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ASSIMILATION OF LOW MOLECULAR WEIGHT ALCOHOLS FOR THE PRODUCTION OF LIPIDS BY SOME SOIL FUNGI

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Aspergillus terreus Thom and *Fusarium solani* (Mart) Sacc. have the ability to utilize ethanol, methanol and i. propanol as sole sources of carbon for lipid biosynthesis. 100g of dry mycelia of *A. terreus* grown on ethanol and methanol-containing media was confirmed to contain 22.22 g and 21.43 g lipids, respectively, compared with 25.29 g on glucose-medium. *F. solani* on the other hand could assimilate ethanol more easily than glucose. The organism had got the power to accumulate 20.71% and 18.49% on ethanol and glucose-media successively. Maximal formation of the non-polar lipids was obtained when methanol was the only carbon source. On the other hand, glucose was the best carbon source for polar lipid accumulation. Components of both polar and non-polar lipids on glucose-medium did not differ from those synthesized on both ethanol and methanol-media, although they differ quantitatively. Unsaturated fatty acids were the prevailing acids in lipids of the two organisms, however lipids of *A. terreus* are characterized by more unsaturation.

Key words: Assimilation, Alcohol, Lipid.

INTRODUCTION

Conversion of low molecular weight alcohols to useful products by bacteria and yeasts are of prime interest of many investigators. These products include extracellular polysaccharides (Huq *et al.*, [5], lipids (Suzuki *et al.*, [10] Rattray and Hambelton, [8] and Jwanny and Rashad, [6]) and enzymes (Allias *et al.*, [1]). Filamentous fungi was confirmed to utilize low molecular weight alcohols for the production of proteins (Goncharova *et al.*, [4] and Ghareib and Mousa, [3].

This work was devoted to elucidate the efficiency of certain fungi to synthesize lipids from different low molecular weight alcohols and to investigate composition of the produced lipids.

MATERIALS AND METHODS

Fungi used in this investigation were previously isolated from soil samples collected from certain localities of Egypt and identified by the Commonwealth Mycological Institute, England. The organisms were maintained on Czapek's agar medium and monthly subcultured. Spore suspensions were obtained from 8-day old cultures and used to inoculate different fermentation media. The basal ingredients had the following composition (g/100ml): NH_4NO_3, 0.3; $NaH_2PO_4 . 2H_2O$, 0.073; K_2SO_4, 0.11; $MgSO_4 . 7H_2$), 0.05; $ZnSO_4 . 7H_2O$, 0.005 and $FeCl_3 . 6H_2O$, 0.016. These constituents were distributed between conical flasks of 250 ml capacity and autoclaved. Filter-sterilized solutions of glucose in a ratio of 3% were added to a group of flasks. In another trials equimolecular weights of different alcohols were separately added to other groups under asceptic conditions just prior to inoculation. Initial pH of all flasks was adjusted to 6.2 after sterilization. The flasks were then inoculated, incubated at 25° for 9 days after which the fungal growth was harvested and different estimations were carried out.

Lipids were extracted from the fresh mycelia by the method of Nichols [7] and purified according to the technique adopted by Folch *et al.* [2]. Non-polar lipids were fractionated and detected as stated previously (Ghareib, [3]. Percentage of different fractions were estimated as described by Selim and Mousa [9]. Polar lipids were separated and fractionated as described by Ghareib [3]. Fatty acids were estimated as methyl esters using Gas Liquid Chromatographic procedure.

RESULTS AND DISCUSSION

Twelve different species belonging to five genera were separately grown for 9 days in batch cultures using media with ethanol, methanol, i. propanol and *n*-butanol as the only sources of carbon. The experimental data (Table 1) reveal the variant behaviour of the investigated fungi towards utilization of different low molecular weight alcohols. None of the twelve species could assimilate butanol as a sole source of carbon. This was in complete accordance with the previous findings of Ghareib and Mousa [3]. Availability of the other alcohols for fungal growth was in the following order: ethanol > methanol > propanol.

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Fusarium solani was the most potential in assimilation of the utilizable alcohols. It could produce 12.3, 3.1 and 0.8 g mycelial dry weights when cultured on litre of ethanol, methanol and propanol containing media respecitvely. Moreover, *Aspergillus terreus* could synthesize 8.0, 1.3 and 0.6 g dry biomass when grown on a litre of the previously stated media. *A. tamarii* was confirmed as utilizer of the three alcohols but not to the levels recorded before. *A. niger, A. ustus* and *Cunninghamella echinulata* haven't the ability to assimilate any of the investigated alcohols. Rest of the experimented fungi utilized ethanol and/or methanol except *A. fumigatus* which assimilate ethanol and propanol.

Felt formation and lipid biosynthesis by *A. terreus* and *F. solani were studied* at different periods of incubation. The data (Table 2) show that maximal yields of dry mycelia were obtained on glucose-medium at all periods of incubation. Ethanol and methanol supports good fungal

Table 1 Mycelial dry weights of fungi grown on low molecular weight alcohol-containing media.

