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Note

Production of Single Cell Protein from Vegetable Oils

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Production of single cell protein from rape-seed oil and solid fraction of palm oil was studied. All strains tested were able to use these oils as a carbon substrate. Growth rates and growth yields for some strains were perfectly acceptable for industrial applications. According to the growth rates (doubling time in hours), the best strains on rape-seed oil were *Candida rugosa, Candida deformans, Candida lipolytica* and *Geotrichum candidum.*

On the solid fraction of palm oil, the best strain was *Candida rugosa.* Growth yields (g of dry matter or protein produced/g of substrate metabolized) were very impressive for all strains tested: an average of 0.8 of dry matter/per g of substrate and 0.35 of protein/g of substrate.

Better utilization of fertilizers, improvement of genetic crosses, and *in vitro* culture techniques[1] have greatly increased the production of vegetable oils, especially rape-seed and palm oil. Table 1 summarizes the evolution of world production of some oil-seeds for the last 10 years.[2] This great production increase has opened up new subjects for research. The most exciting idea is to promote non-conventional protein production in the oilseed-producing developing countries by using oils and by-products as carbon sources for yeasts, bacteria, or fungi.

Palm oil has already been investigated for *Candida blankii* production by Nakahara *et al.*[3] The solid fraction of this oil (stearin) was also used as a carbon source for different yeast strains. More recently, the possibility of using "soapstock", a by-product of oil refining, for SCP production was studied by Ba *et al.*[4] Martinet *et al.*[5] used ammonium or sodium soaps from several fats as substrates for this purpose. Besides vegetable oils, other authors attempted to use other substrates. Hottinger *et al.*[6] and Burkolder *et al.*[7] produced *Candida lipolytica* and *Geotrichum candidum* on fish oils.

The aim of this work was to select the best strain among nine lipolytic yeasts able to use rape-seed oil or palm oil as a substrate in order to produce single cell protein.

Materials and Methods

Strains The following strains were obtained from

Table 1. Evolution of production of some vegetable oils (in 10^3 tons).

Vegetable oils	1970	1975	1978	1979	1980	1981
Soya oil	6089	8325	12425	13130	14605	14530
Rape-seed oil	1925	2713	2987	3508	3295	3685
Sunflower oil	3802	3989	4612	4918	5555	4600
Palm oil	1743	2910	3421	3946	4400	4590

the Centraalbureau voor Schimmelcultures (C.B.S.) Delft, the Netherlands and were chosen for their high lipolytic activity: *Candida curvata* CBS 570 *Candida rugosa* CBS 613 *Candida deformans* CBS 2071 *Candida parapsilosis* CBS 604 *Cryptococcus uniguttilatus* CBS 1730 *Geotrichum candidum* CBS 17853 *Trichosporum cutaneum* CBS 2466 *Rhodotorula pilimanae* CBS 5804. The *Candida lipolytica* YB 423 12 strain was obtained from the Northern Utilization, Research and Development Division, US Department of Agriculture, Peoria, Illinois.

Culture conditions Cultures were carried out on a liquid medium containing 6.7 $g \cdot l^{-1}$ of Yeast Nitrogen Base (YNB) and the substrate (rape-seed oil or the solid fraction of palm oil at various concentrations). The media were sterilized by autoclaving at 110°C for 40 min; the yeast nitrogen base was sterilized by filtration. The cultures were made in Erlenmeyer flasks filled to 1/10 of their volume and placed in a temperature controlled room at 28°C; aeration was ensured by constant shaking at 80 oscillations/min and 7.5 cm amplitude. The utilization of rape-seed oil did not require the use of any emulsifier. For dispersing the solid fraction of palm oil in the aqueous medium, we had to add polyethylene glycol (P.E.G. 600) as an emulsifier on account of this substrate's content of palmitic acid, a saturated fatty acid.

