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Study of Mycoprotein Production Using *Fusarium oxysporum* PTCC 5115 and Reduction of its RNA Content

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Abstract: In this research, production of mycoprotein by *Fusarium oxysporum* (PTCC 5115) was investigated. In order to achieve the highest production yield, cultivation parameters including temperature, agitation speed, carbon and nitrogen sources were optimized by applying the Taguchi design. The optimum conditions obtained for mycoprotein production were: 25°C, 150 rpm, 5 g of glucose per liter and 3.4 g of ammonium dihydrogen phosphate per liter which resulted in the product containing 42% (w/w) crude protein. RNA content was reduced to an acceptable level through heat treatment of fungal biomass at 65°C for 15 min. This heat shock procedure reduced RNA level to less than 1%, which is recognized as an acceptable level for human food grade products.

Key words: *Fusarium oxysporum*, mycoprotein, RNA reduction

Introduction

Mycoprotein is the generic name given to the ribonucleic acid-reduced biomass comprising the hyphae (cells) of *F. venenatum*, initially identified as *F. graminearum*. (Wiebe, 2004). Production of mycoprotein was introduced in the UK in 1985 under the brand name "Quorn" and was used as a meat substitute in foods. The Food and Drug Administration (FDA) approved the use of mycoprotein as a food ingredient in 1998 (Rodger, 2001). Mycoprotein is produced by continuous aerobic fermentation of food-grade carbohydrate substrates by *F. venenatum*, using suitable cofactors (Miller and Dwyer, 2001). The nucleic acid content of fungi may be as high as Fungi may comprise 6% to 11%. In humans, the purine bases in the nucleic acids are metabolized to insoluble uric acid. This can lead to the formation of kidney and gall stones and other disorders including gout. The United Nations Protein Advisory Group recommends that humans consume no more than two grams of nucleic acids per day. Thus after harvest, the mycelium is heat processed to reduce RNA content to safe levels (Hunter, 2001).

On the basis of the dry cell weight, mycoprotein supplies approximately 50% protein, 13% lipid and 25% fiber (Miller and Dwyer, 2001). Mycoprotein's nutritional benefits arise from its chemical composition. The cell walls of hyphae are sources of dietary fiber (chitin and β-1,3 and 1,6 glucan); cell membranes are sources of the polyunsaturated fats and the cytoplasm is a source of high-quality protein. The fiber component of mycoprotein may function as a prebiotic in the lower gut. The fatty acid composition of mycoprotein is much more similar to vegetable than animal fat. The amino acid composition of mycoprotein shows the presence of all the essential amino acids (Rodger, 2001). The consumption of mycoprotein significantly reduces total and Low Density Lipoprotein (LDL) cholesterol levels and raises High Density Lipoprotein (HDL) levels in the serum (Turnbull *et al*., 1992). Mycoprotein consumption also reduces glycemia and insulinemia (Turnbull and Ward, 1995).

According to literature *F. graminearum*, *F. oxysporum* and *F. solani* are edible strains of *Fusarium* (Ward, 1998). In this study *F. oxysporum* (PTCC 5115) was used to investigate mycoprotein production. Conditions for the optimum production of mycoprotein were determined and heat treatment was used for the reduction of RNA levels in the biomass. The results were then compared with those of *F. venenatum*.

Materials and Methods

Organism and medium: The filamentous fungus *F. oxysporum* (PTCC 5115) was maintained at 4°C on agar-solidified Vogel slants. The defined medium of Vogel (Vogel, 1956) was used with glucose as the carbon source. Vogel medium consisted of: 10 g glucose, 2.6 g Na_3 citrate. $2H_2O$, 2.52 g KNO_3, 2.88 g (NH_4) H_2PO_4, 1.6 g KH_2PO_4, 0.2 g $MgSO_4$. $7H_2O$, 0.1 g $CaCl_2$ $2H_2O$, 2.5 ml of biotin solution and 5 ml of trace elements per liter. The trace elements solution consisted of 0.1 g citric acid. H_2O, 0.1 g $ZnSO_4$. $7H_2O$, 0.02 g Fe $(NH_4)_2$ $(SO_4)_2$. $6H_2O$, 5 mg $CuSO_4$. $5H_2O$, 1 mg $MnSO_4$. H_2O, 1 mg H_3BO_3, 1 mg Na_2MoO_4. $2H_2O$ per 100 mL. The pH of the medium was adjusted to 5.8.

