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SINGLE CELL PROTEIN PRODUCTION FROM BEET PULP BY MIXED CULTURE

By

K. M. GHANEM
Botany Department, Faculty of Science, Alexandria University, Alexandria, Egypt

إنتاج بروتينات وحيدة الخلية من لب البنجر باستخدام المزارع المختلطة

خـــالد محمـــد غـــانم

تم تنمية عدة مزارع مختلطة على منبت غذائي يحوي لب البنجر كمصدر كربوني وحيد وكانت مزرعة مختلطة من فطر « تريكود يرما ريزييای » وخميرة « كليفيروميسيس ماركسيانيس » أكثرهم كفاءة من حيث تخليق البروتين (٥١٪من الوزن الجاف) ومقدرة على تحويل لب البنجر إلى بروتين (٣٩٪) وباستخدام تلك المزرعة أمكن مضاعفة كمية لب البنجر وكذلك حذف مستخلص الخميرة من المنبت الغذائي وتم الوصول بتخليق البروتين إلى ٥٤٪ من الوزن الجاف بالمقارنة إلى ٤٩٪ بروتين باستخدام مزارع وحيدة من « تريكود يرما ريزييای » وبتحليل البروتين الناتج وجد إنه يحتوي على جميع الأحماض الأمينية بكميات عالية تماثل الكميات القياسية للبروتينات حسب مواصفات منظمة الأغذية العالمية « فاو » وقريبة من الكميات الموجودة في فول الصويا مما يدعم امكانية استخدامة كعلف .

Key Words: Single cell protein, Beet pulp.

ABSTRACT

A mixed culture of *Trichoderma reesei* and *Kluyveromyces marxianus* was found to be more efficient for single cell protein (SCP) production (51%) from beet pulp (BP) than the other tested mixed cultures containing *T. reesei* or a monoculture of *T. reesei* (49%). The extent of SCP production by the *T. reesei - K. marxianus* co-culture varied in response to different levels of both yeast extract and BP in the basal medium. Under optimum conditions the protein yield reached to a maximum value (54%) and the corresponding efficiency of BP conversion into proteins was 42%. The protein obtained contained all essential amino acids and compared favourably with the profiles of both the FAO guideline and of soy bean oil meal.

INTRODUCTION

Most experiments on single cell protein (SCP) production from cellulosic wastes have been carried out with monocultures (Peitersen, 1975a). However, some attention has been given to mixed microbial systems which have some advantages for efficient biodegradation and protein production from complex lignocellulosic substrates (De La Torre, 1982). Various different combinations of microorganisms acting as mixed cultures have been found to increase protein yields (Callihan and Dunlap, 1971, 1973, Peitersen, 1975a, b, Kristensen, 1978, Molina *et al*, 1983).

In Egypt, surplus quantities of beet pulp (BP), a by-product of sugar production from sugar beet, are available. The author (Ghanem *et al*, in press) has succeeded in obtaining high protein yield (49.3%) using treated BP as the sole carbon source by a monoculture of *Trichoderma reesei*. The present article aims to further increase the protein outputs from BP, using mixed cultures.

MATERIALS AND METHODS

Beet pulp (BP):

The crude BP was kindly supplied by the Delta Sugar Company, Kafr El-Sheikh, Egypt. The dried BP was physically treated by milling in Wiley mill and passed through a 60 gauge mesh sieve, followed by chemical pretreatment with 3% NaOH, to provide the most suitable BP for microbiological studies with *T. reesei* (Ghanem *et al*, in press).

