid accumulation in the cell mass. By increasing the growth period from 7 to 10 days, more accumulation of lipid was observed. However, after 10 days no such enhancement in lipid content of lipid was found. This illustrates that an increase in growth time may have forced the fungus to utilize its fat reserve for its growth. Salt is found to inhibit the accumulation of lipids in the cell mass. Only 6.8 $\pm$ 0.3 to 9.4 $\pm$ 0.3%, w/w of lipid was accumulated in the cell mass grown in the media containing both dextrose and NaCl, particularly in the cell mass of the medium containing 4%, w/v NaCl (6.8 $\pm$ 0.3%, w/w). The presence of 2%, w/v, glucose in Medium C2 (Table 5) helped to accumulate the maximum quantity of lipids (27.3 $\pm$ 2.8%, w/w) of all media (medium A, B and C) investigated.

3.3. Protein and Carbohydrate Content

The maximum quantity of protein (38.7 $\pm$ 2.4%, w/w) with almost the same quantity of TC (38.9 $\pm$ 2.5%, w/w), refer to Table 1, was obtained from biomass grown for 7 days in Medium A containing 2%, w/w, glucose. In Medium B, the maximum amount of TC accumulated (64.7 $\pm$ 2.1%, w/w) and therefore much less protein accumulation (Table 3) took place. However, when comparing the same result with that obtained in the medium containing dextrose and salt, protein content was found to be

Table 1
Compositional Characteristics of *Cladosporium sp.* (Grown in Medium-A) and the isolated lipid

Glucose content (%, w/v)	Growth period (days)	Biomass (g/L, on dry basis)	Composition of Fungus (on dry basis)			Lipid Composition (%, w/w)						Residual glucose in medium (%, w/v)
			Lipid content (%, w/w)	Protein content (%, w/w)	Carbo. content (%, w/w)	TG	DG	MG	FFA	PL	Po. L	
01	07	04.32 ± 0.3	10.3 ± 1.2	24.0 ± 1.4	56.4 ± 2.3	49.0 ± 2.7	6.5 ± 0.4	4.5 ± 0.2	4.7 ± 0.2	0.5 ± 0.1	11.6 ± 0.6	0.0
	10	03.73 ± 0.3	19.2 ± 1.3	22.4 ± 2.3	50.3 ± 2.8	53.9 ± 2.4	7.4 ± 0.5	2.7 ± 0.2	6.2 ± 0.3	1.3 ± 0.1	15.0 ± 0.6	0.0
	13	02.75 ± 0.2	20.4 ± 1.5	34.2 ± 2.4	40.4 ± 2.6	55.4 ± 2.3	8.1 ± 0.6	2.7 ± 0.3	7.6 ± 0.3	2.4 ± 0.2	14.5 ± 0.5	0.0
02	07	06.84 ± 0.2	16.5 ± 1.4	38.7 ± 2.4	38.9 ± 2.5	50.9 ± 2.6	6.9 ± 0.4	3.2 ± 0.3	5.6 ± 0.2	0.7 ± 0.2	15.4 ± 0.4	0.0
	10	06.76 ± 0.2	19.4 ± 1.4	22.2 ± 2.5	49.2 ± 2.4	54.4 ± 2.0	7.5 ± 0.4	2.1 ± 0.2	6.5 ± 0.2	2.1 ± 0.2	13.7 ± 0.5	0.0
	13	06.55 ± 0.3	21.1 ± 1.4	30.5 ± 2.7	46.4 ± 2.4	56.6 ± 2.5	8.4 ± 0.4	1.9 ± 0.2	8.5 ± 0.2	4.3 ± 0.2	12.6 ± 0.4	0.0
05	07	12.45 ± 0.4	18.5 ± 1.3	27.3 ± 2.3	46.8 ± 2.6	53.7 ± 2.2	7.4 ± 0.5	2.3 ± 0.2	6.4 ± 0.3	0.9 ± 0.1	16.3 ± 0.5	1.1 ± 0.3
	10	14.97 ± 0.6	21.1 ± 1.3	28.4 ± 2.2	41.3 ± 2.3	53.0 ± 2.6	6.6 ± 0.5	2.6 ± 0.3	5.6 ± 0.2	1.0 ± 0.1	15.7 ± 0.5	0.6 ± 0.2
	13	15.01 ± 0.6	22.2 ± 1.3	31.2 ± 2.3	37.5 ± 2.4	58.7 ± 2.5	4.0 ± 0.4	3.1 ± 0.3	8.5 ± 0.2	1.1 ± 0.1	13.6 ± 0.4	0.1 ± 0.0
10	07	11.95 ± 0.9	15.3 ± 0.8	23.0 ± 2.0	54.4 ± 2.6	49.3 ± 2.4	5.8 ± 0.4	1.7 ± 0.2	5.0 ± 0.2	1.1 ± 0.2	16.3 ± 0.3	4.2 ± 0.5
	10	15.74 ± 0.8	18.0 ± 1.4	24.4 ± 2.1	48.8 ± 2.6	52.6 ± 2.1	6.7 ± 0.3	2.2 ± 0.3	5.4 ± 0.2	0.6 ± 0.1	14.6 ± 0.4	2.3 ± 0.3
	13	18.86 ± 1.1	20.6 ± 1.8	19.9 ± 2.1	52.2 ± 2.4	52.0 ± 2.9	6.6 ± 0.3	3.2 ± 0.3	5.6 ± 0.2	4.5 ± 0.2	11.3 ± 0.6	1.4 ± 0.2