Analytical methods In an aqueous solution the lipids form an emulsion, making the culture medium more or less opalescent. It is therefore impossible to determine the optical density of such a medium directly without first eliminating the fats; the same applies to the determination of dry matter. Special washing of the cells was thus required. For this, an aliquot of the culture medium was mixed with 0.5 volume of ethanol. The hydroalcoholic mixture was filtered under reduced pressure (Millipore 0.45 μm). Rinsing with alcohol removed the water and a final rinsing with hexane dried the filter. The yeast cells remaining on the filter were resuspended in water and the optical densities and dry matter were measured.

The growth rate was defined to be the time necessary for the population to double (doubling time in hours). It was measured by plotting optical density (on a logarithmic scale) versus time. It was determined during the exponential growth phase. Protein content was determined by the Strickland biuret method.[8] Dry matter was determined after drying at 108°C, until constant weight was reached. After extraction of the lipids from the culture medium, the excess ethanol of the aqueous fraction was eliminated under reduced pressure. After acidification with HCl (4N), lipids were extracted by hexane and the residue was weighed for residual substrate determination.

Amino-acids were analysed after hydrolysis of a sample of lyophilized cells in 6N NCl under N2 (oven 100°C, 24 h). The hydrolysate was filtered and evaporated under reduced pressure in a water bath (35°C). The residue was recovered by a citrate (pH 2.2) buffer solution. 20 μl of this solution was injected into a Durrum D500 apparatus to determine the amino acids by HPLC on ion exchange resin.

Results

Growth rates Growth rates were determined on palm oil and rapeseed oil and pH values were regulated at 6.5 with phosphate buffer (0.2 M) and at 3.5 with tartrate phosphate buffer (0.2 M). Results are given in Table 2. The results obtained confirm that for all strains tested, better growth was obtained at pH 6.5 than at pH 3.5. These results seem normal because of the usual optimum pH for the activity of lipases. Nevertheless, it should be noted that at pH 6.5, sterilization of the medium is necessary and would increase the final cost of the product. In contrast, industrial experience in other fields shows that yeasts can be produced without sterilization of the growth medium at pH 3.5. Under our conditions at pH 3.5, the best strains are *Candida rugosa* and *Geotrichum candidum* for rape-seed oil and *Candida rugosa* and *Candida lipolytica* for the palm oil solid fraction as substrate. We must notice that growth was not as favorable on the palm oil solid fraction as on rapeseed oil, except for slow-growing strains such as *Cryptococcus uniguttilatus*.

Growth yields Growth yields are expressed as dry matter and protein production corresponding to substrate actually consumed (g of dry matter or proteins per g of substrate consumed). Results are summarized in Table 3. All experiments were carried out on a non-buffered medium. The initial pH was 6.5 and the final pH was around 3. Several verifications showed that yields depended little on the medium pH. Results shown in Table 3 give a good estimation of what can be obtained under industrial conditions at pH 3.5. The yields were not very different for the two substrates. The small differences between some strains did not seem significant.

Table 2. Growth rates on YNB rape-seed oil and YNB—solid fraction of palm oil (in hours).

Strains \ Substrate	pH 6.5		pH 3.5	
	Rape-seed oil 0.5%	Palm oil 0.5% PEG 0.05%	Rape-seed oil 0.5%	Palm oil 0.5% PEG 0.05%
Candida curvata CBS 570	4	3.5	16	no growth
Candida rugosa CBS 613	1.5	7	3	7
Candida deformans CBS 2071	2.5	3	5	10
Candida lipolytica YB 423 12	2	4	5	7.5
Candida parapsilosis CBS 604	3.5	4.5	8	no growth
Cryptococcus uniguttilatus CBS 1730	8	8	no growth	10
Geotrichum candidum CBS 178 53	2.5	8	2.5	18
Trichosporum cutaneum CBS 2466	3	10	no growth	16
Rhodotorula pilimanae CBS 5804	4	9	8	9

Table 3. Growth yields from rape-seed oil and the solid fraction of palm oil.

