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Effect of carbon and nitrogen sources on the chemical composition of lipids of *Rhizopus delemar*

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Summary: Soy bean extract with glucose or oleic acid in the growth medium of *Rhizopus delemar* affected the production of higher values of biomass, total lipids and total glycerides than when ammonium nitrate was used as a source of nitrogen. The highest amounts of biomass (12.35 g/l) and total lipids (310 mg/g dry biomass) were attained in glucose-grown mycelia. The high proportions of total glycerides and unsaturated fatty acids in lipids of *Rhizopus delemar* suggest the utilization of such lipid material for nutritional and industrial purposes.

Zusammenfassung: Extrakte aus Sojamehl mit Zusatz von Glucose bzw. Ölsäure dienten als Wachstumsmedium für *Rhizopus delemar.* Ein Einfluß der Zusätze auf die Produktion von Biomasse, Gesamtlipiden und Gesamtglyzeriden wurde beobachtet, wenn Ammoniumnitrat als Stickstoffquelle diente. Die größten Mengen an Biomasse (12,4 g/l) und an Gesamtlipiden (310 mg/g trockene Biomasse) wurden in auf Glucose gewachsenen Myzelien erhalten. Die großen Anteile an Gesamtglyzeriden und an ungesättigten Fettsäuren in den Lipiden von *Rhizopus delemar* legen nahe, solches Lipidmaterial für Ernährungs- und industrielle Zwecke zu verwenden.

Key words: Rhizopus delemar, lipids production, carbon and nitrogen sources, composition of lipids

Introduction

During the last decade there has been a considerable increase in oils and fats consumption both by man and in industry. The increase in oils and fats production from crops is expensive, scarce and limited. Fourtunately, during the last few decades, a new biotechnological industry has been developed creating unlimited prospects for their ample production on a large scale commercial culture of microorganisms.

In fungi, it has been reported that the type of growth medium and growth intervals (time of harvesting) affect both lipid production and fatty acid composition (1–3).

Numerous reports have indicated lipid production by other fungi species of the phycomyces group (2, 4, 5). However, no previous attempts were made in utilizing *Rhizopus delemar* in lipids production. The aim of the present study is to determine the efficiency of the mold *Rhizopus delemar* to convert oleic acid or glucose which are produced as byproducts of oil and sugar industries to single-cell oil. The chemical composition of the produced lipid is of interest in determining the suitability of such lipid for nutritional purposes.

Experimental

Cultivation of Rhizopus delemar

The spore suspension (1×10^6 cells/ml) was prepared from 10 days malt agar slants of *Rhizopus delemar* CBS 327.47 growth at 28 °C. Soy bean meal extract employed for the cultivation medium was prepared by boiling 75 g defatted soy bean meal with 1000 ml 0.5 % NaOH solution for 1 h, neutralized with 0.1 NHCl and insoluble residue was filtered off. Two per cent of glucose or 0.6 g oleic acid as sodium oleate, 0.1 % KH_2PO_4 and 0.05 % $MgSO_4$ were added to the soy bean meal extract. In some experiments, soy bean meal extract was replaced by 0.25 % NH_4NO_3. The pH was adjusted to 5.6 and 500 ml of different media were put in 2 l flasks and sterilized at 120 °C for 20 min. The flasks were inoculated with 10 ml of the spore suspension and cultivation was carried out at 27 °C with constant shaking (125 rpm). Samples were analyzed every 24 h.

Extraction and analysis of total lipids

Lipid extraction was achieved by the method of Folch et al. (6). Total lipids were fractionated into various lipid classes by chromatography on thin layer plates coated with Silica gel G (type 60) (200 × 200 × 0.5 mm). The plates were developed in petroleum ether/ether/acetic acid (70 : 30 :2, v/v/v) and sprayed with 50 % H_2SO_4. Visualization was made by heating the plates at 180 °C for 10 min. The charred spots were measured with a densitometer (Shimadzu CS-190) by reflectance scanning. This method revealed a positive relationship between the amount of spotted sample in the range of 10–50 µg and light absorbance. Total lipids were transesterified with 0.3 M sodium methoxide and analysed by gas chromatography (7).

Results and Discussion

Effect of various media and culture aging on biomass, total lipids and total glycerides production by Rhizopus delemar

Among the three media used (Table 1), glucose plus soy bean meal extract was associated with the highest biomass, 12.35 g dry biomass/l and total lipids (3830 mg/l). On the other hand, when glucose was replaced by oleic acid as a carbon source in the medium containing soy bean meal extract, both biomass and total lipids produced by the mold decreased considerably. However, the lowest yield of biomass and total lipids was obtained when soy bean meal extract was replaced by ammonium nitrate in the previous medium. Glucose and soy bean meal extract enhanced growth and lipase production by *Rhizopus delemar* more than other carbon and nitrogen sources (8).

Total lipids extracted from one day fungal growth expressed as a percentage of dry biomass was also influenced by medium ingredients. The highest yield (72 %) was obtained from medium containing inorganic nitrogen followed by soy bean meal extract plus oleic acid medium (60 %), while the lowest value was detected in the medium containing soy bean

Table 1. Biomass, lipids and total glycerides produced by *Rhizopus delemar*, grown on various media for different intervals at 28 °C with shaking (125 rpm).