TG: Triglyceride, DG: Diglyceride, MG: Monoglyceride, FFA: Free Fatty Acid, Carbo. : Carbohydrate, PL: Phospholipid, Po. L: Total polar lipid.

Table 2
Effect of Carbon Source (Glucose) Content on the Fatty Acid Composition of Lipids from *Cladosporium sp.* Grown in Medium A

Glucose content (%, w/v)	Growth period (days)	Fatty acid composition (%, w/w)													
		14:0	14:1	16:0	16:1	16:2	18:0	18:1	18:2	18:3 (α)	18:4	20:4	20:5	22:5	22:6
1	07	0.5 ± 0.1	2.0 ± 0.4	19.6 ± 2.2	0.5 ± 0.1	0.4 ± 0.1	4.9 ± 0.9	28.2 ± 2.2	38.7 ± 2.5	1.8 ± 0.3	0.2 ± 0.03	–	1.3 ± 0.2	–	0.2 ± 0.03
	10	0.4 ± 0.1	–	19.4 ± 2.2	0.5 ± 0.1	–	5.3 ± 1.2	28.4 ± 2.2	39.4 ± 2.8	0.9 ± 0.3	–	2.1 ± 0.3	–	0.8 ± 0.2	1.8 ± 0.5
	13	–	–	26.0 ± 3.0	0.7 ± 0.2	–	8.2 ± 1.5	23.6 ± 2.2	27.3 ± 2.4	0.9 ± 0.2	–	2.9 ± 0.4	2.4 ± 0.4	2.3 ± 0.3	5.1 ± 0.4
2	07	0.2 ± 0.1	–	22.7 ± 2.4	–	–	4.8 ± 1.2	29.1 ± 1.6	37.5 ± 2.6	1.3 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	1.7 ± 0.3	0.4 ± 0.1	0.9 ± 0.3
	10	–	0.2 ± 0.01	22.3 ± 2.4	0.9 ± 0.3	0.1 ± 0.1	5.1 ± 1.1	30.5 ± 2.4	38.4 ± 2.4	1.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.3	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
	13	0.3 ± 0.0	0.4 ± 0.01	20.7 ± 2.5	0.8 ± 0.3	0.6 ± 0.2	4.4 ± 1.3	27.5 ± 2.4	41.5 ± 2.3	1.1 ± 0.1	0.5 ± 0.03	0.3 ± 0.04	0.4 ± 0.03	0.2 ± 0.03	0.5 ± 0.2
5	07	–	0.2 ± 0.01	27.2 ± 2.6	0.9 ± 0.2	–	7.2 ± 1.8	30.3 ± 3.2	30.6 ± 2.6	1.0 ± 0.3	0.3 ± 0.1	0.1 ± 0.0	0.6 ± 0.1	0.1 ± 0.0	0.3 ± 0.2
	10	–	0.2 ± 0.06	25.1 ± 2.4	0.9 ± 0.1	0.1 ± 0.04	6.7 ± 1.2	33.0 ± 3.5	31.2 ± 2.3	0.8 ± 0.2	0.3 ± 0.1	0.1 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
	13	0.2 ± 0.06	–	21.6 ± 2.2	0.7 ± 0.2	–	5.7 ± 1.1	33.4 ± 3.2	33.4 ± 3.2	0.8 ± 0.3	–	0.2 ± 0.01	–	2.5 ± 0.3	1.3 ± 0.1
