Int J Pharm Bio Sci 2015 Jan; 6(1): (B) 1016 - 1020

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

A STUDY ON THE APPLICATION OF KLUYVEROMYCES MARXIANUS BIOMASS FORMULATIONS AS FISH FEED TO BLACK MOLLIES

MEERA BABU*, SHANTA PREMA RAJ AND GOPINATH L

Department of Biotechnology, Dr. M. G. R. Educational and Research Institute, Maduravoyal, Chennai- 600 095.

ABSTRACT

Biomass of *Kluyveromyces marxianus* was used to prepare feed formulations for ornamental fish-black mollies (*Poecilia latipinna*). The cytotoxicity of the fungal biomass was studied using 3T3 cell lines. Three different formulations of the biomass were used in the feeding trial. Formulation I was prepared as 100% dry cells, formulation II was prepared as 50% dry cells+50%starch and Formulation III was prepared by adding amylase enzyme to the formulation II. Commercially available feed was used as formulation IV. Seven days old mollies were selected for the feeding trial and fed with formulations for 90 days. Average weight increases was noted at regular intervals and the efficiency of the formulations in increasing mean body weight was studied. The results suggest that dried biomass of *Kluyveromyces marxianus* was efficient in increasing the mean body weight of Black mollies and hence it can be used as an alternative for commercial feed additives.

KEYWORDS: **Kluyveromyces marxianus, fish feed, Poecilia latipinna, feed trial, 3T3 cell lines.**

MEERA BABU
Department of Biotechnology, Dr. M.G. R. Educational and Research Institute, Maduravoyal, Chennai 600 095.

***Corresponding author**

INTRODUCTION

Protein is considered as a vital nutrient for animals, as it is for humans. With the increasing demand in meat, poultry and sea food products, the need for their increased production of the same has reached its peak. It is quoted that a 0.5T bullock synthesizes less than 0.5kg of protein in every 24-hours, whereas, 0.5T of soybeans produces the equivalent of 40kg protein in every 24-hours and 0.5T of yeast generates 50T in the same time[1]. Such a significant capacity of the microbes makes the production of microbial protein a fast growing sector. Yeasts are considered as a good source of proteins or amino acids as their 40% of dry weight consists of protein[2]. Especially, the *Kluyveromyces* species have been most widely studied for SCP production[3]. In addition, yeast products are widely utilized as feed additives for ruminant animals in many parts of the world. Yeast cell wall contains chitin, mannan and immuno- stimulants[4]. *Saccharomyces cerevisiae* contains 45% protein, 8% fat, 13% ash, 10% water and 23% fibre and carbohydrate. It has an excellent amino acid profile being deficient only in methionine. Dairy and beef producers and ruminant nutritionists worldwide believe that, yeast products enhance dry matter (DM) intake and overall animal performance. In addition, yeast products are modestly priced and hence economic barriers to their use are low. Since animal feed accounts for 60 - 70% operational cost in aquaculture, any feed formulation that is cost efficient and meets the requirement of fish should be the focus of nutritionists for fish culture business to be more profitable[4].The quality of different feedstuff is dependent on their amino acids profile and proteins digestibility. Amino acid profile of fish meal has all the essential amino acid components (histidine, arginine, tryptophan, lysine, leusine, isoleusine, phenylalanine, valine, methionine and threonine) at a higher level than other feedstuff. The addition of fish meal to animal diets increases feed conversion efficiency and growth through better food palatability and nutrient uptake[6].In view of the above said facts the current study was designed to study the effect of fungal biomass on Black mollies in order to increase their growth and body weight.

MATERIALS AND METHODS

Production of Kluyveromyces marxianus biomass

The fungal strain *Kluyveromyces marxianus* was subcultured on potato dextrose agar plates (pH5.6) and stored at 37°C for further use. The strain was previously isolated by the authors as mentioned in Babu *et al*[7]. Five days old culture was used for the production of biomass by inoculating the fungal spores in to the production medium and incubating it at room temperature for 5 days. After incubation, the fungal biomass was harvested by filtration using Whatmann No1 filter paper, aseptically. The filtered biomass was further lyophilized to obtain dry cells of *Kluyveromyces marxianus* (yeast cells).

