International Journal of Current Science Research
www.drbgrpublications.in

Volume: 3; Issue: 2; February-2017; pp 1106-1112. ISSN: 2454-5422

# Effect of marine yeast *Debaryomyces hansenii* on the growth, survival and reproduction of *Artemia parthenogenetica*

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## Abstract

The brine shrimp *Artemia* is playing an important role in aquaculture. The demand for large quantities of high quality *Artemia* biomass and cyst is increasing exponentially. In the present study marine yeast was taken as a good probiotic which improves the growth of *Artemia and the* coconut water was used as a medium for the culture of marine yeast *Debaryomyces hansenni*. After 48 hours yeast were harvested and mixed with rice bran, then fed to *Artemia* at different concentrations. The marine yeast cultured in coconut water medium supported growth, survival and reproduction of *Artemia.*

**Keywords:** *Debaryomyces hansenii*, Probiotic, Coconut water, *Artemia parthenogenetica*

## Introduction

The brine shrimp *Artemia* is playing an important role in aquaculture (Bhat, 1993). The demand for large quantities of high quality *Artemia* biomass and cyst is increasing exponentially (Agarwal, 1992). In the present study coconut water is used as the culture medium for the growth of *Debaryomyces hansenii*. After 48 hours yeast was harvested and mixed with rice bran, then fed to *Artemia parthenogenetica* at different concentrations. The marine yeast cultured in coconut water medium supported growth, survival and reproduction of *Artemia.*

Aquaculture has enormous global economic implications for the 21st century (Leger and Sorgeloos, 1985). In the present study marine yeast was taken as a good probiotic which improves the growth of *Artemia and the* coconut water was used as a medium for the culture of marine yeast *Debaryomyces hansenni*. After 48 hours yeast were harvested and mixed with rice bran, then fed to *Artemia* at different concentrations. Yeast added feed supported maximum growth, survival and reproduction of *Artemia***.**

The following are the major objectives of this study:

1. Utilization of waste coconut water as a raw material for the culture of marine yeast *D.hansenii.*

2. To identify the optimum salinity, pH and temperature which supports maximum growth of yeast.

3. To study the effect of various concentrations of *D.hansennii* on the growth survival and reproduction of *Artemia parthenogenetica.*

4. To select the suitable marine yeast as a basic concentration.

## Materials and Methods

Natural *Artemia* population is found in about 360 sites on the five conditions of the world (Vanhaecke *et al*., 1984). These natural *Artemia* populations are found in salt lakes – coastal or Inland, sulphate or carbonate rich waters, costal lagoons and man – made salt pans of tropical, sub-tropical and temperate climate regions. Brine shrimps are non selective filter feeders (Reeve, 1963) and feed on particulate matter of biological origin such as organic detritus from mangrove water as well as on small living organisms of the appropriate size such as microalgae and bacteria.

The experimental animal selected was *A. parthenogenetica*. The brine shrimp, *A. parthenogenetica* is a locally available Indian strain with high reproductive potential. The adults of *A. parthenogenetia* were collected from the man managed salt pans of Thamaraikulam about 12km south east of Nagercoil. The animals were collected from the salt pans and were released into plastic trough (25 litres) containing 80 ppt culture medium in the laboratory. The

culture medium was prepared by dissolving commercially available solar salt at the rate of 80gm/litre. The salinity was measured with a salinity refractometer. Every morning and evening rice bran suspension was given as feed to the culture stock. The freshly released nauplii from the stock culture were used for the study. Yeast is a protein source and it may induce the survival, growth and reproduction of *Artemia.* In the present study, the yeast *Debaryomyces* spp. is used as supplementary diet.

*Debaryomyces* is a genus of yeast, which belongs to the family *Saccharomycetaceae.* They reproduce by multilateral budding. Pseudomycelium may be formed. Ascus formation is by conjugation, between separate cells. Fermentation occurs in some species of *Debaryomyces hansenii, Debaryomyces marama, Debaryomyces polymorphus, Debaryomyces tammarii, Debaryomyces vanriji* isolated from soil, food, water etc.

