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IMPROVING FISH FEED BY YEAST SOLID STATE FERMENTATION

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Abstract: A formulated fish feed was fermented employing two strains of *Saccharomyces cerevisiae* in solid state fermentation (SSF) at their corresponding optimized growth conditions. The strain Sn-1Y enhanced protein content of fermented feed up to 31.68% after 72 hours. While Sn-5Y showed increase of protein content up to 32.90% after 48 hours. Total carbohydrate content of the feed showed a declining trend up to 54.54% for Sn-1Y and 55.39% in case of Sn-5Y after 168 hours of fermentation. Maximum reduction (42.50 and 16.25%) of free amino acids occurred for Sn-1Y and Sn-5Y after 72 and 48 hours of incubation, respectively. The present study showed that the fermentation of the fish feed by *S. cerevisiae* may improve its quality. Application of such feeds may economize the development of aquaculture in this country.

Key words: Fish feed, yeast fermentation, *Saccharomyces cerevisiae,* feed protein content, feed amino acid content.

INTRODUCTION

Aquaculture is one of the fast growing food producing sector in the world. Growth of this sector requires increased production of fish feed. Fish meal is one of the important feed ingredients due to its adequate amino acid, essential fatty acids and mineral profile (Zhou *et al.,* 2004; Craig and McLean, 2006). However, due to stagnant supply of fish meal, prices will inevitably increase with demand (Watanabe, 2002; Lunger *et al.,* 2007). This situation necessitates significant reduction in the dependence of the aqua feed industry upon fish meal supplies by searching and evaluating suitable and less expensive alternative protein sources (Hardy and Tacon, 2002; Lunge *et al.,* 2006). Many plant feedstuffs have received attention in recent years. However, due to amino acid unbalances,

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presence of anti-nutritional factors and low palatability, a high level of fish meal replacement with plant feedstuffs had generally not been well accepted (Lunger *et al.,* 2006).

Single cell protein (SCP) is used widely in feed now-a-days which include microalgae, bacteria and yeast biomass. These non-conventional alternative protein sources are being attempted as feed ingredients for fish due to their nutritional richness in terms of proteins, vitamins, pigments and complex carbohydrates. Among SCP, yeasts have been considered more suitable for aquafeed (Tacon, 1994). Several studies have shown that yeasts can successfully replace part of dietary fish meal in feed of different fish species (Matty and Smith, 1978; Oliva-Tales and Goncalves, 2001; Olvera-Novoa *et al.,* 2002). Yeasts also work as immunostimulant (Gannam and Schrock, 1999; Sakai, 1999) and probiotics (Gatesoupe, 1999). In some studies, supplementation of yeast containing diets with limiting amino acids has been shown to improve fish growth performance (Murray and Marchant, 1986). However, search of diversity of the microbial strains and that of the substrates which may be used for the cultivation of SCP yielding organisms suffice to allow continuing the efforts for more fruitful outcome. The present investigation was intended to ferment a formulated fish feed employing two strains of *Saccharomyces cerevisiae* to incorporate SCP. The information add further for improving the development of aquaculture in the developing country.

MATERIALS AND METHODS

Two already isolated strains of *S. cerevisae* designated as Sn-1Y and Sn-5Y, preserved in Microbial Biotechnology Laboratory, Department of Zoology, University of the Punjab (Quaid-e-Azam campus), Lahore were employed for this study.

Optimization of growth conditions

The yeast strains were revived in yeast selective medium containing yeast extract, 0.3g; chloramphenicol, 0.5g; agar agar, 1.5g and distilled water, 100 ml (Merck 1996-97) and then optimized for temperature (34, 37 and 45°C), oxygen demand (aerobic and anaerobic), effect of pH (5, 6 and 7) and inoculum size (1, 5 and 10%) while cultivating in yeast selective broth.

Solid state fermentation of fish feed

For solid state fermentation, an apparatus was designed and installed according to Hofrichter *et al.* (1999). Twenty grams of the formulated fish feed (Table I) was taken in each glass container (12cm length x 6cm diameter) and autoclaved. Then 2ml of sterilized distilled water and 12 ml of already prepared inoculum was added, mouth of the container was re-closed with the screwed plastic lid and incubated at respective optimum condition of each yeast isolate. Samples were taken after 24, 48, 72, 120 and 168 hours under aseptic conditions. Fermentation jar was stirred and sterile distilled water was daily added to replenish 70% (v/w) water content. Control jars were processed similarly but without yeast inoculation.

