魚粕の圧搾汁による酵母の培養

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Cultivation of Yeast in Medium Containing Liquid from Fish Waste Juice*1

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Attempts were made to examine whether the liquid obtained from the mackerel waste juice could be utilized as a nutrient source by yeast or not. Yeast, *Saccharomyces serevisiae* IFO 2114, grew sufficiently in 75% sea water containing 2.5% liquid. Moreover, 1% glucose or molasses added as a carbon source into the culture medium accelerated growth of the yeast. The stationary phase was observed after 30 h from beginning of the yeast culture. The nitrogen and phosphorus contents in the culture medium decreased with the yeast growth. These results indicate the possibility of a large scale cultlure for yeast by use of the liquid prepared from fish waste juice.

Previous studies on the utilization of fish waste demonstrated that the scrap meal prepared from fish waste was useful as fish feed rich in a protein and lipid by application of the microbial function.[1–4] However, in the process of manufacturing of scrap meal from fish waste, a considerable amount of pressed juice is produced. The fish waste juice contains lipids, water soluble substances such as nitrogenous and phosphorus compounds, and sediments. If it is thrown away without proper treatment, it may become a source of environmental pollution. Accordingly, it is necessary to find out a suitable way to utilize the pressed waste juice.

In the present study, the liquid which contains water soluble substances was separated from the fish waste juice, and it was investigated whether the liquid could be utilized as a nutrient source by yeast or not.

Materials and Methods

Experiment I

Experiment I was conducted to examine the optimum levels of liquid and glucose added into a culture medium.

1. Liquid. Liquid was obtained by centrifuging the juice pressed out from mackerel waste at 2,000 rpm for 15 min and stored in a refrigerator.

2. Yeast Seed. *Saccharomyces cerevisiae* IFO 2114 cultured on a MY agar slant medium[5] was inoculated into 20 m*l* MY broth medium in a 100 m*l* test tube and cultured at 28–30°C for 36 h by shaking in a water bath with reciprocal shaker.

3. Culture Media. Thirty nine test tubes of 100 m*l* capacity were divided into 3 groups with 13 each. To each test tube of the first group, 0.5 g glucose was added and 1.0 g glucose was added to each of the second group. No glucose was added to that of the third group. Then, from 0 to 3.0 m*l* of the liquid with a cumulative increase of 0.25 m*l* was added to each test tube of 3 groups. The volume of medium in each test tube of 3 groups was adjusted exactly to 50 m*l* by addition of the required amount of 75% sea water and sterilized.

4. Propagation. The yeast seed was inoculated to each medium at a rate of 5 million cells per m*l* and cultured at 28–30°C for 36 h by shaking. After the culture, the test tubes were stored immediately in a refrigerator until the yeast cells were counted by a hemacytometer. Before counting, the test tubes containing the yeast cultured were violently shaked to make a homogeneous distribution of cells in the medium.

Experiment II

In experiment II, The cultivation time for the best growth of the yeast was examined. The forty test tubes of 100 m*l* capacity were divided into 5 groups, and 50 m*l* of medium was added into each test tube and then sterilized. These media employed were 75% sea water containing

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Table 1. Growth of the yeast, *Saccharomyces cerevisiae* cultured in 75% sea water containing liquid from fish waste juice and glucose

Conc. of liquid (%)	Conc. of glucose (%) 0	1.0	2.0
	$\times 10^6$ cells/m*l*	$\times 10^6$ cells/m*l*	$\times 10^6$ cells/m*l*
0	9.2	20.8	26.4
0.5	16.2	30.6	42.1
1.0	15.4	42.2	52.8
1.5	20.4	61.1	71.6
2.0	23.3	65.3	93.5
2.5	27.2	84.4	106.6
3.0	24.5	79.3	92.5
3.5	25.2	78.8	87.2
4.0	28.6	78.4	95.2
4.5	31.0	87.2	96.0
5.0	23.4	79.0	92.2
5.5	19.6	54.8	104.8
6.0	17.2	37.2	82.3

1.0 or 2.0% glucose, and 75% sea water containing 2.5% liquid with and without 1.0 or 2.0% glucose, respectively. The test tubes were set in a water bath for shaking culture, and one test tube from each group was taken out at 6 h-intervals. The preparations of the liquid and yeast seed, and the methods of incubation, propagation and cell counting were the same as described in experiment I.

Experiment III

The effect of molasses added in place of glucose into the medium on growth of the yeast was investigated in this experiment. Furthermore, the changes of nitrogen and phosphorus levels in the medium during the culture were examined. Two flasks of 3 *l* capacity were used. The media (1.5 *l*) were 75% sea water containing 2.5% liquid, and 75% sea water containing 2.5% liquid and 1.0% molasses. The preparations of the liquid and yeast seed, and the methods of incubation, propagation and cell counting were the identical to those in experiment I. The cultivation was continued for 48 h and the sampling was done at 6 h-intervals.

