생명과학회지
Korean J. Life Science, 6(2), 135-141, 1996

Development of Yeast Strains as Feed for Aquaculture : Possible Yeast Strains

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Abstract

Possible yeast strains that could be used as feed for aquaculture were studied. It was shown that the maximum specific growth rate and the biomass yield of *Kluyveromyces fragilis* yeast and *Candida utilis* yeast under optimum pH and temperature were much higher than those of *Saccharomyces cerevisiae* yeast which had been as established yeast diet for rotifer culture. Hence, this work was focussed on the growth characteristics of the two yeasts through flask cultures for mass production.

With 5% inoculum dosage, the best values of μ_{max} and OD_{max} were obtained with on 2.5% fructose medium and 2% YE medium for *K. fragilis* and *C. utilis*, respectively, where the values of μ_{max} and OD_{max} were found to be 0.73 hr^{-1} and 3.00 for *K. fragilis* and 0.59 hr^{-1} and 2.80 for *C. utilis*. It was also found that the lag phase of the growth incresed with increasing initial zinc and NaCl concentrations and decreased with increasing inoculum dosage. Both yeasts could survive relatively well at 3.5% NaCl concentration, and only *C. utilis* yeast could utilize zinc.

Introduction

Providing with large volumes of quality microalgae as live feed in seedling productions of marine animals is very important, since they have been used as an essential food for the larval stages of fish and organisms of shellfish[1]. However, intensive culture and harvesting of unicellular algae is expensive and labor intensive. Thus, replacement of algae with cheap alternative foods would significantly reduce operating costs of aquaculture hatcheries.

Yeasts have been considered as an algal substitute for several species of filter-feeders such as rotifers[2-3], *Artemia*[4-6] and bivalve molluscs[7-8] because of their small particle size, high protein content as single cell proteins and relatively low production costs. In this respect, baker's yeast (*Saccharomyces cerevisiae*), especially enriched with fish oil (ω-3 yeast), has been used widely for rotifer culture as a substitute of *Chlorella* so far. Single cell proteins have been also suggested for use as a potential primary source of nitrogen in fish feeds[9]. *S. cerevisiae* has been sometimes used in fish feeds at levels near 5% of the diet and has been regarded as a source of stable and readily available B vitamines[10].

It is becoming increasingly evident that the development of low-cost, high-quality protein feedstuffs is critical for the future success of the aquaculture industry[11]. For feeds of fish and bivalves, there have been, howe-

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ver, only few reported studies on the nutritional value of yeasts other than baker's yeast[7,12-14]. Thus, developing new yeast strains as feed in aquaculture has been presented in this work.

Materials and Methods

Microorganisms

Cultures of *Saccharomyces cerevisiae* DBY747 (supplied from Cell & Molecular Biology Lab., Seoul National University), *Kluyveromyces fragilis* (ATCC 36534) and *Candida utilis* (ATCC 9950) used in this study were maintained on YEPD agar slants which contained : dextrose, 2% ; yeast extract, 0.5% ; peptone, 2% ; and agar, 2%. The yeast cells were grown aerobically in shaken tubes at 30℃ using a rotary shaker at 180 rpm, and harvested at the late-log phase. The 2% (v/v) of tube cultures were aseptically inoculated into the flasks. The yeast cultures were regularly checked under microscope in order to eliminate any possibility of contamination.

Medium Composition

To obtain optimum composition of the medium for each yeast cell, the following media were used : 1) Three YE media (various concentrations of dextrose, 1%, 2%, and 3% with yeast extract, 0.5%), 2) Four fructose media (various concentrations of fructose, 1%, 2%, 2.5%, and 3% with yeast extract, 0.5%), and 3) Two complex media (two different concentrations of dextrose, 1% and 2% with K_2HPO_4, 2.8mM ; KH_2PO_4, 12.8mM ; NH_4Cl, 75mM ; Na_2SO_4, 11.5mM ; $MgCl_2$, 125mM ; citric acid, 1.0mM ; and biotin, 4μg/L). The initial pH of each medium was adjusted to 4.5 with HCl.

Analyses

Samples from the flask cultures were analyzed for the concentration of yeasts. The cell concentration was measured spectrophotometrically at a 620 nm wavelength using 752 UV Grating Spectrophotometer. Samples of three yeasts were diluted in order to confine the absorbance readings to the range 0.1−0.7 optical densities (OD), as required by the Lambert-Beer law. To relate the measured OD to the dry-weight yeast concentrations (DCW), several samples of the three yeasts were taken at different times over the course of flask cultures. For the measurement of the dry weight of yeast cells, the washed solids after centrifuged twice at 5,000 rpm for 10 min were transferred into an aluminium dish and dried overnight at 100℃. The resulting graphs are shown in Fig. 1. The maximum specific growth rate, μ_{max}, was calculated from the slope on a semi-log graph of OD vs. cultivation time. All the measurements were performed in triplicates.