Chemicals and reagents

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) was purchased from Invitrogen, USA. Acridine orange was obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

Cell growth inhibition studies by MTT assay

Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, 3T3 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200µl of Delbucco's Minimum Essential Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics (streptomycin, penicillin-G, kanamycin, amphotericin B). Then culture supernatant was removed and DMEM containing various concentrations (10–100µl) of test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10µl, 5mg/ml) at 37°C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments[8].

Cell viability (%) = Mean OD/Control OD x 100

Formulation of fish feed using the active dry cells of K. marxianus

The Black molly (*Poecilia latipinna*) fishes were obtained from local market and reared in clean water. The pH of the water was maintained at 7.Seven day old mollies were separated in 4 groups of 20 fishes each and treated as follows,

Group 1 :100% dry cells of *K. marxianus* (Formulation1)
Group 2 :50% dry cells + 50% starch (Formulation2)
Group 3 :1% amylase enzyme + 50% dry cells + 50% starch (Formulation3)
Group 4 : Standard feed (Commercially available)

The mollies were fed with the above said formulations twice a day at an amount of 7% of body weight for a period of 90days. The initial body weight was measured then periodically tested for every 30 days for a time period of 90 days at which the fishes attained maximum growth. The feed trial was done in triplicates.

RESULTS AND DISCUSSION

Cell viability and toxicity studies (3T3 cell lines)

The yeast dry cells were tested for their cytotoxicity, if any, by MTT assay. The result of the assay shows that the yeast cells had significantly low toxicity even at higher concentrations. The cell viability was 78% at highest concentration of the cells used (Fig. 1, 2). The cytotoxicity was in the range of 8-21%, which is acceptably less.

Figure 1
Cell viability and toxicity caused by dry K. marxianus cells on 3T3cell lines