Organisms	Dry weight (g/100 ml)			
	Ethanol	Methanol	Propanol	Butanol
Alternaira alternata (Fr.) Keiseler	–	0.15	–	–
Aspergillus fumigatus Fresenius	0.15	–	0.05	–
A. niger Van Tiegh	–	–	–	–
A. nidulans (Eidam) Wint	0.13	–	–	–
A. tamarii Kita	0.67	0.12	0.02	–
A. terreus Thom	0.80	0.13	0.06	–
A. ochraceus Wilhelm	0.08	0.05	–	–
A. ustus (Bainier Thom & Church	–	–	–	–
Fusarium accuminatum Ell. & Ev.	0.64	0.43	–	–
F. solani (Mart.) Sacc.	1.23	0.31	0.08	–
Cunninghamella echinulata (Thaxt.) Thaxt. ex. Blakeslee	–	–	–	–
Penicillium chrysogenum Thom	0.18	0.10	–	–

Table 2 Behaviour of *Aspergillus terreus* and *Fusarium solani* at different periods of incubation on glucose and alcohol-containing media.

Fungus	Incubation period days	Glucose			Ethanol			Methanol			Propanol		
		Felt	Lipid		Felt	Lipid		Felt	Lipid		Felt	Lipid	
			g	%		g	%		g	%		g	%
A. terreus	5	1.98	0.29	14.60	0.48	0.06	12.50	0.06	–	–	0.00	0.00	0.00
	7	2.83	0.56	19.80	0.66	0.11	16.67	0.09	–	–	0.04	–	–
	9	3.48	0.88	25.29	0.81	0.18	22.22	0.14	0.03	21.43	0.06	–	–
	11	3.95	0.91	23.04	1.12	0.24	21.43	0.20	0.03	15.00	0.09	–	–
	13	4.20	0.91	21.67	1.25	0.25	20.00	0.24	0.03	12.50	0.13	0.02	15.38
F. solani	5	0.80	0.09	11.25	0.65	0.09	13.85	0.10	–	–	0.00	0.00	0.00
	7	1.18	0.19	16.10	0.96	0.17	17.71	0.19	0.02	10.53	0.00	0.00	0.00
	9	1.46	0.27	18.49	1.22	0.25	20.49	0.31	0.04	12.90	0.0	–	–
	11	1.60	0.29	18.13	1.40	0.29	20.71	0.36	0.04	11.11	0.12	0.01	8.33
	13	1.65	0.27	16.36	1.36	0.27	19.85	0.42	0.04	9.50	0.12	0.01	8.33

Results are given in g/100 ml of medium.

growth however ethanol was more available. Propanol on the other hand was found unsuitable as sole source of carbon for growth of the two experimented organisms. Concerning lipid formation it is apparent that *A. terreus* could synthesize 25.29%, 22.22% and 21.43% lipids from the mycelial dry weight after 9 days of incubation on glucose, ethanol and methanol-containing media successively. Lipid production by *F. solani* reached the maximum on ethanol and glucose-media amounting to 20.71% and 18.49% at the 11*th* and 9*th* days of incubation, respectively. Moreover, the organism had got the power to synthesize 12.9% lipids of the dry biomass at the 9*th* day of incubation when methanol was the only source of carbon.

Lipids produced after 9 days of incubation on glucose, ethanol and methanol-containing media were analyzed and the results are given. Maximal contents of *A. terreus* and *F. solani* non-polar lipids (Table 3) were obtained on methanol-media. Triglycerides were the major components of lipids of the two fungi. The different glycerides represent more than half content the non-polar lipids. Free fatty acids and free sterols were detected at moderate levels. With respect to polar lipids, Table 4 demonstrate that optimal contents were recorded on glucose followed by ethanol. Decrease of polar lipid on methanol-medium is in agreement with the findings of Rattray and Hambleton [8] using *Hansenula polymorpha.* Generally there is no major differences between components of the polar lipids obtained on glucose and ethanol-media. An exception to this generalization was the presence of cerobroside (C) in polar lipid of *A. terreus* on glucose-medium. Polar lipids synthesized on methanol-media are characterized by an increase in phosphatidylcholin (PC) level in lipid of *A. terreus,* and levels of phosphatidylserine (PS) and PC at the expense of phosphatidylethanolamine (PE) in lipid of *F. solani.*

Table 5 represent the fatty acid pattern of lipids produced on different media. These lipids were characterized by aboundance of unsaturated fatty acids. However more unsaturation was recorded in *A. terreus* lipids. Myris-

Table 3. Non-polar lipid components of *A. terreus* and *F. solani* grown on glucose and alcohol-media.

