Lipid accumulation by a cellulolytic strain of *Aspergillus niger*

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Abstract. Lipid accumulation by a cellulolytic mold, *Aspergillus niger*, was studied. The amount of lipid accumulated ranged from 13.6–16.6% on various carbon sources, namely glucose, xylose, avicel (microcrystalline cellulose) and bagasse (a natural lignocellulosic substrate). Neutral lipids, phospholipids and glycolipids of the mycelia varied from 41.0–46.2%, 34.9–38.4% and 18.7–22.6% of total lipids, respectively. Unsaturated fatty acids comprised around 80% of total fatty materials with linoleic and oleic acid predominating. Of the four nitrogen sources tested, NH_4Cl was the best source for lipid synthesis from cellulose (bagasse). Optimum temperature range for growth and lipid synthesis was 25–30 °C.
Key words. Aspergillus niger; cellulose; lipid; fatty acids.

A great deal of work has been performed on lipid-accumulating yeast strains because a number of them are capable of accumulating large amounts of intracellular lipids[1], and the lipids produced usually have a great similarity to plant oils. With the aim of producing substitutes for some of the more expensive types of oil, it is important to gain an increased understanding of fatty acid biosynthesis in other microorganisms too. The potential for the bioconversion of cellulose present in agricultural, industrial and forest residues by cellulolytic microorganisms has been widely explored as a possible means of production of valuable substances such as enzymes, glucose, ethanol and other organic solvents, and protein. The lipids of filamentous fungi have not been as thoroughly studied as those of bacteria and yeasts. We reported earlier the isolation of a cellulolytic mold, *Aspergillus niger* AS-101, which utilizes different lignocellulosic substrates efficiently, in submerged as well as in solid state culture, for the production of cellulase and of single cell protein[2–5]. Properties of the cellulase enzyme complex have also been described elsewhere[6,7]. This paper reports studies on the lipid accumulation of *Aspergillus niger* AS-101 when grown in shake-flasks on various carbon sources including bagasse, a natural cellulosic substrate.

Materials and methods

Microorganism. Aspergillus niger AS-101 was isolated from decomposing substrates in our laboratory[2]. The culture was maintained at 4 °C by subculturing every month on potato dextrose agar slants.

Cultural conditions. The basal medium[3] contained g/l: KH_2PO_4, 2.0 g; $(NH_4)_2SO_4$, 1.8 g; $MgSO_4 \cdot H_2O$, 0.3 g; $CaCl_2 \cdot 2H_2O$, 0.3 g; $MnSO_4 \cdot H_2O$, 1.56 mg; $ZnSO_4 \cdot 7H_2O$, 1.4 mg; $CoCl_2 \cdot 6H_2O$, 2.66 mg; yeast extract, 0.5 g; and carbon source, 10 g. The pH of the medium was adjusted to 5.0 before sterilization.

Erlenmeyer flasks (250 ml) containing 50 ml medium were inoculated with 2% of a conidial suspension containing 5×10^6 conidia per ml. Flasks were incubated for 6 days at 30 °C on a rotary shaker (120 rpm). After incubation the contents of the flask were filtered on Whatman No. 1 filter paper that had previously been dried to a constant weight in an oven at 80 °C. The filter paper and mycelium were similarly dried to constant weight and the dry weight (biomass) determined by subtraction.

Analytical methods. Residual sugars and cellulose content were determined by the colorimetric methods of Miller[8] and Updegraff[9], respectively. Total lipid content was estimated as described by Evans and Ratledge[10] with slight modification. Lipids were extracted by homogenizing the mycelial biomass in chloroform/methanol (2:1) solution. After filtration, cells were extracted for 3 h in methanol/chloroform/water (2:1:1) solution. The extracts were pooled, dried over anhydrous $MgSO_4$ and evaporated to dryness. The lipids were redissolved in diethyl ether and transferred to a preweighed vial. The ether was evaporated and lipid material was dried and weighed. Fatty acid methyl esters[11] were prepared in methanol-sulphuric acid and identified using a Gas Chromatograph, Chemito 3800. Identification of sample components was performed by comparison of their relative retention time with that of authentic standards. Colorimetric methods were used to estimate total phospholipids[12] and glycolipids[13]. Solvents used for all analysis were of reagent or HPLC grade. Unsaturation index (UI) was calculated as described earlier[14], using the following formula:

$$UI = [1\,(\%\,\text{monounsaturate}) + 2\,(\%\,\text{diunsaturate}) + 3\,(\%\,\text{triunsaturate})]/100$$