Results and Discussion

It has been known that yeasts may cause a variety of diseases of the skin (dermatomycoses), systematic mycoses, otomycoses, onychomycoses, etc. mostly by species of *Candida, Cryptococcus, Torulopsis, Trichosporon,* and *Pityrosporum*[15]. Therefore, possible strains as a yeast diet were selected by paper research preliminarily.

Possible Yeast Strains

K. fragilis has been used for several years for the production of single cell protein from whey and it has been one of the few microorganisms classified as GRAS (Generally Recognized As Safe for human consumption)[16]. *K. fragilis* yeast has been also investigated to develop alternative sources of dietary protein in human nutrition [17]. As a food additive, *C. utilis* yeast has found to have relatively high concentration of essential amino acids in the yeast protein[18].

Since both yeasts were not pathogenic definitely, their values of μ_{max} and the maximum optical density (OD_{max}) under optimum temperature and pH were compared to those of *S. cerevisiae* yeast that had been an established yeast diet for rotifer culture. The results obtained through flask cultures are shown in Table 1,

Table 1. The values of the maximum specific growth rate (μ_{max}) and the maximum optical density (OD_{max}) for three yeasts

Conditions	Species	*K. fragilis*		*C. utilis*		*S. cerevisiae*	
		μ_{max} (hr^{-1})	OD_{max}	μ_{max} (hr^{-1})	OD_{max}	μ_{max} (hr^{-1})	OD_{max}
Temp. (℃)	25	0.32± 0.01	3.70± 0.04	0.26± 0.01	2.50± 0.01	0.20± 0.03	1.90± 0.12
	30	0.54± 0.01	3.70± 0.03	0.45± 0.05	2.70± 0.01	0.27± 0.04	1.80± 0.05
	37	0.58± 0.01	3.70± 0.05	0.39± 0.01	2.70± 0.04	0.12± 0.10	1.55± 0.14
pH	4	0.51± 0.01	3.70± 0.02	0.51± 0.01	2.60± 0.07	0.13± 0.02	1.70± 0.07
	4.5	0.49± 0.01	3.80± 0.03	0.44± 0.07	2.70± 0.04	0.15± 0.01	2.00± 0.06
	5	0.58± 0.01	3.70± 0.08	0.58± 1.01	2.70± 0.08	0.13± 0.01	1.75± 0.01
	6	0.53± 0.01	3.70± 0.08	0.38± 0.05	2.60± 0.05	0.13± 0.02	1.65± 0.10

Table 2. The values of μ_{max} and OD_{max} on various media

Medium	Species	*K. fragilis*		*C. utilis*	
		μ_{max}(hr^{-1})	OD_{max}	μ_{max}(hr^{-1})	OD_{max}
YE medium	1% dextrose	0.61± 0.01	2.80± 0.06	0.57± 0.02	2.50± 0.01
	2% dextrose	0.68± 0.05	2.70± 0.07	0.59± 0.04	2.80± 0.15
	3% dextrose	0.70± 0.05	2.90± 0.02	0.53± 0.05	2.80± 0.10
Fructose medium	1% fructose	0.60± 0.05	2.90± 0.01	n.e.	n.e.
	2% fructose	0.67± 0.06	2.80± 0.05	0.47± 0.03	2.29± 0.11
	2.5% fructose	0.73± 0.01	3.00± 0.05	n.e.	n.e.
	3% fructose	0.55± 0.08	3.00± 0.10	0.48± 0.03	2.20± 0.10
Complex medium	1% dextrose	n.e.	n.e.	0.51± 0.05	2.30± 0.10
	2% dextrose	n.e.	n.e.	0.39± 0.04	2.20± 0.04

* n.e. : not experimented

where OD_{max} implies the maximum biomass since values of OD were found to relate to those of DCW as seen in Fig. 1. From the table, the optimum conditions of yeast cultures were obtained ; that were 37℃ and pH 5 for *K. fragilis*, 30℃ and pH 5 for *C. utilis*, and 30℃ and pH 4.5 for *S. cerevisiae*, respectively. At the optimum conditions, the values of μ_{max} and OD_{max} of *K. fragilis* and *C. utilis* were much higher than those of *S. cerevisiae*. This fact means that the two yeasts have the advantage of mass production over *S. cerevisiae* yeast because they grow faster and produce more single cell proteins than *S. cerevisiae* yeast does. From the results, *K. fragilis* yeast and *C. utilis* yeast were considered to be possible strains as feed in aquaculture, and the growth characteristics of them were mainly focussed in this study.