Maintenance and cultivation:

The pure stock cultures were maintained on glucose-peptone agar slants with transfers at monthly intervals. Cultivation was carried out with a medium, previously found to be the most suitable for SCP by *T. reesei* (Ghanem *et al*,), the medium contained (g/l): NaOH-pretreated milled BP, 2; $(NH_4)_2SO_4$, 3; KH_2PO_4, 2; $MgSO_4.7H_2O$, 0.3; $CaCl_2.6H_2O$, 0.3; yeast extract, 1; $FeSO_4.5H_2O$, 0.01, and pH = 5.0. The organisms were allowed to grow in 100 ml. portions of the medium dispensed in

250 ml. Erlenmeyer flasks. The flasks were sterilized by autoclaving at 121°C for 15 min. inoculated with 8 ml. spore suspension obtained from 5 days old cultures of *T. reesei* plus 2 ml. of 48 hr. old cultures of the tested yeasts and incubated at 30°C under shaked conditions (200 shakes/min. amplitude 7 cm) for 7 days.

Analyses:

The fermentation residue (residual BP + microbial growth of *T. reesei* and the yeast) was separated by centrifugation at 4000 rpm for 20 min, washed with distilled water and dried at 30°C for constant weight, this residue being referred to as the dry weight. The dried residue was analysed for its content of crude protein content (SCP) by the micro Kjeldahl technique (total nitrogen x 6.25), and amino acid constituents using a Beckman Amino Acid Analyzer.

RESULTS AND DISCUSSION

SCP production by mixed culture:

Four different species of yeasts in addition to *T. reesei* were tested. The results (Table 1) revealed that the mixed culture of *T. reesei* and *K. marxianus* offered a combination that gave as high as 51% yield of protein and efficiently converted about 39.4% of the supplemented BP into SCP. This might be attributed to the availability of some nutrients such as amino acids and vitamins in the fermentation medium, provided either by the living or lyzed dead cells of *K. marxianus,* which served to stimulate the metabolic activities of *T. reesei.* In this respect, it was reported that the protein yields from cellulosic wastes were increased using mixed cultures (Peitersen, 1975b, Callihan and Dunlap, 1971, 1973).

Table 1
Dry weights and SCP production by the tested mixed cultures

Microbial System	Final pH	* Dry Weight mg.	SCP		** Conversion Efficiency
			mg.	% to Dry Wt.	
Monoculture of:					
Trichoderma reesei (control)	5.1	1450	714.9	49.3	35.7
Mixed culture of:					
T. reesei + *Kluyveromyces marxianus*	5.1	1547	788.5	51.0	39.4
T. reesei + *Candida utilis*	4.8	1521	762.0	50.1	38.1
T. reesei + *Saccharomyces cerevisiae*	5.0	1536	758.8	49.4	37.9
T. reesei + *Saccharomyces vuarum*	4.9	1467	724.7	49.9	36.2

* Dry weight = residual BP + microbial growth

** Conversion efficiency $= \frac{\text{mg. protein}}{\text{mg BP in 100 ml. medium}} \times 100$

As the basal medium has 1g/l. yeast extract, the increased activities of *T. reesei* in mixed culture can be attributed to the continuous consumption of the released reducing sugars during the fermentation of the cellulosic waste (BP) from the medium by *K. marxianus* yeast resulting in the low level of reducing sugars (detected as few milligrams/liter) which in turn increases the synthesis and activity of cellulases of *T. reesei.* In this respect, it has been reported that regulation of cellulases by catabolite repression can be avoided by using mixed cultures (De La Torre, 1982).

Effect of yeast extract levels:

Different concentrations (0.0-2 g/l.) of yeast extract were tested. The results (Table 2) indicated that the highest level of protein was recorded in the absence of yeast extract, while its presence favoured a small increase in growth yields. These results suggested that *K. marxianus* in mixed culture provided nutrients to the mould and that the catabolite repression of cellulases of *T. reesei* may be avoided. Thus, the yeast extract in the basal medium can be substituted by *K. marxianus.*

Table 2
Dry weight and SCP production by the mixed culture of *T. reesei* and *K. marxianus* as influenced by different yeast extract levels.