The yeast *Debaryomyces hansenii* was mixed with rice bran in different proportions and the prepared diets have been tested for their suitability to culture the brine shrimp, *Artemia parthenogenetica.*

### Effect of salinity on the growth of *D. hansenii*

*D.hansenii* was cultured at different salinities in coconut water. Maximum growth occured in control (O.D - 1.99) whereas minimum growth was recorded at 25% salinity (O.D - 0.20).

### Effect of pH on the growth of *D. hansenii*

*D. hanseni* was cultured at different pH in coconut water. It showed maximum growth at the pH 4 (O.D-1.99) and the minimum growth at the pH 10 (O.D-1.66). It was interesting to note that *D. hansenii* showed maximum growth at the pH – 4 i.e. the pH of the coconut water used for the culture of marine yeast.

### Effect of temperature on the growth of *D. hansenii*

*D. hansenii* was cultured at different temperatures in coconut water. This yeast showed optimum growth at 25$^{o}$C (O.D -1.58) and minimum growth at 40$^{o}$C (O.D – 0.20).

## Result and Discussion

Developing a low cost culture medium for raising marine yeast using commonly available organic waste coconut water with objectives to recover nutrients from waste, to develop suitable yeast feed for *A. parthenogenetica* and also to reduce the culture cost is the aim of this study. In the present investigation a systematic analysis of the marine yeasts on different salinity, pH and temperature levels were attempted in order to estimate the most conducive levels of these media for the growth of marine yeasts. It was observed that, the growth was enhanced by the nutrient content of the coconut water and considerable changes were observed in different salinity, pH and temperature levels.

Considering the different salinity levels attempted on *D.hansenii,* the control gives the optimum population level (O.D-1.99) in. Additional levels of salinity only bring a decline in the population density. This was confirmed that high salinity levels may produce stress to the organism due to osmotic imbalance, Osmotic regulation requires active mechanisms such as permeability of water, water elimination of retention and salt retention or secretion (Marian, 1991).

The different levels attempted by changing the pH levels on the yeast the population density is optimum in the control (O.D-1.99). $P^H$ of the raw coconut water is 4. Thus the coconut water can be used as it is for better growth of yeast *D. hansenii.* In the case of temperature, the study revealed that raw coconut water kept at $25^0$ C temperature yields maximum production of these yeasts.

Survival 74.4% was showed by *D. hansenii* at 0.4 mg concentration. Minimum survival was noted at 1.0 mg concentration. This may be due to the higher dose which caused reduced acceptability of the feed, probably due to reduced digestability. In the present investigation *Artemia* fed with *D. hansenii* yeast produced higher growth rate 11.8 ± 0.83 mm at 0.4 mg/g concentration (Table: 1).

Growth was low when rice bran alone was used and high in all the tested animals on application of the yeast. Ovary development was faster ($8.4^{th}$ day) at 0.4 mg/g concentration (Table: 2). The young ones produced by *Artemia* fed *D. hansenii* were 178 ±1.20.

Table : 1 Growth (length in mm) of ***Artemia parthenogenetica*** fed with different concentrations of **fresh feed** (***Debaryomyces hansenii*** cultured in coconut water) – reared from naupliar stage onwards. Each value is the mean (± S.D) of five animals.