Growth Characteristics

The growth characteristics of the two yeasts, *K. fragilis* and *C. utilis*, were investigated in order to obtain the best conditions of cultivation for mass culture. For this purpose, flask cultures were executed at optimum temperature and pH that had been already obtained.

1. Optimum composition of the medium

In general, YE medium has been used for the cultivation of yeasts, but nine media including YE medium were tested for the best growth of the two yeasts in this

study, and the results were tabulated in Table 2. The best growth were obtained on 2.5%-fructose medium for *K. fragilis* yeast and on 2%-YE medium for *C. utilis* yeast, respectively. These results are in agreement with the reports of Cruz-Guerrero *et al.*[19] and Fournier *et al.*[20] The values of μ_{max} and OD_{max} were found to be 0.73 hr^{-1} and 3.00 for *K. fragilis* and 0.59 hr^{-1} and 2.80 for *C. utilis*, respectively.

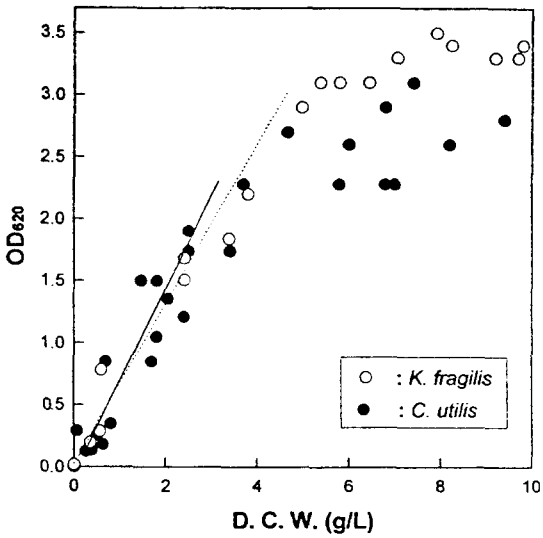

Fig. 1. Calibration curves of optical density vs. dry cell weight

2. Effect of inoculum dosage

The inoculum dosages of 1, 2, 3, 5, and 10% (v/v) were tested to investigate its effect on the growth of each yeast. Increasing the inoculum dosage decreased the length of the lag phase for both yeasts as shown in Table 3. This means that it needs time to adapt in order to produce enzymes to allow its metabolisation when the cell is confronted with a new environment. In Fig. 2, the effect of inoculum dosage on μ_{max} and OD_{max} is shown, where the 5% inoculum dosage gave the best results. This could be explained by reducing the outward diffusion from the cells at higher inoculum dosages [18]. However, at much higher inoculum dosages, an adverse effect occurred on yeast cells.

Table 3. The effect of inoculum dosage on the lag phase

Inoculum dosage	Length of lag phase (hr)	
(%, v/v)	*K. fragilis*	*C. utilis*
1%	1.7±0.1	1.4±0.1
2%	1.0±0.1	1.4±0.1
3%	0.8±0.1	1.5±0.1
5%	0.5±0.1	0.6±0.1
10%	0.4±0.1	0.5±0.1

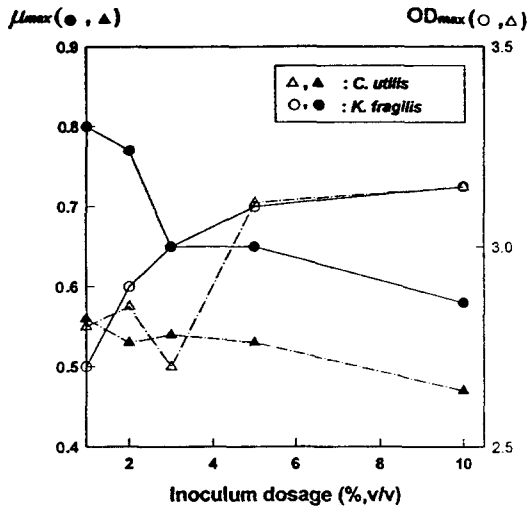

Fig. 2. The effect of inoculum dosage on μ_{max} & OD_{max}

3. Effect of zinc concentration

Zinc has known to be a cofactor for a number of metalloenzymes, such as hexokinase, alcohol dehydrogenase, and glutamate dehydrogenase[18]. It was also reported that with zinc in the medium the protein content of the culture biomass was significantly increased thereby increasing the efficiency of the single cell protein production process[18]. In this respect, 10, 15, 20, 25 μM of zinc were added to the medium. In case of *C. utilis* yeast, increasing the concentration of zinc decreased the value of μ_{max}, and its effect on OD_{max} was some-

what less as shown in Table 4. In contrast to *C. utilis* yeast, zinc had toxic effect somewhat on *K. fragilis* yeast, and this yeast could not survive at the concentrations over 20 μM. It is presumed that *C. utilis* yeast has different metabolic pathway from that of *K. fragilis* yeast[21-22].