Table I: Percent composition of formulated fish feed ingredients.

Sr. No.	Feed ingredients	Quantity (%)
1	Molasses	4.0
2	Fish meal	5.0
3	Rice polish	34.3
4	Table salt	1.0
5	Vitamin premix	1.0
6	Ground nut oil cake	53.7
7	Dicalcium phosphate	1.0

Colony forming unit of fermented fish feed

Fermented feed (1.0g) was suspended in 9ml sterile physiological saline and then serially diluted for few times more. From each dilution 100µl was spread over 20ml of nutrient agar medium. After 24 hours of incubation at 37°C, colonies were counted for CFU calculation. Only those plates were taken into account for which the CFU count fell within the range of 30 to 300.

Biochemical analysis of food

Fresh feed, control as well as fermented (0.5g) was taken and homogenized in 4.5ml saline solution. Following centrifugation at 5000 rpm at 4°C for 10 minutes, the supernatant was processed for the estimation of total carbohydrate, protein and amino acids according to the

methods of Dubois *et al.* (1956); Lowry *et al.* (1951) and Jayarraman (1981), respectively.

Statistical analysis

Statistical analysis was performed according to program SPSS 12. For comparison of two and more than two groups, Student "t" test and single factor analysis of variance were applied, respectively.

RESULTS

Both yeast strains showed best growth at 34ºC with pH 5 after 24 hours of incubation. Under optimum temperature, pH and aeration, the strains Sn-1Y and Sn-5Y showed highest growths with 1% and 5% inocula, respectively (Table II). Total carbohydrate contents of the fermented feed showed 55.39% and 54.54% decreases after 168 hours of incubation for Sn-1Y and Sn-5Y yeasts, respectively. Amino acid contents of the fermented feed showed reductions of varying levels as compared to the control in case of Sn-1Y, while the feed fermented with Sn-5Y did not differ from control regarding the amino acids contents (Table III).

Table II: Optimization of growth conditions of the *S. cerevisiae* strains.

Parameters		**Yeast strain**	
		Sn-1Y	**Sn-5Y**
Temperature	34°C	$0.89^{a}\pm0.123$	$0.997^{a}\pm0.070$
	37 °C	$0.61^{a}\pm0.120$	$0.699^{b}\pm0.064$
	45 °C	$0.03^{b}\pm0.007$	$0.034^{c}\pm0.005$
pH	5	$0.91^{a}\pm0.026$	$0.946^{a}\pm0.030$
	6	$0.90^{b}\pm0.017$	$0.926^{a}\pm0.016$
	7	$0.18^{b}\pm0.042$	$0.221^{b}\pm0.044$
Inoculum size	1%	$0.89^{a}\pm0.035$	$0.826^{a}\pm0.033$
	5%	$0.78^{a}\pm0.066$	$0.944^{a}\pm0.063$
	10%	$0.85^{a}\pm0.023$	$0.820^{a}\pm0.098$
Oxygen requirement	+ve	$1.07^{a}\pm0.010$	$1.077^{a}\pm0.011$
	-ve	$0.82^{a}\pm0.002$	$0.806^{a}\pm0.048$

Values represent absorbance at 600nm and Mean± SEM of triplicates. The values without a common alphabet are significantly different from each other in the respective column. Student "t" test and analysis of single factor variance; $p\leq0.05$.

The feed fermented with yeast isolate Sn-1Y showed increase in protein contents and values of the parameter turned out to be 27.46%, 31.46% and 31.68% than the controls after 24, 48 and 72 hours of the SSF, respectively. Sn-5Y fermented feed showed significant increase in protein contents of fermented feed up to 32.90% after 48 hours of incubation (Table IV).

Table III: Effects of solid state fermentation on carbohydrates and amino acid contents of the formulated fish feed

Incubation time (hrs.)	Total carbohydrates (mg/g)			Amino acid (mg/g)		
	Control	Fermented feed		Control	Fermented feed	
		Sn-1Y	Sn-5Y		Sn-1Y	Sn-5Y
24	20.85 ± 0.31	17.08^{*a} ±0.67	16.72^{*a} ±1.23	5.65 ±0.05	5.06^{a} ±0.06	5.00^{*a} ±0.54
48	20.52 ± 0.19	18.02^{*a} ±0.52	14.57^{*a} ±1.80	5.55 ±0.34	3.78^{*a} ±0.14	4.65^{*b} ±0.26
72	20.63 ± 0.29	15.30^{*a} ±0.82	15.11^{*a} ±1.10	5.61 ±0.16	3.23^{*a} ±0.27	5.35^{a} ±0.13
120	20.72 ± 0.28	11.60^{*b} ±0.62	11.34^{*a} ±1.39	5.62 ±0.27	4.36^{b} ±0.50	5.41^{a} ±0.26
168	20.65 ± 0.84	9.39^{*b} ±0.47	9.21^{*b} ±1.00	5.65 ±0.27	4.66^{a} ±0.62	5.59^{a} ±0.21

For details of statistical analysis, see Table I.

