

## Development of large scale cultivation process of locally isolated and commercial aquatic yeast in laboratory scale bioreactors for the production of fish feed

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### Introduction

Single Cell Protein or SCP has been used as protein substitute either for human consumption or animal feed. SCP is basically microorganisms that has a good protein content. One example of SCP is yeast. The fish industry of developed country has long used aquatic yeast known as red yeast (*Phaffia rhodozyma*) in feed formulation. Apart from providing the nitrogen content, the red yeast also provide astaxanthin, a compound that helps development of pigmentation in rainbow trout (Nakano et al, 1999). By using SCP, the amount of fish-meal needed in fish feed can be reduced. Another aspect of the proposed research is to screen for local aquatic yeast strain and to investigate their potential as fish feed. The richness of flora and fauna in Malaysia is very suitable for finding potentially useful industrial microorganism

### Materials and Methods

Methods used includes standard microbiology technique for yeast isolation and cultivation, mutagenesis technique, molecular technique for construction of genomic library and fermentation process for cultivation of the yeast strain.

### Results and Discussion

In this research, five different yeast strains were isolated from several aquatic and food sources. These yeasts have since been identified as belonging to the genus of *Rhodotorula*, *Pichia* and *Candida* respectively. The isolate designated as YL3, putatively known as a haploid strain of *Pichia ohmeri*, is able to tolerate growth temperature of 42 °C and salt osmolarity of 2.5M NaCl. Fermentation studies were carried out to determine the growth kinetics of the yeast. The studies include growth on different carbon source and nitrogen source, different concentration of carbon source as well as growth at different agitation speed using bioreactor system. Due to its ability to grow on methanol, growth studies on methanol were also carried out. Genetic characterization was also initiated by carrying out mutagenesis of the strains. Mutants of YL3 were generated using UV mutagenesis and replica plating technique. Preliminary characterization of these UV-treated mutants lead to the isolation of 7 auxotrophic mutant with different amino acid biosynthesis defects; 20 temperature sensitive (*ts*) mutants ; and 16 osmotic sensitive (*os*) mutants. The stable auxotrophs included two *adenine-deficient*, one *methionine-deficient* and one *histidine-deficient* mutant. Among the *ts* mutation observed, 3 are absolute/tight *ts* mutants and 10 are leaky *ts* mutants.

### Benefits from the study

Technology offered from the research is strain modification and characterization through mutagenesis. The research team acquires the experience to isolate industrial relevant strain, carrying out characterization studies including mutagenesis as well as fermentation studies.

The isolation of auxotrophic mutants will also help developed yeast expression system for the locally isolated yeast strains. The fish feed industry may benefit through the use as a protein supply.

The use of the yeast as single cell protein or SCP utilizing methanol as carbon source should be explored further. Methanol is a by-product of petroleum refinery that does not have much industrial application.

### Project Publications

Characterization of Locally Isolated Yeast Strain Towards Industrial Application. Research and Invention Exhibition UPM 2002, Tan Li Lung, Raha A R, A Ariff and HM Yusof, Bronx medal winner.

Isolation of Auxotrophic, Temperature and Osmotic Sensitive mutants of locally isolated *Pichia ohmeri* strain via ultra violet mutagenesis. Tan Li Lung, Raha A R, A Ariff and HM Yusof, *manuscript prepared*

Name of Graduate	Research Topic	Degree Awarded
1. Ho Hooi Ling	The effect of media formulation and growth condition on growth and plasmid stability of recombinant <i>Lactococcus lactis</i>	Master Sains Bioteknologi
2. Nor Azian Abd Latif	Growth characterization of locally isolated aquatic yeast.	Master Sains Teknologi Fermentasi

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