MICROBIAL BIOMASS AND PROTEIN PRODUCTION FROM WHEY

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SUMMARY: Nineteen different mould fungi, 18 yeasts and 12 bacteria were screened for their growth and single-cell protein content on deproteinized powdered whey. Eight fungi, 8 yeasts and 7 bacteria proved to be most suitable organisms for production of microbial protein. They were further grown on sterilized and non-sterilized unsalted cheese whey.

Key Words: Biomass, whey.

INTRODUCTION

Cheese whey, in Egypt, is treated as a waste and discharged in nature without any treatment which creats pollution problems.

It is a by-product of the dairy industry and contains usually high levels of lactose (4-4% w/w/g), low levels of nitrogenous compounds and small amounts of vitamins and minerals (3,12,19).

Lactose, the main nutrient in whey, can be economically utilized by its conversion to single-cell protein (SCP) or single-cell oil (SCO).

Dried whey has been widely used as a feed for cattle although its nutritional value is low, because of its low organic nitrogen content. The production of microbial protein from whey reduces the BOD value of the effluent by converting lactose to protein. Also Moresi *et al.* (8) reported that the cultivation of *Kluyreromyces fragilis* has the main advantage of making easier the final disposal of whey, since it lowers the COD by more than 90% and converts lactose into microbial biomass. Same observation was reported by Moon *et al.* (7) using yeasts. According to Shahani and Mathur (13) only 56% of the whey solids were utilized for human food and animal feed.

Many studies of microbial protein production from whey have been reported, mostly using yeasts (5, 6, 9, 16-18).

In the studies reported so far, certain yeasts have been used for conversion of whey lactose into biomass. Although there are other lactose utilizing microorganisms, these have not been studied for their conversion efficiency of whey lactose. A major problem in using whey as a fermentation medium has been the fact that relatively few organisms are able to ferment lactose (11). In order to discover more efficient microorganisms for this purpose 19 fungi, 18 yeasts and 12 bacteria were screened in this work to select the most suitable organisms for their biomass and protein content when grown on deproteinized dry whey, sterilized unsalted whey (UsW1), non-sterilized unsalted whey (UsW2) and salted whey.

MATERIALS AND METHODS

Organisms

Used in this investigation are listed in Table 1. All of them were obtained from the culture collection of the Institut für Mikrobiologie der Westfalischen Wilhelms-Universitat Münster, FRG with the exception of *Candida tropicalis*, *Pichia polymorpha* and *Hansenula* sp. which were obtained from the Microbiological Resource Center, Ain Shams University, Cairo, Egypt.

Types of whey used

a) Powdered whey (PW) gratefully donated by Firma Meggle, Reithmering, FRG.

b) Salted whey (SW) and unsalted whey (UsW) were obtained from the National Dairy Industry in Alexandria, Egypt.

Media for screening

A whey-based medium was used which routinely contained (g/l distilled water): whey powder, 50; $(NH_4)_2SO_4$, 3; Na_2HPO_4, 0.2; $MgSO_4.7H_2O$, 0.1; NaCl, 0.1; $CaSO_4.2H_2O$, 0.1; $FeSO_4.7H_2O$, 0.025; $ZnSO_4.7H_2O$, 0.0075; $MnSO_4.4H_2O$, 0.005; $CuSO_4.5H_2O$, 0.001 and, H_3BO_3, 0.0005.

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The initial pH of the medium was adjusted to 5.0 and sterilized by autoclaving (121°C for 15 min). The medium was decanted to remove precipitated protein, dispensed in 250 ml capacity Erlenmeyer flasks (50 ml each) and then re-autoclaved.

Culture conditions

Growth was carried out at 30°C under static conditions for 6 days in the case of fungi and under shaked conditions (180 rpm) for 5 days for yeast and bacteria.

Inoculum

The inoculum was prepared from 2-3 day old cultures. The spores or organisms from a test tube slope were suspended in 5 ml sterile dist. water and then 1 ml suspension was directly inoculated in the Erlenmeyer flasks containing 50 ml medium.

Analytical methods

Dry cell weight:

a. Fungi: From the culture broth (50 ml), the mycelia were filtered on a filter paper (tared after drying at 105°C) and washed twice with dist. water. The filter papers containing the mycelia were dried at 105°C for constant weight.

b. Yeasts and bacteria: The cells were harvested by centrifugation, washed twice with dist. water and the dry weight determined after drying over night at 105°C.