Table IV: Effects of fermentation total protein contents and CFU of yeast/g of fermented fish feed.

Incubation time (hrs.)	Total protein (mg/g)			CFU/g	
	Control	Fermented feed		Fermented feed	
		Sn-1Y	Sn-5Y	Sn-1Y	Sn-5Y
24	12.36 ±0.18	15.76^{*a} ±1.02	15.06^{*b} ±0.28	287 x 10^{4}	2 x 10^{8}
48	12.32 ±0.08	16.20^{*a} ±0.15	16.37^{*a} ±0.51	126 x 10^{6}	315 x 10^{6}
72	12.33 ±0.02	16.23^{*a} ±0.86	14.38^{*b} ±0.67	5.69 x 10^{6}	55 x 10^{6}
120	12.48 ±0.02	14.62^{*a} ±0.62	12.75^{*c} ±0.84	4 x 10^{10}	148 x 10^{8}
168	12.50 ±0.02	15.54^{*b} ±0.86	14.05^{*b} ±0.09	4 x 10^{11}	32 x 10^{10}

For details of statistical analysis, see Table I.

CFU of Sn-1Y/g of 24 hours fermented feed was recorded as 287 x 10^4. The number of cells continued to increase so that at 2nd day, the value reached up to 126 x 10^6. For the 5th and 7th days CFU/g approached the values of 4 x 10^{10} and 4 x 10^{11} respectively. For the isolate Sn-5Y, the CFU/g appeared as 2 x 10^8 after 1st day and after 2nd and 3rd day the number of cells decreased to 315 x 10^6 and 55 x 10^6 respectively. While at days 5th and 7th CFU/g increased again, progressively (Table IV).

DISCUSSION

Solid state fermentation has been reported as a mean of elevating total protein content of different substrates by many workers (Iyayi and Aderolu, 2004; Rofstie *et al*., 2005). This technique has greatest socio-economical potential through the production of fermented feeds (Pandey *et al*., 1999). The present study was aimed at improving protein contents of a formulated fish feed employing SSF by *S. cerevisiae*. The fermentation did enhance protein content of fish feed up to 31.68% and protease up to 17.82% as compared to respective control values. Protein rich fish feed is well known for its growth promoting effect. Significant increase of protein contents of SSF feeds over control values are highly appealing to speculate their fish growth promoting effects. Gelinas and Barrette (2007) used potato starch as fermentation substrate for yeast protein enrichment. After drying, fermented starch was found to contain 11-12% protein, including 7-8% yeast protein. Durand and Chereau (2004) used SSF for single-cell protein production from raw sugar beet pulp employing a mutant strain of *Trichoderma viridae.* A variety of enzymes are produced by microbes such as amylases, cellulases, proteases, lipases and lignocellulases. These enzymes help to degrade the starch, non starch polysaccharides and other polymeric forms of the molecules in the substrate to soluble monomers with a beneficial increase in total carbohydrate and protein contents (Ofuya and Nwanjiuba, 1990; Pandey *et al*., 1999; Iyayi and Losel, 2001). Prakasham *et al*. (2006) investigated the production of alkaline protease under SSF using alkalophilic *Bacillus* sp. Green gram husk supported maximum protease production.

Total carbohydrates of the fermented feed decreased up to 50% for both the yeast isolates. The decreases are attributed to carbohydrate utilizing nature of the growing yeast cells. In other words, microbial cell

mass translated the carbohydrate contents of the feed to protein as total protein contents of the fermented feed increased over the control values. Researchers have shown that ability of the yeast to grow on substrates like wheat bran and sugarcane bagasse (Zhang *et al.,* 2002). Thus such wastes can be helpful in raising SCP. The results of this study have shown that SSF by the yeast isolates is effective for the enhancement of the protein content of formulated fish feed. These results have indicated towards an opportunity for further improving and economizing the aquaculture development in this country.

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