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THE PRODUCTION OF SINGLE-CELL PROTEIN FROM WHEY

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Summary.

The conversion of whey to single-cell protein by yeasts was investigated. The most suitable organism tested was Kluyveromyces marxianus NCYC 1424. The efficiency of whey conversion to biomass was directly related to higher oxygen availability in the medium.

INTRODUCTION.

Cheese whey is a waste by-product of the dairy industry. It contains low levels of non-coagulable protein, but is rich in lactose (Meyrath and Bayer, 1979). Environmental controls have promoted the industry to explore economic disposal methods or pay high water treatment premiums. The low protein content precludes whey from being accepted as a high-grade food or feeding material. One disposal method that has been developed is the membrane separation of whey into a high quality protein concentrate which can be incorporated into food products, and a lactose-containing permeate which can be used to yield single-cell protein (SCP) suitable for animal feed (Horton et al., 1972; Pace et al, 1974; Vringnaud, 1976). However, the present high capital cost of the separation plant means this approach is currently uneconomic for relatively low-volume whey producers. A potentially cheaper means of disposal involving the direct utilization of whole whey to SCP by yeasts has been investigated (Wasserman et al, 1961; Meyrath and Bayer, 1979; Akin et al, 1967), but has not yet been perfected to economic viability.

The long-term aim of this research project is to optimise the direct microbial utilization of whole whey to SCP. The present paper reports the selection of a suitable strain of the lactose-metabolising yeast Kluyveromyces marxianus.

MATERIALS AND METHODS.

Organism. Kluyveromyces marxianus NCYC 1424 was maintained on YM agar slopes at 25°C.

Media. A whey-based medium was used which routinely contained (per litre of distilled water) 50g whey powder plus the following mineral salts: 3g $(NH_4)_2SO_4$; 2g Na_2HPO_4; 0.2g $MgSO_4.7H_2O$; 0.1g NaCl; 0.1g $CaSO_4.2H_2O$; 0.025g $FeSO_4.7H_2O$; 0.0075g $ZnSO_4.7H_2O$; 0.005g $MnSO_4.4H_2O$; 0.001g $CuSO_4.5H_2O$ and 0.0005g H_3BO_3 adjusted to pH 4.5 and sterilized by autoclaving (121°C, 15 minutes). The medium had to be decanted to remove precipitated protein and then re-autoclaved.

Culture conditions. Growth was routinely carried out at 30°C. Liquid cultures were incubated on a rotary shaker in 100 ml baffled conical flasks containing 50 ml of growth medium inoculated from a stock slope, or in aerated 2-litre fermentation vessels (aeration rate = 5 litres air/min) containing 1 litre of growth medium inoculated with 100 ml of whey-grown started culture. The yeast was also grown in a Biotec FL-103 3-litre fermenter linked to a Biotec LP-100 control console: during growth on whey-based medium the temperature was maintained at 30°C, and the pO_2 in the culture was recorded with a membrane-type galvanic oxygen electrode which was calibrated in sterile medium at 30°C. The dissolved oxygen tension in the medium was calculated from the assumption that pO_2 100% of air-saturated medium was 0.209 atm. After the dissolved oxygen had been set at the desired value in a growing culture, it was maintained at this value by automatic adjustment of the stirrer speed.

Analytical methods. Growth was monitored by recording the optical density of removed samples at 500nm. Cells were subsequently harvested by centrifugation, and the supernatant analysed for lactose by the colorimetric dinitrosalicylate method (Miller, 1959), and for ethanol and ethyl acetate by gas chromatography using an 80/100 mesh Porapak Q column. The flame ionization detector for gas chromatography operated under the following conditions: column temperature, 100°C; injector temperature, 40°C; detector temperature, 200°C; carrier gas (N_2) flow rate, 10 ml/sec.

RESULTS AND DISCUSSION.

A preliminary examination of yeasts available from the National Collection of Yeast Cultures to assess growth on a lactose minimal agar selected 47 lactose-metabolising strains of Brettanomyces, Candida, Kluyveromyces, Rhodotorula, and Trichosporon. A subsequent screen to assess growth in shaken conical flasks identified 11 of these yeasts which were able to grow rapidly on minimal media containing dried whey powder as the principal carbon source.

To further quantify the relative rates of growth on lactose, the 11 selected strains were grown in 2-litre fermenter vessels containing 1 litre of whey powder medium aerated at 5 litres air/min. The results (Table 1) indicated that all tested strains of Candida grew well under these growth conditions, some strains of Kluyveromyces grew well whereas others grew more slowly, and strains of Trichosporon grew less rapidly than yeasts of the other two genera. The most rapidly growing yeast with the shortest lag phase under these growth conditions was Kluyveromyces marxianus NCYC 1424; interestingly this yeast was originally isolated in a previous study of whey-metabolising microorganisms (Wasserman et al, 1961).