Results and discussion

Influence of carbon source. The influence of different carbon sources on the growth and fatty acid composition of *A. niger* AS-101 was investigated using $(NH_4)_2SO_4$ as nitrogen source. Biomass dry weight, substrate utilization and lipid yield increased with the fungal growth up to 6 days and thereafter remained more or less constant. The lipid composition of the cells harvested in the sta-

Table 1. Lipid accumulation by *A. niger* AS-101 grown on various carbon sources[a]

Carbon source[b]	Biomass (g/l)	Substrate consumed (%)	Total lipid (%)	% of total lipids Neutral lipids	Phospho-lipids	Glyco-lipids
Glucose	1.9	95	16.6	41.0	38.4	20.6
Xylose	1.7	92	15.2	46.2	32.6	21.2
Avicel[c]	2.0	84	14.9	44.6	36.7	18.7
Bagasse[c]	1.9	84	13.6	42.5	34.9	22.6

[a] Mycelia were harvested after 6 days of cultivation and the values are the mean of three replicates. [b] Carbon sources used at a concentration of 10 g/l. [c] Values for biomass were corrected by the amount of undegraded cellulose.

tionary phase is depicted in table 1. A similar growth and lipid production pattern was found in *Trichoderma reesei*[14]; however, an opposite trend was observed in *A. nidulans*[15] when grown on glucose as carbon source. The total lipid content of *A. niger* AS-101 cultures varied from 13.6% to 16.6% of total biomass, and was largest with glucose as the carbon source (table 1). A satisfactory amount of lipid was produced by *A. niger* AS-101 from avicel (microcrystalline cellulose) and bagasse (a natural lignocellulosic substrate), giving values of 14.9% and 13.6% of total mycelial biomass, respectively. The conversion of cellulosic materials into lipid can be achieved either by the hydrolysis of cellulose separately, followed by the consumption of the resulting sugars by a suitable organism, or ideally by direct conversion by a cellulolytic organism[15]. Our studies indicated that *A. niger* AS-101 can accumulate lipids by directly converting cellulosic materials. Individual lipid classes varied when different carbon sources were used, from 41.0–46.2% (neutral lipids), 34.9–38.4% (phospholipids) and 18.7–22.6% (glycolipids) of the total mycelial lipids.

The analysis of the fatty acids of the mycelial biomass after six days of fermentation is shown in table 2. Unsaturated fatty acids comprised around 80% of the total fatty material. The major unsaturated fatty acids were linoleic (18:2) and oleic (18:1), while major saturated fatty acids were palmitic (16:0) and stearic (18:0). Small amounts of myristic (14:0) palmitoleic (16:1) and marguaric (17:0) acids were also detected. The unsaturation index (UI) ranged from 1.37 to 1.44, a value similar to those reported for other mesophilic fungi[14]. The desaturation step from 18:2 to 18:3 was found not to be very active in *A. niger* AS-101. The biosynthesis of unsaturated fatty acids seems to follow the same pathway (stearic acid → oleic acid → linoleic acid → linolenic acid) as in yeast[16]. No significant differences were observed in fatty acid composition and UI of the lipids of *A. niger* AS-101 grown on different carbon sources. The stability in fatty acid composition regardless of the carbon source utilized is in agreement with results reported for *Candida*[16]. However, a slightly higher level of linoleic acid (18:2) was obtained using cellulose as carbon source; the values obtained were also higher than those reported by earlier workers in non-cellulolytic strains of *A. niger*[17,18]. Discrepancies which have been observed in fatty acid composition may be due to variations in strains of the organism used and in growth conditions.

Influence of nitrogen source. Bagasse was employed as the sole carbon source in the subsequent studies, in view of our aim of producing lipid from cellulosic materials. The effect of different nitrogen sources on the lipid accumulation and fatty acid composition was studied and the data are presented in table 3. The lipid content and fatty acid composition varied with the nitrogen source used. Of the four nitrogen sources tested, NH_4Cl proved to be the best source for lipid synthesis. A significant decrease in linoleic acid (18:2) and an increase in oleic acid were observed with KNO_3 as nitrogen source, and this resulted in decreased unsaturation index. Urea was a poor nitrogen source for lipid synthesis; however, cultures with urea had slightly higher levels of oleic and linolenic acids. NH_4Cl was the best source for linoleic acid synthesis (54% of total lipid), followed by $(NH_4)_2SO_4$ (51% of total lipid). The nitrogen source used might affect the fatty acid composition because of differences in the uptake rates of different forms of nitrogen, or in its catabolism. NH_4^+ might enhance linoleic acid production by stimulating cofactors needed for the desaturation step

Table 2. Fatty acid composition of mycelial lipid of *Aspergillus niger* AS-101 grown on various carbon sources

Carbon source	Relative fatty acid composition[b] (%, w/w) 16:0	18:0	18:1	18:2	18:3	UI[c]
Glucose	8.3	5.2	23.5	50.0	6.0	1.42
Xylose	7.9	6.4	24.6	48.6	5.2	1.37
Avicel	9.2	6.3	23.1	51.8	5.9	1.44
Bagasse	10.4	4.7	25.9	51.0	4.8	1.42

[a] Values are the mean of three replicates. [b] Small amounts of other fatty acids, 14:0, 16:1, 17:0 and 20:0 were also detected. [c] Unsaturation index.