10	07	0.3 ± 0.1	–	28.5 ± 3.0	0.5 ± 0.05	0.3 ± 0.05	8.0 ± 1.0	29.3 ± 2.3	29.2 ± 2.4	0.6 ± 0.1	0.2 ± 0.05	0.1 ± 0.02	0.1 ± 0.05	0.5 ± 0.1	0.1 ± 0.05
	10	0.2 ± 0.1	–	28.5 ± 2.4	0.7 ± 0.1	0.2 ± 0.1	7.1 ± 1.1	29.0 ± 2.0	31.6 ± 2.4	0.6 ± 0.1	0.1 ± 0.05	0.1 ± 0.02	0.4 ± 0.04	0.3 ± 0.1	1.0 ± 0.2
	13	0.3 ± 0.1	–	25.4 ± 3.2	0.6 ± 0.1	–	8.7 ± 0.8	30.7 ± 2.2	30.9 ± 2.9	1.1 ± 0.1	0.2 ± 0.1	–	0.2 ± 0.04	1.3 ± 0.5	0.2 ± 0.1

Table 3
Compositional Characteristics of *Cladosporium sp.* (Grown in Medium B for 7 days) and the isolated lipids

Dextrose content (%, w/v)	Biomass (g/L, on dry basis)	Composition of Fungus (on dry basis)			Lipid composition (%, w/w)						Residual sugar in medium (%, w/v)
		Lipid content (%, w/w)	Protein content (%, w/w)	Carbo. content (%, w/w)	TG	DG	MG	FFA	PL	Po. L	
1	6.31 ± 0.2	12.5 ± 0.4	18.6 ± 1.3	60.7 ± 2.2	51.5 ± 2.5	06.5 ± 1.2	2.6 ± 0.2	04.3 ± 0.2	2.2 ± 0.2	10.6 ± 0.4	0.2 ± 0.03
2	6.89 ± 0.3	14.2 ± 0.5	13.2 ± 1.5	64.7 ± 2.7	55.6 ± 2.6	07.6 ± 1.2	4.5 ± 0.3	05.5 ± 0.2	2.5 ± 0.2	11.6 ± 0.5	0.9 ± 0.50
5	6.35 ± 0.2	16.6 ± 0.5	11.6 ± 1.2	64.7 ± 2.1	60.9 ± 2.8	08.5 ± 1.3	5.9 ± 0.2	10.6 ± 0.3	0.6 ± 0.1	07.7 ± 0.4	1.1 ± 0.09
2*	7.00 ± 0.3	08.5 ± 0.3	20.8 ± 1.4	54.9 ± 2.3	45.0 ± 2.3	14.5 ± 2.1	4.2 ± 0.2	05.2 ± 0.3	2.1 ± 0.2	07.4 ± 0.4	–
2**	7.41 ± 0.2	09.4 ± 0.3	22.5 ± 1.3	53.1 ± 2.3	48.5 ± 2.5	18.5 ± 2.3	7.6 ± 0.3	08.3 ± 0.2	2.8 ± 0.3	06.4 ± 0.5	–
2***	5.84 ± 0.2	06.8 ± 0.3	28.7 ± 1.2	49.5 ± 2.3	43.0 ± 2.2	13.0 ± 1.8	3.9 ± 0.2	04.8 ± 0.2	1.8 ± 0.2	07.2 ± 0.5	–

All the abbreviations are as mentioned earlier. * Containing 1%, w/w NaCl; ** Containing 2%, w/w NaCl; ***Containing 4%, w/w NaCl.