Analytical Methods

Total nitrogen and total phosphorus in the medium were estimated by modified Kjeldahl method,[6] and by Hansen and Robinson method,[7] respectively, after removal of the yeast cell by filter.

Results and Discussion

As shown in Table 1, the yeast grew in proportion to the concentration of the liquid in the culture medium and the maximum growth was observed at 2.5% level of the liquid. Furthermore, supplemented glucose at a level of 1.0 or 2.0% in the medium accelerated growth of the yeast.

Table 2 shows the relationship between the yeast growth and the cultivation time. The stationary phase during a culture of the yeast was observed after 30 h in each medium. A combination of the liquid and glucose yielded better growth than in the case of the liquid or the glucose only.

The replacement of glucose by molasses did not almost have an adverse influence on growth of the yeast, as shown in Fig. 1. The nitrogen and phosphorus contents in the medium gradually decreased with the yeast growth. This result indicates that the yeast utilized the nitrogen and phosphorus in the medium as nutrient sources for its growth.

Contents of nitrogen and phosphorus in the

Tnble 2. Cultivation time and growth of the yeast in various media

Time of culture (h)	Without liquid		2.5% liquid		
	1% glucose $\times 10^6$ cells/m*l*	2% glucose $\times 10^6$ cells/m*l*	without glucose $\times 10^6$ cells/m*l*	1% glucose $\times 10^6$ cells/m*l*	2% glucose $\times 10^6$ cells/m*l*
0	5.0	5.0	5.0	5.0	5.0
6	7.3	7.7	6.1	10.7	9.8
12	12.7	15.1	10.8	26.3	24.2
18	20.7	22.2	18.0	40.7	49.6
24	22.4	27.4	24.3	62.3	71.1
30	24.9	30.1	27.5	65.1	82.3
36	24.1	30.4	27.1	67.4	87.0
42	23.6	28.9	27.8	69.4	85.2
48	23.3	29.4	27.4	60.1	83.0

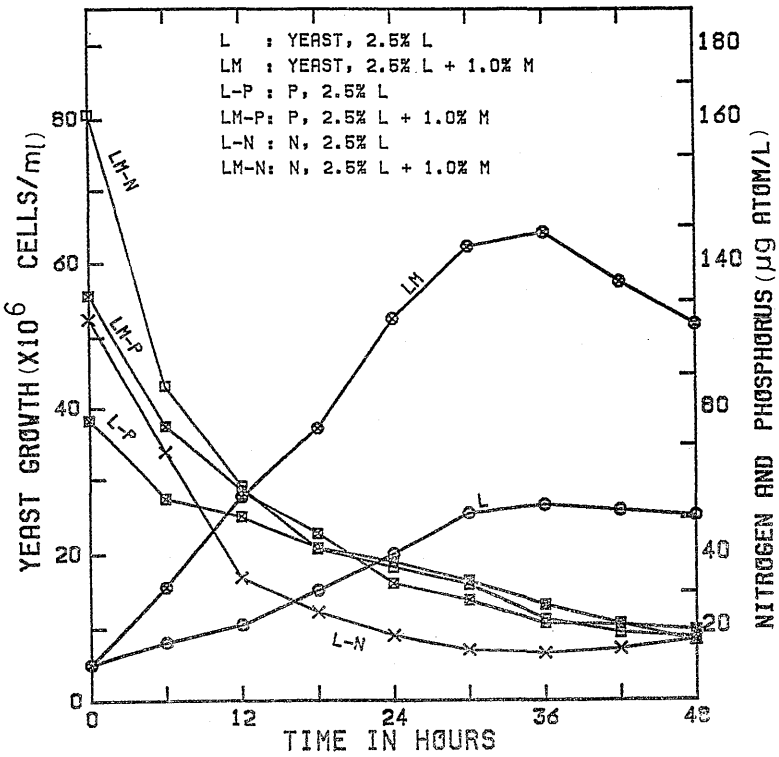

Fig. 1. Relationship between population growth of the yeast and phosphorus or nitrogen level in the culture media.

medium after 36 hours' cultivation are considered to be a safety level enough to discard without pretreatment.

From these findings, it was found that *Sacch. cerevisiae* IFO 2114 showed the good growth, when cultured in diluted sea water containing 2.5% liquid from fish waste juice and 1.0% molasses for 36 h at 28 to 30°C. The results obtained from the present study also suggest the possibility of a large scale culture for yeast by use of liquid from fish waste juice.

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