Table 3. Effect of different nitrogen sources on lipid accumulation and fatty acid composition of *A. niger* AS-101[a]

Nitrogen source[b]	Biomass (g/l)	Lipid (%)	Relative fatty acid composition[c] (%) 16:0	18:0	18:1	18:2	18:3	UI[d]
$(NH_4)_2SO_4$	1.9	13.5	10.5	4.9	25.6	51.0	4.6	1.41
NH_4Cl	2.4	15.2	9.8	4.3	24.5	54.3	4.3	1.46
KNO_3	1.6	10.9	7.7	7.4	33.4	40.7	3.5	1.25
Urea	1.5	9.6	6.7	3.8	27.3	48.5	5.0	1.46

[a] Values are means of three replicates. Bagasse (10 g/l) was the carbon source. [b] The initial concentration of the nitrogen source was 400 mg N/l. [c] Small amounts of other fatty acids, 14:0, 16:1, 17:0 and 20:0, were also detected. [d] Unsaturation index.

Table 4. Effect of temperature on the lipid accumulation and fatty acid composition of *A. niger* AS-101[a]

Temperature (°C)	Biomass (g/l)	Lipid (%)	Relative fatty acid composition[b] (%) 16:0	18:0	18:1	18:2	18:3	UI[c]
20	1.4	12.2	8.6	6.2	26.7	50.0	3.9	1.38
25	1.8	14.5	7.2	4.9	25.5	53.5	4.8	1.47
30	2.0	15.4	10.0	4.5	25.1	54.0	4.2	1.46
35	1.6	11.9	7.5	5.9	24.1	51.4	4.0	1.26

[a] Values are the mean of three replicates. Bagasse (10 g/l) and NH_4Cl (400 mg N/l) were the carbon and nitrogen sources, respectively. [b] Small amounts of other fatty acids, 14:0, 16:1, 17:0 and 20:0 were also detected. [c] Unsaturation index.

from 18:1 to 18:2[19]. The intracellular NH_4^+ concentration has also been reported to affect the regulation of lipid biosynthesis in yeast[19].

Influence of temperature. A. niger AS-101 was grown at 20, 25, 30 and 35 °C in a medium containing bagasse and NH_4Cl as carbon and nitrogen sources, respectively. The mold grown at 25 °C and 30 °C exhibited almost comparable values for lipid content and fatty acid composition (table 4). The yields were decreased when growth was below 25 °C or above 30 °C. This suggested an optimum temperature range of 25–30 °C for culturing this strain, to achieve a maximum lipid yield from cellulose. Earlier observations showed a similar temperature range for the maximum production of cellulase enzyme by this strain[3,4].

Aspergillus niger AS-101 grown on various carbon sources including natural cellulosic residues was able to accumulate lipids to a level of about 15%. An average fatty acid profile showed reasonable agreement with published values from non-cellulolytic strains of this organism. This ability indicates the possibility of using this organism for the conversion of widely-available lignocellulosic materials into single cell oil in addition to single cell protein.

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Age- and sex-related differences in the content of prothymosin α in rat tissues

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Abstract. Differences in the tissue content of prothymosin α during the early postnatal development of male and female rats are reported. Thymus and spleen have been found to contain significantly higher amounts of prothymosin α in the newborn and prepubertal animals, as compared to adults, whereas liver has been found to contain low levels of prothymosin α throughout development. These findings indicate a functional association of prothymosin α with the proliferating lymphoid tissues of the young rat.
Key words. Prothymosin α; thymosin α_1; development.

Prothymosin α (ProTα), a highly acidic polypeptide (pI 3.5) of 109–111 residues[1], mainly produced in the mammalian thymus and spleen[2–5], has been implicated in functions related to the immune system[6–9], and in intracellular regulatory events associated with cell proliferation[10–13].

The primary sequence of ProTα[7,10,11,14–17] includes the immunoactive peptide thymosin α_1 ($T\alpha_1$)[18], which