Table 4
Fatty Acid Composition of Lipids from *Cladosporium sp.* Grown (for 7 Days) in Medium B

Dextrose Content (%, w/v)	Fatty Acid Composition (%, w/w)											
	14:1	16:0	16:1	18:0	18:1	18:2	18:3 (α)	18:4	20:4	20:5	22:5	22:6
1	0.1 ± 0.0	23.7 ± 1.8	0.5 ± 0.2	09.4 ± 1.9	37.6 ± 1.9	25.5 ± 2.2	1.3 ± 0.20	0.2 ± 0.1	0.2 ± 0.3	0.2 ± 0.01	0.6 ± 0.1	0.4 ± 0.2
2	–	28.6 ± 1.1	0.6 ± 0.1	10.3 ± 2.4	37.2 ± 2.3	19.3 ± 1.4	1.2 ± 0.10	0.4 ± 0.1	–	0.7 ± 0.30	0.4 ± 0.1	0.3 ± 0.2
5	–	27.7 ± 1.7	0.7 ± 0.3	10.5 ± 1.4	38.3 ± 2.2	19.5 ± 1.8	1.1 ± 0.10	0.5 ± 0.1	–	1.0 ± 0.06	0.5 ± 0.2	0.4 ± 0.1
2*	0.6 ± 0.2	25.2 ± 1.5	0.7 ± 0.1	07.4 ± 0.9	40.9 ± 3.1	20.2 ± 1.2	0.2 ± 0.05	–	–	0.4 ± 0.02	–	–
2**	–	34.0 ± 2.4	–	07.8 ± 1.5	25.7 ± 1.6	16.9 ± 1.4	–	–	–	–	–	–
2***	–	03.9 ± 0.9	–	05.5 ± 1.2	73.7 ± 3.7	10.1 ± 1.3	–	–	–	–	–	–

* Containing 1%, w/w NaCl; ** Containing 2%, w/w NaCl; ***Containing 4%, w/w NaCl.

Table 5
Compositional characteristics of *Cladosporium sp.* (Grown in Medium C for 13 Days) and the isolated lipids

Glucose content (%, w/v)	Biomass (g/L, on dry basis)	Composition of Fungus (on dry basis)			Lipid composition (%, w/w)						Residual sugar in medium (%, w/v)
		Lipid (%, w/w)	Protein (%, w/w)	Carbo. content (%, w/w)	TG	DG	MG	FFA	PL	Po. L	
–	09.10 ± 0.3	17.5 ± 2.3	22.9 ± 2.3	51.5 ± 3.2	50.6 ± 3.3	4.5 ± 0.2	2.4 ± 0.1	6.4 ± 0.2	1.6 ± 0.1	11.4 ± 1.2	0.9 ± 0.2
2	10.25 ± 0.3	27.3 ± 2.8	24.4 ± 2.8	47.9 ± 3.0	59.2 ± 3.2	8.7 ± 1.2	1.5 ± 0.1	9.5 ± 0.3	1.5 ± 0.1	15.3 ± 1.6	1.1 ± 0.4

All the abbreviations are as mentioned earlier.