Inoculum preparation: Vogel medium was used in inoculum preparations. Fermentation was conducted in flasks, each containing 100 mL of medium, inoculated with 5% (v/v) of fungal suspension and incubated at

Table 1: Results of experiments (L_9 orthogonal array)

Experiment	Temperature (°C)	Agitation Speed (rpm)	Glucose (g/l)	Ammonium Dihydrogen Phosphate (g/l)	Cell Dry Weight (g/l)	Crude Protein (g/100)
1	20	150	5	2.4	4.5	41.1
2	20	200	10	2.9	5.7	35.9
3	20	250	15	3.4	5.5	37.2
4	25	150	10	3.4	7.2	43.5
5	25	200	15	2.4	8.2	42.2
6	25	250	5	2.9	3.8	39.6
7	30	150	15	2.9	8	41.3
8	30	200	5	3.4	3.9	43.2
9	30	250	10	2.4	6.1	38.0

Table 2: Analysis of variance (ANOVA)

Factor	df	s	v	f	s	P (%)
Temperature	2	1.064	0.559	-	1.064	38.145
Agitation Speed	2	0.976	0.513	-	0.976	34.997
Glucose	2	0.358	0.188	-	0.358	12.844
Ammonium Dihydrogen Phosphate	2	0.39	0.204	-	0.39	14.009
Error	0					
Total	8	2.789				100.00%

df = Degree of Freedom, s = Sum of Sqrs, v = Variance, f = F-ratio, s = Pure Sum, P = Percent

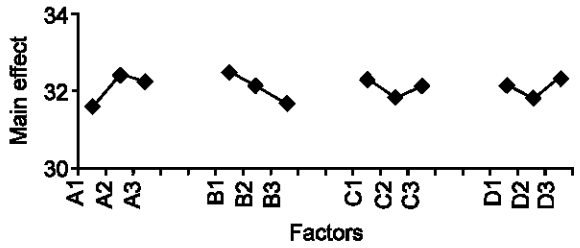

Fig. 1: Main effect of factors on production of mycoprotein, A = Temperature (20, 25, 30°C), B = agitation speed (150, 200, 250 rpm), C = Glucose (5, 10, 15 g/l), D = Ammonium dihydrogen phosphate (2.4, 2.9, 3.4 g/l)

25°C on a rotatory shaker at 200 rpm. After achieving maximum specific growth rate (μ_{max}), the flasks were stored at 4°C.

Experimental design: Optimum culture conditions for mycoprotein production were determined using the Taguchi design with the L_9 orthogonal array. In this design the optimization parameters were used at three levels as follows: temperature (20, 25, 30°C), agitation speed (150, 200, 250 rpm), glucose as carbon source (5, 10, 15 g/l) and ammonium dihydrogen phosphate [$(NH_4 H_2PO_4)$] as nitrogen source (2.4, 2.9, 3.4 g/l).

RNA reduction: Fungal biomass suspensions (20 mL) were subjected to heat treatment at 65°C and 72°C for 5-30 min. At these temperatures, RNA is degraded into monomers by ribonuclease which can then diffuse out of the cells (Wiebe, 2002; Shojaosadati *et al.*, 1999).

Analytical methods: Crude protein was measured using the microkjeldahl technique. The crude protein content of the biomass was calculated as 6.25 times the total nitrogen and protein content (w/w), represented by g protein per 100 g total dry solids (Lang, 1958).

The RNA, carbohydrate and total lipids content were measured using standard methods (Chomczynski and Sacchi, 1987; Dubois *et al.*, 1956; Barnes and Blackstock, 1973).

Ash, amino acids and fatty acids content were determined according to A.O.A.C (Association of Official Analytical Chemists) (1997).

Statistical analyses: All experiments were performed in triplicate. Qualitek-4 statistical software using analysis of variance (ANOVA) was used for determination of optimal conditions. T-test was used to compare amino acids of the mycoprotein with the FAO (Food and Agriculture Organization) reference protein and amino acids of soybean meal at the 0.05 significant level.

Results

Results of experiments and analysis of variance (ANOVA) are presented in Table 1 and 2. According to these results the optimal conditions for achivement of the highest crude protein yield were 25°C, 150 rpm, 5 g of glucose per liter and 3.4 g of ammonium dihydrogen phosphate per liter (Fig. 1). Under these optimum condition, 5 g of biomass per liter in the batch cultivation mode was obtained, where the crude protein concentration comprised approximately 42% (w/w) of dry solids.

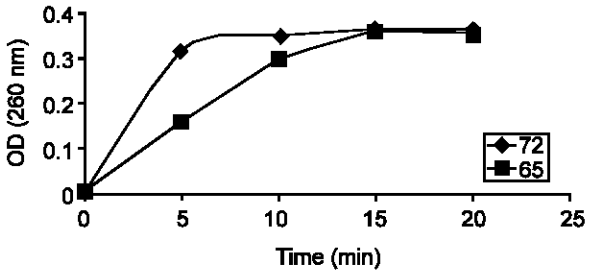

Fig. 2: Effect of temperature and duration of heat treatment on the release of nucleotides

Table 3: Amino acid composition of *Fusarium oxysporum*

Amino Acid	g per 100 g protein
Aspartic acid	9.81
Glutamic acid	17.48
Serine	4.35
Glycine	7.11
Histidine	2.40
Arginine	6.41
Threonine	9.28
Alanine	5.77
Proline	6.82
Tyrosine	1.37
Valine	6.06
Methionine	1.45
Isoleucine	4.63
Leucine	6.20
Phenylalanine	4.20
Lysine	6.66

According to Fig. 2, maximum reduction of RNA was achieved at 65°C for 15 min and 72°C for 10 min. Results indicated that weight loss of biomass at 65°C for 15 min (18.6%) is lower than that at 72°C for 10 min (36.7%). After heat treatment of biomass at 65°C for 15 min, RNA levels reduced from 7% in the original biomass sample to 1% in the heat treated sample.