Strains \ Yields	Rape-seed oil 0.2%		Solid fraction of palm oil 0.2%	
	Dry matter (g/g of substrate)	Proteins	Dry matter (g/g of substrate)	Proteins
Candida curvata CBS 570	0.85	0.30	0.75	0.36
Candida rugosa CBS 613	0.75	0.30	0.65	0.26
Candida deformans CBS 2071	0.75	0.30	0.85	0.35
Candida lipolytica YB 423 12	0.68	0.31	0.66	0.21
Candida parapsilosis CBS 604	0.90	0.27	0.94	0.45
Cryptococcus uniguttilatus CBS 1730	0.70	0.25	1.00	0.40
Geotrichum candidum CBS 178 53	0.78	0.29	1.00	0.34
Trichosporum cutaneum CBS 2466	0.80	0.30	0.85	0.45
Rhodotorula pilimanae CBS 5804	0.85	0.36	1.00	0.73

Amino-acids composition Amino-acids contents of the strains are given in Table 4. Each amino-acid is expressed as a percentage of total amino-acids. As usual, all yeasts tested have high lysine contents whereas their methionine contents are rather low.

Discussion

In previous work we showed that ammonium or sodium soaps from fats were excellent substrates for yeast production. Vegetable oils (TG) have the same potential although

Table 4. Amino acid contents of yeast grown on YNB-glucose (Percentage of amino acid (w/w) in the total.

Strains \ Amino acids	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ileu	Tyr	Leu	Phe	His	Lys	Arg	Trp
Candida curvata CBS 570	10	6	6	12	4	5	6	2	6	2	5	5	8	5	2	8	6	2
Candida rugosa CBS 613	12	6	8	12	4	6	8	1	7	2	4	3	7	4	2	7	5	2
Candida deformans CBS 2071	10	6	6	12	4	5	8	1	6	2	4	3	7	4	2	9	9	2
Candida lipolytica YB 423 12	11	6	6	12	5	6	8	1	6	2	5	3	8	4	2	8	5	2
Candida parapsilosis CBS 604	11	6	6	13	4	5	7	1	6	2	5	3	8	4	2	9	6	2
Cryptococcus uniguttilatus CBS 1730	10	6	6	12	4	5	7	1	6	2	5	5	8	5	2	7	6	2
Geotrichum candidum CBS 178 53	10	6	6	14	5	5	6	1	5	1	5	6	7	5	2	8	6	2
Trichosporum cutaneum CBS 2466	9	5	5	12	5	6	8	1	5	2	4	7	7	5	2	7	7	3
Rhodotorula pilimanae CBS 5804	9	5	5	12	5	5	7	1	5	2	4	4	8	3	5	6	11	3
Egg	9	5	6	12	5	3	4	2	7	4	8	4	9	6	2	7	6	2

dry matter yields were not as favorable (0.8 instead of 1.0). It is possible to carry out the fermentation at pH 3.5 with some strains, which represents a great advantage for an industrial process since no medium sterilization would be required. It is easier to work with liquid oil than with a solid fatty substrate. Nevertheless, the direct utilization of the solid part of palm oil is possible. *Candida rugosa*, with a generation time of 7 h and yields of 0.65 dry matter and 0.26 proteins, is perfectly acceptable for such an industrial application.

Let us remember that the palm tree, owing to the well adapted agricultural techniques using the new hybrid species of *Elais guineesis*, produces 5 tons per ha under optimal conditions at Lame in the Ivory Coast and its production may reach 7 or 8 tons per ha under the exceptional growing conditions at San-Alberto in Colombia.[2] In Malaysia, the national production average is about 4 tons per ha. Following fractionation of the oils, 1.6 tons of the solid fraction (stearin) per ha would be available to produce 1 to 1.5 tons of yeasts, *i.e.* 500 to 750 kg of SCP per ha. The palm tree may thus become indirectly one of the best proteaginous plants.

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