| **Concentration (mg)** | | | | | | |
|---|---|---|---|---|---|---|
| Time (days) | Control | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
| $6^{th}$ | 1 ± 0.0 | 1.1 ± 0.22 | 1 ± 0.0 | 1.1 ± 0.22 | 1.3 ± 0.27 | 1.6 ± 0.40 |
| $8^{th}$ | 1.7 ± 0.27 | 2.2 ± 0.27 | 2.3 ± 0.27 | 2.4 ± 0.22 | 2.5 ± 0 | 2.2 ± 0.27 |
| 10th | 2.3 ± 0.57 | 3.7 ± 0.90 | 3.3 ± 0.57 | 3.7 ± 0.57 | 4.9 ± 0.41 | 3.9 ± 0.41 |
| 12th | 4.9 ± 0.74 | 7.8 ± 0.57 | 8.2 ± 0.57 | 6.8 ± 0.27 | 6.8 ± 0.27 | 5.9 ± 0.65 |
| 14th | 5 ± 0.70 | 8.2 ± 0.57 | 8.6 ± 0.65 | 8.2 ± 0.57 | 7.7 ± 1.03 | 6.6 ± 0.41 |
| 16th | 7.7 ± 1.20 | 9.2 ± 0.57 | 9.6 ± 0.96 | 9.3 ± 0.90 | 7.8 ± 0.57 | 8.4 ± 0.65 |
| 18th | 7.7 ± 1.20 | 9.2 ± 0.57 | 9.6 ± 0.96 | 9.3 ± 0.90 | 7.8 ± 0.57 | 8.4 ± 0.65 |
| 20th | 7.9 ± 1.38 | 9.7 ± 0.57 | 9.9 ± 0.82 | 9.6 ± 1.08 | 8 ± 0.5 | 8.7 ± 0.57 |
| 22nd | 8.3 ± 1.25 | 10.1 ± 0.54 | 10.4 ± 0.82 | 9.9 ± 1.29 | 8.4 ± 0.41 | 9.1 ± 0.41 |
| 24th | 8.3 ± 1.25 | 10.1 ± 0.54 | 10.4 ± 0.82 | 9.9 ± 0.82 | 9.7 ± 0.57 | 9.1 ± 0.41 |
| 26th | 8.5 ± 1.45 | 10.4 ± 0.41 | 10.7 ± 0.57 | 10.0 ± 1.06 | 8.8 ± 0.27 | 9.4 ± 0.41 |
| 28th | 8.8 ± 0.27 | 9.1 ± 0.41 | 11.8 ± 0.83 | 10.4 ± 0.41 | 8.8 ± 0.27 | 9.9 ± 1.29 |
| 30th | 8.8 ± 0.27 | 9.4 ± 0.41 | 11.8 ± 0.83 | 10.4 ± 0.41 | 9.4 ± 0.41 | 9.9 ± 1.29 |

Table : 2 Maturation period and Reproduction of *Artemia parthenogenetica* fed with different concentrations of fresh feed *(Debaryomyces hansenii* cultured in coconut water). Each value is the mean (± S.D) of five animals.

| Concentration (mg/g) | Maturation (days) | Brood I | Brood II | Brood III | Brood IV | Brood V |
|---|---|---|---|---|---|---|
| Control | 12.0 ± 0.70 | 20.4 ± 0.54 | 29.3 ± 1.24 | 34.0 ± 1.20 | - | - |
| 0.2 | 9.4±0.89 | 36.6±0.89 | 54.3±2.0 | 100.0±0.90 | 152.0±1.26 | 166.0±0.71 |
| 0.4 | 8.4±0.89 | 62.2±1.30 | 71.8±1.30 | 138.0±1.28 | 178.0±1.20 | 176.0±0.98 |
| 0.6 | 9.2±0.89 | 51.0±2.12 | 58.3±1.24 | 82.0±0.90 | 120.0±0.90 | 146.0±1.10 |
| 0.8 | 9.1±0.70 | 49.8±1.92 | 55.3±1.20 | 59.0±1.40 | 117.0±0.90 | 94.0±1.48 |
| 1 | 9.0±0.89 | 47.2±2.07 | 52.3±1.24 | 55.0±1.24 | 105.0±1.10 | 82.0±0.98 |

The marine yeast feeds are very good feed for the culture of *A.parthenogenetica* (Appelbaum,1979). It involves very easy preparation procedure, less cost, good palatability and odour (Furukawa,1972). This support the following report of Patel, 1984.

Comparison of the survival, growth and fecundity of *Artemia* fed with yeast with that of those supplied with rice bran revealed that yeasts are the better feed for the following reasons.

*Artemia* provided with yeast cultured in the coconut water in the present study attained a maximum length of 11.8 ± 0.83 mm within a short period of 30 days. The survival percentage was only 33% in those culture supplied with rice bran whereas in the yeast supplemented culture it was 74.4%.The fecundity in the yeast fed animals in terms of either the number of broods per animal or the number of off springs per animal was higher when fed with the yeast species than that of rice bran fed cultures.

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