Table 6
Fatty Acid Composition of Lipids from *Cladosporium sp.* Grown (for 13 Days) in Medium C

Glucose content (%, w/v)	Fatty Acid Composition (%, w/w)											
	14:0	14:1	16:0	16:1	16:2	18:0	18:1	18:2	18:3 (α)	18:4	20:5	22:6
–	0.5 ± 0.1	0.3 ± 0.1	25.7 ± 2.2	0.9 ± 0.3	0.2 ± 0.1	10.6 ± 1.2	35.9 ± 3.0	23.4 ± 2.2	1.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
2	0.7 ± 0.2	–	26.5 ± 2.3	0.7 ± 0.1	–	10.5 ± 1.1	35.9 ± 3.2	24.5 ± 2.3	1.3 ± 0.1	0.3 ± 0.1	–	–

higher. It was also noticed that protein accumulation in the cell mass decreased (18.6 ± 1.3, 13.2 ± 1.5 & 11.6 ± 1.2 %, w/w) with an increase in dextrose concentration from 1 to 5%, w/v, in the medium, whereas it increased (20.8 ± 1.4, 22.5 ± 1.3 & 28.7 ± 1.2%, w/w) with increasing concentration of NaCl from 1 to 4%, w/v in the medium. No appreciable changes in protein or carbohydrate contents were noticed in Medium C2 (24.4 ± 2.8 and 47.9 ± 3.0%, w/w) as compared to Medium C1 (22.9 ± 2.3 and 51.5 ± 3.2%, w/w).

3.4. Lipid Profile

TG was obtained as the major constituent (Table 1, 3 and 5) among all the components assayed (TG, DG, MG, FFA, Po. L and PL) for lipid samples. The total content of other components (DG, MG, FFA, Po. L and PL) comprised of 27.8 to 35.7, 26.2 to 43.6 and 26.3 to 36.5%, w/w of lipids in Medium-A, B and C respectively. Some higher quantities of DG (13.0 ± 1.8 to 18.5 ± 2.3%, w/w) were found in Medium-B containing both dextrose and salt as compared to Medium A or C.

3.5. Fatty acid Composition

Palmitic acid ($C_{16:0}$, 19.4 ± 2.2 to 28.6 ± 1.1%, w/w), Oleic acid ($C_{18:1}$, 23.6 ± 2.2 to 38.3 ± 2.2%, w/w) and Linoleic acid ($C_{18:2}$, 19.3 ± 1.4 to 41.5 ± 2.3%, w/w) were found to be the major fatty acids in all lipid fractions (Table 2, 4 and 6). Among these, Medium-A accumulated the maximum amount of $C_{18:2}$ fatty acid, whereas Medium-B and C showed the maximum accumulation of $C_{18:1}$. Interestingly, lipid fraction obtained from the Potato- Dextrose medium (containing 2% dextrose and 4% NaCl) accumulated 73.7 ± 3.7%, w/w of $C_{18:1}$ fatty acid. Under this growth condition the fungus may appear to be an excellent source of oleic acid. A closer observation shows a higher quantity (12.7%, w/w) of long chain PUFA ($C_{18:4}$, $C_{20:4}$, $C_{20:5}$, $C_{22:5}$ and $C_{22:6}$) accumulated in the lipid fraction obtained from the biomass grown in Medium-A containing 1%, w/v glucose, for 13 days. Another interesting observation was that the fatty acid composition had a ratio of (refer to Table 2) saturated, monounsaturated and polyunsaturated fatty acid ranging from 25.0 to 36.8, 24.3 to 34.1 and 31.1 to 45.1%, w/w respectively, which is quite close to the ideal dietary fatty acid composition i.e. saturated, mono unsaturated, poly unsaturated (1:1:1, w/w/w).

4. CONCLUSION

The present study shows that *Cladosporium sp*. may accumulate as much as 27.3 ± 2.8%, w/w of lipids and more than 50%, w/w protein. It may also accumulate a high (73.7 ± 3.7%, w/w) proportion of oleic under specific growth conditions. A higher accumulation of oleic acid suggests it may appear to be a promising source. The authors believe that further investigation is needed before commercial exploration. The fatty acids were also found to contain 12.7%, w/w long chain PUFA. This high fatty acid ratio encourages further work on the presence of mycotoxins in the lipids.

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