Fungus	Carbon sources	Non-polar lipid (%)	Fractions (% of total non-polar lipid)							
			Mono glycerides	Di glycerides	Free sterols	Free fatty acids	Tri glycerides	Methyl esters	Sterol esters	Glycerol esters
A. terreus	Glucose	78.2	4.4	4.7	13.5	15.2	46.4	1.8	9.1	4.9
	Ethanol	79.1	4.6	6.3	11.6	13.4	43.5	5.5	8.2	6.8
	Methanol	82.4	1.5	6.7	13.3	15.8	44.4	8.5	9.8	0.0
F. solani	Glucose	77.4	5.3	4.6	13.0	15.5	44.8	4.9	7.2	4.7
	Ethanol	78.3	4.8	6.6	10.9	13.9	43.9	7.8	6.9	5.2
	Methanol	81.9	5.9	6.7	12.6	16.4	40.9	7.3	10.2	0.0

Table 4. Polar lipid composition of *A. terreus* and *F. solani* grown on glucose and alcohol-media.

Fungus	Carbon sources	Polar lipid (%)	Fractions (% off total polar lipid)							
			PS	PI	PC	PE	C	PA	PME	HY
A. terreus	Glucose	21.8	20.1	16.4	18.2	12.2	4.3	16.7	6.4	5.7
	Ethanol	20.9	20.9	16.6	18.9	13.7	0.0	17.8	6.0	6.1
	Methanol	17.6	20.2	18.8	20.0	10.1	0.0	16.0	8.7	6.2
F. solani	Glucose	22.6	16.4	20.5	17.0	21.3	0.0	9.6	7.1	8.1
	Ethanol	21.7	16.5	19.0	17.3	22.4	0.0	10.6	8.2	6.0
	Methanol	18.1	20.9	21.3	19.7	14.2	0.0	10.6	9.3	4.0

PS: Phosphatidylserine; PI: Phosphatidylinositol; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; C: Cerobroside; PA: Phosphatidic acid; PMI :Phosphatidyl N.methyl ethanolamine; HY: Hydrocradon.

Table 5. Fatty acid pattern of lipids produced by *A. terreus* and *F. solani*.

Fungus	Carbon source	Fatty acids (%)										
		Capric	Lauric	Myristic	Palmitic	Palmito-leic	Stearic	Oleic	Linoleic	Linole-nic	Arachi-dic	Behinic
A. terreus	Glucose	1.0	3.1	17.7	10.9	8.3	5.4	21.4	14.5	8.2	5.7	3.8
	Ethanol	0.0	3.7	17.4	11.1	8.3	5.9	20.6	14.3	8.0	6.6	4.1
	Methanol	0.5	2.9	17.9	11.3	8.2	5.7	21.3	14.1	8.0	6.1	4.0
F. solani	Glucose	0.0	2.6	9.6	16.7	6.0	9.4	16.1	23.7	5.4	5.6	4.9
	Ethanol	0.0	3.0	10.0	16.9	6.0	9.6	16.1	22.9	5.1	5.2	5.2
	Methanol	0.0	2.4	9.6	17.0	6.1	9.1	16.4	22.6	5.4	5.9	5.5

tic, oleic and linoleic acids were the dominant acids in lipids of *A. terreus*. Moreover palmitic, oleic and linoleic acids were the prevailing fatty acids of *F. solani* lipids.

REFERENCES

1. J.J. Allias, A. Louktibi and J. Baratti Agric, Biol. Chem. **47**, 1509 (1983).
2. J. Folch, M. Lees, and G.H. Sloane-Stanley, J. Biol. Chem., **226**, 497 (1957).
3. M. Ghareib and N. Mousa, Proc. Egypt. Bot. Soc. **4** (Ismilia Conf.) 11 (1985), Acta Microbiol. Hung., **33**, 27 (1985), J. Fac. Educ. (Ain Shams Univ.), **9**, 117 (1986-b).
4. I.A. Goncharvoa, A.G. Lobanok and V.G. Basitzkaya, Vestsi. Akad. Navuk. BSSR, Ser Biyal. Navuki, **4**, 64 (1976).
5. M.N. Huq, B.J. Ralph and P.A. Richard, Aus. J. Boil. Sci. **31**, 311 (1978).
6. E.W. Jwanny and M.M. Rashad, Egypt. J. Food Sic., **12**, 21 (1984).
7. B.W. Nichols, *New Biochemical Separations,* A.T. James, L.T. Morris (ed.) (VanNostrand, New York, (1964) p. 321.
8. J.B. Rattray and J.E. Hambleton, Can. J. Microbiol. **26**, 190 (1980).
9. M.S.N. Selim and N. Mousa, Proc. Egypt. Bot. Soc. **3** (Mansura Conf.), 189 (1982).
10. O. Sużuki, Y. Jigmi and S. Nakasato, Agric. Biol. Chem., **43**, 1343 (1979).