Yeast extract level (g/l.)	Dry Weight mg.	SCP		Conversion Efficiency
		mg.	% to Dry Wt.	
0.0	1515	789.0	52.0	39.5
0.5	1532	786.1	51.3	39.3
1.0	1547	788.5	51.0	39.4
1.5	1551	787.8	50.8	39.4
2.0	1556	785.9	50.5	39.3

Effect of BP level:

The basal medium, lacking 1 g/l. yeast extract, was modified by adding different levels of NaOH-pretreated milled BP ranging from 10 to 60 g/l. The results (Table 3) revealed that level of 4% BP resulted in maximum protein yields (54%) combined with highest recorded efficiency in conversion of BP into SCP (41.8%) with the mixed culture. It has subsequently been found (Ghanem, *et al,* in press) that 2% BP was optimum for SCP production by a monoculture of *T. reesei.*

Table 3

Dry weight and SCP production by mixed culture of *T. reesei* and *K. marxianus* as influenced by BP levels.

BP Level g/100 ml.	Final pH	Dry Weight (mg.)	SCP		Conversion Efficiency
			mg.	% to Dry wt.	
1	5.0	519	260.5	50.2	26.1
2 (basal)	5.1	1517	789.0	52.0	39.5
3	5.1	2295	1212.2	52.8	40.4
4	5.2	3096	1672.1	54.0	41.8
5	5.2	3816	2030.1	53.2	40.6
6	5.2	4465	2340.0	52.4	39.0

Amino acids composition:

The good nutritional quality of the fungal proteins obtained from the mixed culture has been assessed through the GLC analyses of the amino acids of the fermentation products (unfermented BP + microbial biomass) obtained under the tested cultural conditions. The results (Table 4) indicated that the tested proteins showed an amino acid profile that compared favourably with both the FAO reference, and soy bean oil meal values or higher, with the exception of methionine and lysine which showed lower values.

When the amino acid profile of the mixed cuture (*T. reesei* + *K. marxianus*) was compared with that of the monoculture (*T. reesei*), obtained under comparable conditions, the values of some amino acids (leucine, phenylalanine, threonine, valine, aspartic, glutamic and proline) were higher in mixed culture than in monoculture, while some (crystine, methionine, tyrosine, glycine, histidine and serine) were of comparable values in both samples. This finding is in accordance with the results of other workers (Molina *et al,* 1983).

The previous data indicated that using a mixed culture of *T. reesei* and *K. marxianus* for SCP production from BP is more efficient than using a monoculture of *T. reesei.* Thus using the mixed culture the protein yields were increased from 49.3 to 54%, while the conversion efficiency of BP into proteins was raised from 35.7 to 41.8%. On the other hand, the yeast extract in the basal medium could be substituted by the presence of *K. marxianus* in the mixed culture.

Table 4

Amino acid content (g/100g protein) of a monoculture of *T. reesei* and a mixed culture of *T. reesei* and *K. marxianus* as compared with the FAO reference and soy bean oil meal protein.

Amino Acid	Monoculture (*T. reesei*)	Mixed Culture (*T. reesei* + *K. marxianus*)	FAO	Soy Bean Oil Meal
Essential:				
Cystine	4.8	4.5	2.0	1.4
Isoleucine	9.6	5.2	4.2	5.7
Leucine	7.1	7.9	4.8	7.7
Lysine	4.2	3.7	4.2	6.5
Methionine	0.9	0.8	2.2	1.4
Phenylalanine	3.8	5.4	2.8	5.1
Threonine	5.0	6.2	2.8	4.0
Tyrosine	4.6	4.4	2.8	2.7
Valine	6.9	8.0	4.2	5.0
Non-essential:				
Alanine	6.5	5.8	—	—
Arginine	4.8	3.3	—	—
Aspartic acid	9.5	10.0	—	—
Glutamic acid	8.5	13.2	—	—
Glycine	5.5	5.3	—	—
Histidine	3.8	3.8	—	—
Proline	4.8	5.7	—	—
Serine	5.3	5.1	—	—

The results obtained indicated the potential value of a mixed culture of *T. reesei* and *K. marxianus*, compared to a monoculture of *T. reesei*, for improving the nutritional value of the Egyptian beet pulp. These results obtained have initiated a series of systematic studies aimed at making fermented BP one of the important available foodstocks in Egypt.

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