Estimation of protein in biomass: The protein percentage of the biomass was estimated by micro-Kjeldahl's method as proposed by Mc Kenzie and Wallace (4).

RESULTS AND DISCUSSION

The organisms which were able to grow on powdered whey (PW) are shown in Table 1. The fungi showed the best biomass production followed by some yeasts and bacteria.

Preliminary tests in our laboratory (not published) showed that among the organisms chosen in this survey 3 fungi, 4 yeasts and 3 bacteria (Table 2) had relatively high protein content when cultivated on (PW). These were further experimented with for growth studies and protein content when utilizing unsalted and salted, sterilized and non-sterilized cheese whey.

Table 1: Growth of lactose-metabolizing microorganisms on powdered whey.

a- Bacteria
Bacillus amyloiquefaciens, B. cereus, B. firmus, B. lentus, B. macerans, B. megaterium, B. subtilis.
b- Yeasts
Candida melivii, C. oleophila, C. parapsilosis, C. tropicalis, Hansenula sp., Pichia polymorpha, Saccharomyces uvarum, Schizosaccharomyces pombe.
c-Fungi
Aspergillus carbonarius, A. flavus, A. glaucus, A. ostianus, A. parasiticum, A. terreus, Neurospora (-), Neurospora (+).

Table 2: Growth and crude protein content of some microorganisms when utilizing powdered whey (PW), sterilized unsalted whey (UsW1) and non-sterilized unsalted whey (UsW2).

Organisms	Dry wt (mg/100 ml)			Crude protein (%)		
	PW	UsW1	UsW2	PW	UsW1	UsW2
a- Bacteria						
B. amyloliquefaciens	434	96	1678	77.0	21.0	19.0
B. macerans	524	410	1730	37.0	18.4	51.0
B. megaterium	464	104	1882	64.0	1.2	47.5
b-Yeasts						
C. melivii	572	1522	1882	56.9	48.1	49.5
C. oleophila	308	554	1882	36.3	42.8	48.0
C. parapsilosis	618	416	1936	41.1	42.2	54.7
Hansenula sp.	296	1362	1476	49.0	55.7	47.2
c- Fungi						
A. ostianus	708	524	1532	47.3	40.5	45.8
Neurospora (-)	850	1546	1914	34.9	28.5	45.6
Neurospora (+)	838	1246	1644	30.9	32.8	48.6

It should be mentioned that none of the organisms under test were able to grow on the salted whey (SW).

From Table 2, it could be concluded that the sterilized unsalted whey (UsW1) supported better biomass production with all yeasts and fungi (except for *C. parapsilosis*).

The types of whey used showed no significant effect on the crude protein content of fungi and yeasts. On the other hand, drastic decrease in protein content was detected among the bacteria when using UsW1.

The present investigation showed also that some bacteria and yeasts were more promising than fungi in their crude protein content when grown on PW. It has been reported that bacteria have more rapid growth and metabolic rates than yeasts; they have simpler nutritional requirements and their use should eliminate the contamination which often occurs in yeast propagations (1). Cell yields of 59.8% were obtained by using *Aeromonas hydrophila* RH 726 with cottage cheese as a substrate.

Few studies have been carried on fungi (3,15). Fungi have relatively low growth rates and process control is more difficult than in the case of yeasts or bacteria. However, there are several advantages in using fungal cells (14), their amino acid profile is better, the recovery of biomass from the culture broth is much easier, their filamentous structure facilitates production of texturized foodstuffs without extraction and spinning, and they are, as yeasts, already accepted as foods in many parts of the world.

The purpose of using non-sterilized unsalted whey (UsW2) was due to an economical point of view namely to lower the cost of process, especially in our country.

This whey (UsW2) clearly improved the biomass production of the organism used which might be due to the presence of the natural microflora of milk. Moulin *et al.* (10) reported that traditional industrial fermentations are not run under sterile conditions because of costs. Complex flora are involved; reasons for the balance of phase mixed cultures are not always clear.

It should be pointed out that cell cytotoxicity tests, especially with fungi and some bacteria must be performed in order to use microorganisms as feed. For example *Bacillus cereus* was reported to produce toxins in milk and cream under strongly aerated conditions (2).

It also would be of considerable value if an environmentally safe microorganism could utilize the lactose of whey permeate and generate potentially profitable quantities of a commercially useful by-product. However, most microorganisms have limited or no β-galactosidase activity, which prevents them from making effective use of lactose as a carbon source.

Further investigations on the production of single-cell oil (SCO) is being undertaken to make full use of an important waste, whey, in Egypt.

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