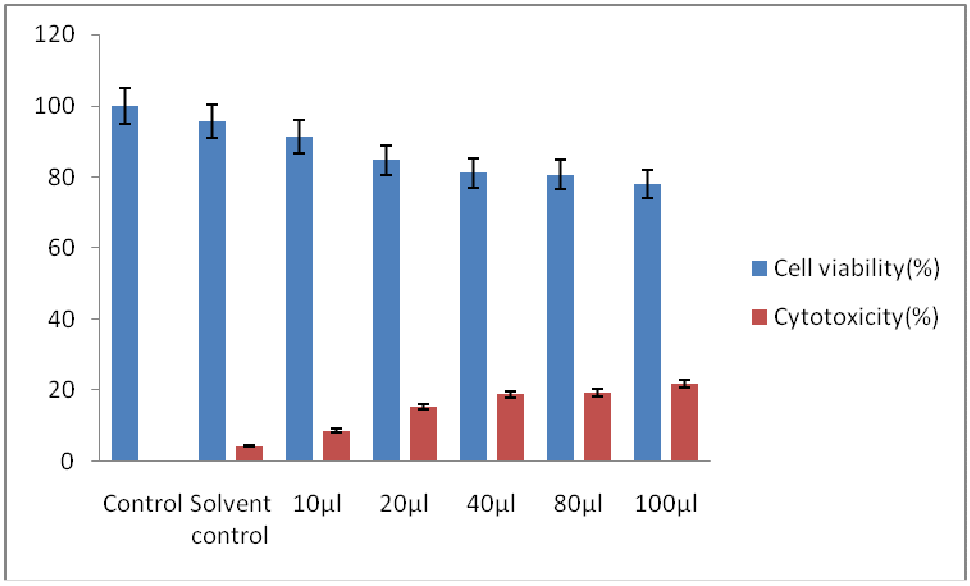
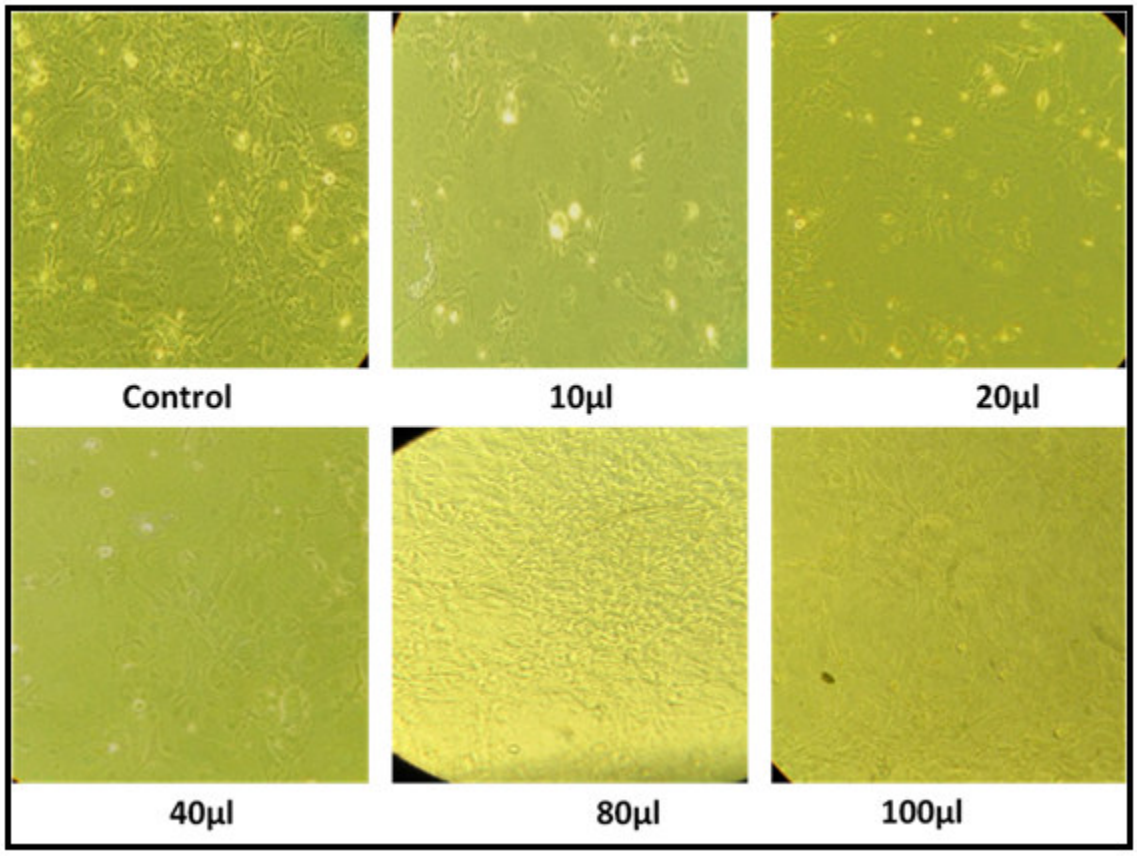


Figure 2
Effect of dry K. marxianus cells on 3T3cell lines



FEED TRIAL IN BLACK MOLLIES - Poecilia latipinna

The results of fish feeding trials are given in Table 1. Formulations 1, 2, and 3 had the ability in maintaining the body weight of the fishes as did the standard feed. The purpose of introducing starch and amylase in the feed was to check whether amylase could give any additional benefits by degrading starch and providing readily available reducing sugar to the fishes. But there were no difference in the body weights obtained using formulations 2 and 3. The reason may be that starch in formulation 2 had been degraded by amylase positive bacteria of the gut of fishes nullifying the effect of the addition of amylase in formulation 3. Hence the additional supply of sugar had compensated 50% of extra dry cells in formulation 2. In contrast, Li and Gatlin found that the hybrid striped bass fed the diets supplemented with 1% and 2% dried brewer's yeast (Brewtech®) had up to 20% more weight gain compared to fish fed with basal diet[9]. However, in concordance with our report, there was no significant difference in percent weight gain (WG), among the treatments in *Tilapia* feeding on torula yeast[10]. The results of the current study confirm to the findings of Montgomery and Gerking who used algae as a source of dietary proteins[11]. Since algae has high protein content, they have a beneficial effect on growth, feed utilization and physiological conditions[12]. Furthermore, the presence of yeast in good levels in fish diet makes it rich in amino acids, phosphorus and calcium.