The 11 strains differed widely in the capacity to accumulate ethanol and ethyl acetate as products of the fermentation of lactose. With the exception of Candida pseudotropicalis NCYC 143, strains of Candida and Trichosporon showed less tendency to ferment lactose than strains of Kluyveromyces.

On the basis of these data, Kluyveromyces marxianus NCYC 1424 was chosen for further growth studies using a Biotec FL-103 fermenter vessel linked to a Biotec LP-100 control console, allowing monitored adjustments to the temperature, rate of aeration and speed of agitation of the growing culture. The whey powder medium was agitated by a multi-blade impeller initially operated at 500 rev/min and oxygenated by forced aeration at rates appropriate to maintain monitored pO_2 values of 50 to 80%. The results obtained when K. marxianus NCYC 1424 was grown on whey-based medium at pO_2 50% (Figure 1) suggested that after an initial lag phase of 1-2 hours, lactose was rapidly metabolised with a concomitant increase in biomass until the medium was completely depleted of detectable lactose approximately 9 hours after inoculation of the yeast. The level of ethanol in the medium showed a diphasic pattern, initially increasing to a maximum level of 0.447% v/v 5 hours after inoculation of the yeast, and then subsequently progressively decreasing until no detectable level

was recorded 9 hours after inoculation. This pattern of results is typical of a facultatively fermentative yeast (van Dijken et al, 1984). The initial production of ethanol could be due to insufficient oxygen being available in the medium to support maximum respiratory metabolism. The alternative possibility that K. marxianus NCYC 1424 could be a Crabtree-positive yeast that performs an alcoholic fermentation in spite of aerobic conditions when there is a high level of metabolizable carbohydrate present (Ephrussi et al, 1956) is unlikely since previous studies have shown that the Crabtree effect is non-existent in Kluyveromyces spp. (Moulin et al, 1981). In terms of using K. marxianus NCYC 1424 growing on whey as a source of SCP such a pattern of metabolism is disadvantageous because although the ethanol produced during the initial phase of growth is subsequently assimilated by the yeast as the level of available lactose decreases, this represents an energetically less efficient method of lactose utilization than direct respiratory metabolism and hence a potential loss of biomass. Also, some of the alcohol is carried out of the fermenter in the exit gas stream and thus lost as a potential source of biomass.

The results obtained when K. marxianus NCYC 1424 was grown on whey-based medium at higher pO_2 values up to 80% (Table 2) showed a consistent series of trends. As the pO_2 increased from 50% to 80%, the biomass production rate (g biomass/1 h^{-1}) increased progressively whereas both the lactose utilization rate (g lactose used/1 h^{-1}) and the amount of ethanol produced during the initial fermentative phase of growth decreased progressively. Overall, the Y_{sub} increased from 0.318 at pO_2 50% to 0.430 at pO_2 80%. These data suggest that higher oxygen availability in the medium encourages a pattern of lactose metabolism by K. marxianus NCYC 1424 geared predominantly to respiration, hence optimising biomass production. Thus this yeast, like the closely-related strain K. fragilis (Moulin et al, 1981) exhibits a Pasteur effect (Fiechter et al, 1981). The molecular basis of ethanol production by K. marxianus NCYC 1424 grown in aerobic culture most probably reflects an oxygen limitation factor: even growth of this yeast on whey-based medium at pO_2 80% resulted in a certain amount of ethanol being produced, some of which will be sparged out of the medium in the exit gas stream and hence lost as a potential source of SCP.

Past experience both with yeast grown on whey (Akin et al, 1967), and molasses (Harrison, 1967) suggests that ethanol production can be

reduced and biomass yield concomitantly increased by lowering the concentration of carbohydrate initially present in the growth medium. Preliminary results suggest that a similar result can be obtained with K. marxianus NCYC 1424 grown on whey-based medium, although the effect requires further characterization.

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Table 1. Growth of lactose-metabolizing yeasts on whey medium in aerated 2-litre flasks.

Microorganism	NCYC culture no.	lag phase (min)	$\mu(h^{-1})$
Candida kefyr	1441	180	0.630
Candida pseudotropicalis	6	320	0.444
	143	280	0.577
	188	240	0.502
	744	250	0.577
Kluyveromyces marxianus	827	460	0.161
	1424	115	0.693
	1425	160	0.462
	1426	340	0.182
Trichosporon beigelli	444	250	0.277
	1432	225	0.288

Table 2. Growth of K. marxianus NCYC 1424 in the Biotec FL-103 fermenter at different dissolved oxygen tensions.

	pO_2 50%	pO_2 65%	pO_2 80%
Max. lactose utilization (g/l h^{-1})	5.20	5.05	4.81
Max. diol yield (g/l h^{-1})	1.17	1.46	1.67
Max. EtOH accumulation (% v/v)	0.447	0.2403	0.1792
Y_{sub} (g biomass produced) / (g lactose utilized)	0.318	0.356	0.430

Figure 1. Growth of K. marxianus NCYC 1424 in the Biotec FL-103 fermenter at pO_2 50%.