Fraction	Soy bean meal extract plus glucose				Soy bean meal extract plus oleic acid				Ammonium nitrate plus oleic acid			
	Incubation time (days)				Incubation time (days)				Incubation time (days)			
	1	2	3	4	1	2	3	4	1	2	3	4
Dry biomass g/l	5.78	7.76	12.35	11.17	3.34	4.57	5.78	7.26	1.40	1.82	1.89	2.55
Total lipids mg/l	2570	2800	3830	3670	2000	1520	1149	1721	1007	614	675	298
Total lipids mg/g dry biomass	452.2	359.7	310.4	328.3	599.0	333.0	199.0	237.0	718.0	337.0	358.0	117.0
polar lipids (%)	6.5	5.4	5.5	4.2	12.9	5.6	8.3	6.8	0.7	0.4	0.40	0.76
MG (%)	3.5	5.0	5.9	1.9	nd	nd	nd	nd	nd	nd	nd	nd
DG (%)	10.3	6.1	11.1	12.3	6.9	4.2	9.5	6.3	5.6	3.8	1.2	5.7
Sterols (%)	12.7	8.3	10.2	5.8	15.1	8.2	5.0	7.1	6.0	5.1	3.0	5.2
FFA (%)	14.6	16.3	18.3	17.6	11.4	24.0	18.1	20.2	65.0	65.0	60.3	53.2
TG (%)	52.5	58.8	49.0	58.1	53.7	58.0	59.1	59.6	22.6	25.6	35.1	36.2
Total glycerides (%)	66.4	70.0	66.1	72.4	60.6	62.2	68.1	65.9	28.8	29.4	36.2	40.8

MG = monoglycerides
TG = triglycerides
DG = diglycerides
nd = not detected
FFA = free fatty acids

Table 2. Fatty acid composition of total lipids extracted from *Rhizopus delemar* grown on various media for different intervals at 28 °C with shaking (125 rpm).

Fraction	Soy bean meal extract plus glucose				Soy bean meal extract plus oleic acid				Ammonium nitrate plus oleic acid			
	Incubation time (days)				Incubation time (days)				Incubation time (days)			
	1	2	3	4	1	2	3	4	1	2	3	4
C 14:0	15.2	4.3	6.9	1.8	8.8	1.7	1.0	1.9	nd	nd	nd	nd
C 16:0	19.6	12.2	12.8	7.3	7.7	11.2	15.0	7.5	5.7	4.8	6.3	6.4
C 16:1	0.4	0.6	0.3	2.8	20.3	11.2	6.2	5.5	4.1	2.3	5.3	2.4
C 18:0	2.9	1.5	2.0	3.4	0.3	0.1	0.5	0.8	0.8	nd	nd	1.7
C 18:1	19.2	20.7	41.0	42.1	39.8	56.0	57.7	62.0	80.3	89.7	86.9	86.5
C 18:2	36.0	57.9	31.5	36.9	23.1	19.7	19.6	22.3	9.1	3.1	1.5	3.0
C 18:3	6.6	2.7	5.3	5.7	nd	nd	nd	nd	nd	nd	nd	nd
Total unsaturated fatty acids	62.2	82.0	78.2	88.1	84.5	86.9	83.5	89.8	93.5	95.2	93.7	91.9

nd = not detected

meal extract and glucose (45 %). Previous investigations (2, 4, 5) carried out on other species of the phycomyces group grown on glucose in batch culture revealed a yield similar to that obtained by us when the mold was grown on glucose. Lipids and biomass yields progressively increased with the time of incubation only in soy bean meal extract medium which contained glucose up to the third day, then declined afterwards. Meanwhile, the yields declined in the other two media after the first day of incubation.

The source of nitrogen greatly affected the percentage of total glycerides in lipid extracts. In this respect, organic nitrogen increased total glycerides synthesised. In contrast inorganic nitrogen depressed their yield.

Effect of various media and culture aging on the fatty acid composition of total lipid extracts

Table 2 shows the fatty acid composition of total lipids extracted from *Rhizopus delemar* grown on the previously described media for different time intervals. Growing the mold on glucose as the sole carbon source for one day produced lipids which contained linoleic acid (36 %) as the major fatty acid, while replacing glucose with oleic acid produced fungal growth rich in oleic acid.

Similarly the source of nitrogen affected fungal fatty acid pattern. Inorganic nitrogen increased oleic acid content approximately twofold that produced with organic nitrogen. The source of nitrogen greatly affected the total unsaturated fatty acid content of total lipids. Inorganic nitrogen increased the degree of unsaturation of the total lipid extracts relative to that obtained using organic nitrogen. The fatty acid pattern synthesized in different fungi was reported to vary according to the nitrogen source (3, 9). The degree of total lipid unsaturation was not only affected by a nitrogen source but also by culture aging, since an incubation period longer than one day increased the amounts of unsaturated fatty acids at the expense of saturated fatty acid, particularly oleic acid.

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