The amino acid (g per 100 g protein) and the fatty acid (g per 100 g lipid) composition of mycoprotein are shown in Table 3 and 4. Ratio of unsaturated to saturated fatty acids after heat treatment was 3.1 to 1. The overall biochemical composition of the mycoprotein (w/w) is as follows: 42% crude protein, 0.75% RNA, 16.7% carbohydrate, 12% lipid and 5.3% ash.

Discussion

Under optimal conditions, fungal biomass containing 42% (w/w) crude protein was obtained. Optimum temperature for mycoprotein production was 25°C, lower and higher temperatures decreased protein production (Fig. 1). According to the data (Fig. 1) increase in the agitation speed led to a decrease in the rate of protein production and consequently product yield, due to damage of mycelial biomass during mechanical agitation (Moo-Young *et al.*, 1993). Results of experiments show that an agitation speed of 150 rpm is an optimal value for *F. oxysporum* fermentation which is quite sensitive to high agitation rates, consistent with adequate mixing and oxygen supply. The C:N ratio also affect the protein production, so that lower glucose levels and higher ammonium dihydrogen phosphate levels resulted in higher protein production (Fig. 1).

Heat treatment at 65°C for 15 min reduced the RNA content of the biomass to less than 1% which is regarded as a safe level for human consumption. This is in agreement with previous studies of *F. venenatum* (Wiebe, 2002).

In Table 5, the amino acid composition of mycoprotein is compared with that of the soybean meal and FAO

Table 4: Fatty acid composition of Fusarium oxysporum

Fatty acid	g per 100 g lipid
Myristic acid (C14:0)	0.19
Palmitic acid (C16:0)	13.2
Palmitoleic acid (C16:1)	0.89
Stearic acid (C18:0)	8.98
Oleic acid (C18:1)	37.9
Linoleic acid (C18:2)	31.6
γ-Linolenic acid (C18:3)	0.08
Arachidic acid (C20:0)	0.88
α-Linolenic acid (C18:3)	4.47
Behenic acid (C22:0)	0.53

Table 5: Amino acid composition of various proteins (g per 100 g protein)

Amino Acid	Mycoprotein	Soybean Meal	FAO Reference
Threonine	9.28	4	2.8
Valine	6.00	5	4.2
Cystine	-	1.4	2.0
Methionine	1.45	1.4	2.2
Isoleucine	4.63	5.4	4.2
Leucine	6.20	7.7	4.8
Tyrosine	1.37	2.7	2.8
Phenylalanine	4.20	5.1	2.8
Lysine	6.7	6.5	4.2

Table 6: Amino acid composition of Fusarium oxysporum and Fusarium venenatum (g per 100 g protein)

Amino Acid	*Fusarium oxysporum*	*Fusarium venenatum*
Alanine	5.77	6.3
Arginine	6.41	7.3
Aspartic acid	9.81	10.3
Cystine	-	0.8
Glutamic acid	17.48	12.5
Glycine	7.11	4.3
Histidine	2.40	3.5
Isoleucine	4.63	5.2
Leucine	6.20	8.6
Lysine	6.66	8.3
Methionine	1.45	2.1
Phenylalanine	4.20	4.9
Proline	6.82	4.5
Serine	4.35	5.1
Threonine	9.28	5.5
Tryptophan	-	1.6
Tyrosine	1.37	4.0
Valine	6.06	6.2

reference protein (Moo-Young *et al*., 1993). Threonine, valine, isoleucine, leucine, phenylalanine and lysine content of mycoprotein are significantly higher than that of the FAO reference and the threonine and valine contents are more than that of the soybean meal. Amino acid compositions of *F. oxysporum* and *F. venenatum* (Rodger, 2001) were compared (Table 6); glutamic acid, glycine, proline and threonine levels in *F. oxysporum* are significantly higher than those in *F. venenatum*. Experimental analysis showed that threonine content of *F. oxysporum* was very high. The lipid and ash content of *F. oxysporum* were in agreement with previous studies of *F. venenatum*, however, *F. oxysporum*'s carbohydrate content was found to be much higher.

Conclusion: The optimization procedures implemented in this study indicate that food-grade mycoprotein containing relatively high quality protein can be produced by *F. oxysporum*.

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