Table 1
Growth promoting effect of dry cells of K. marxianus on Poecilia latipinna

Days of treatment	Mean body weight (g)			
	Group 1	Group 2	Group 3	Group 4
0	10.4±0.54	10.1±0.87	10.8±0.74	10.4±0.85
30	54±0.97	54.2±0.94	54.6±1.28	53.8±1.04
60	78.1±1.66	78.4±1.28	76.9±1.93	79±1.19
90	98±1.48	97.5±1.64	99.2±2.55	99±2.43

The values are expressed as Mean±SD

CONCLUSION

Thus, the results of the study indicate that dry cells of *Kluyveromyces marxianus* could be an economic and beneficial alternative for the comparatively costlier fish feeds.

REFERENCES

1. Shahzad. MA and Rajoka MI, Single Cell Protein Production from *Aspergillus terreus* and its evaluation in broiler chick. International Journal of Bioscience, Biochemistry and Bioinformatics, 1(2):137-141, (2011).
2. Charlie WS, The subtleties of yeast. Feed Mix, 10(6): 32-33, (2002).
3. Hassan M, Iraj N, Manoochehr T, Improvement of SCP production and BOD removal of whey with mixed yeast culture. Electronic Journal of Biotechnology, 7(3):252-258 (2004).
4. Ovie SO and Eze SS, Utilization of *Saccharomyces cerevisiae* in the partial replacement of fishmeal in *Clarias gariepinus* diets. International Journal of Advanced Agricultural Research, 2-83-88, (2014).
5. Adesulu EA and Mustapha, Use of housefly maggots as fish meal replacement in Tilapia culture. A recent vogue in Nigeria. Proceedings of 5th International Symp. On Tilapia in Aquaculture (ISTA'00), Rio de Janairo, Brazil. Pp. 138-143, (2000).
6. Miles RD and Chapman FA, The benefits of fish meal in aquaculture diets. University of Florida. IFAS Extension. http://edis.ifas.ufl.edu/FA122 (2006).
7. Babu M, Raj SP, Nirmala CB, Deccaraman M and Sagadevan E, Production of Single Cell Protein using *Kluveromyces marxianus* isolated from paneer whey. International Journal of Biology and Advance Research, 05(05):255-257, (2014).
8. Evelyn MLP, Fernando WCA, Ivone AS, Isla VGA, Bastos, Teresinha GS *et al.,* Pharmacological screening and acute toxicity of bark roots of *Guettarda platypoda*. Brazilian Journal of Pharmacognosy, 23(1): 1-21, (2013).
9. Li P and Gatlin DM, Evaluation of brewer's yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops M. saxatilis*). Aquaculture. 219; 681–692 (2003).
10. Novoa OMA, Palcios MCA and Castillo OL, Utilization of torula yeast (*Candida utilis*) as a protein source in diets for tilapia (*Oreochromis mossambicus* Peters) fry. Aquaculture Nutrition. 8; 257- 264, (2002).
11. Montgomery WL and Gerking SD, Marine macroalgae as food for fishes: an evaluation of potential food quality. Environ. Biol. Fish, 5: 143-155, (1980).
12. Mustafa MG and Nakagawa H, A review: dietary benefits of algae as an additive in fish feed. Bamidgeh- The Israeli Journal of Aquaculture. 47 (3-4): 155-162, (1995).