4. Effect of NaCl concentration

The effect of NaCl concentration on the growth of each yeast were studied at various concentrations of 1, 2, 3, 5, and 7% (v/v) in order to use the yeasts as feed for marine animals that live in about 35‰-salinity seawater. Increasing the NaCl concentration increased the length of the lag phase for both yeasts as shown in Table 5. The effect of NaCl concentration on μ_{max} and OD_{max} are also shown in Fig. 3. Even though the effect of NaCl on both yeast cells were distinct, the values of μ_{max} and OD_{max} were not relatively low. This means that cultivating these yeasts at 3.5% NaCl may be considered in order to feed the yeasts as live feed in an isotonic condition.

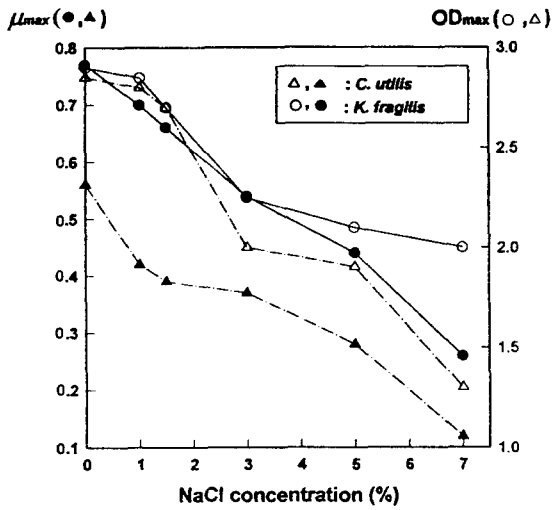

Fig. 3. The effect of NaCl concentration on μ_{max} & OD_{max}

From the all above results, the two yeasts, *K. fragilis* and *C. utilis*, seem to be good as algal substitutes, even though they have not proven to be of consistently high nutritional value. Problems that arise when feeding a yeast monodiet have often been assigned to nutritional deficiencies of the yeast diet[8,23-24]. Because yeast cells are known to have a complex and thick cell envelop, poor digestibility may be an important constraint in the use of this single cell protein as a food source in aquaculture. Further research should be focussed on this problem and some works are actively going.

Table 5. The effect of NaCl concentration on lag phase

NaCl concentration (%, v/v)	Length of lag phase (hr)	
	K. fragilis	*C. utilis*
CONTROL	1.0± 0.1	1.4± 0.1
1%	0.3± 0.1	1.6± 0.1
2%	0.5± 0.1	2.0± 0.1
3%	1.7± 0.1	2.5± 0.1
5%	2.5± 0.1	4.5± 0.1
7%	4.4± 0.1	6.0± 0.1

Table 4. The effect of zinc concentration on μ_{max} and OD_{max}

Species	*K. fragilis*		*C. utilis*	
Zinc concentration(μM)	μ_{max} (hr^{-1})	OD_{max}	μ_{max} (hr^{-1})	OD_{max}
CONTROL	0.73± 0.01	3.00± 0.05	0.59± 0.04	2.80± 0.15
10μM	0.38± 0.04	2.52± 0.09	0.44± 0.05	2.75± 0.02
15μM	0.19± 0.08	2.43± 0.06	0.31± 0.01	2.65± 0.10
20μM	n.d.	n.d.	0.30± 0.06	2.80± 0.08
25μM	n.d.	n.d.	0.25± 0.08	2.85± 0.03

* n.d. : not detected because of no growth

Conclusions

Possible yeast strains were studied with the possibility of developing them as algal substitutes. *K. fragilis* yeast and *C. utilis* yeast have known to be not pathogenic so that they are suitable to be developed as feed for aquaculture. Compared to *S. cerevisiae* yeast that had been an established yeast diet for rotifer culture, the two yeasts showed to have much higher values of the optical density and the specific growth rate. Hence, this work was focussed on the growth characteristics of the two yeasts for mass production.

The dry weight of each yeast cell had different values to the measured optical densities. The best values of μ_{max} and OD_{max} were obtained with 5% inoculum dosage on 2.5% fructose medium and 2% YE medium for *K. fragilis* and *C. utilis*, respectively, where the values of μ_{max} and OD_{max} were found to be 0.73 hr^{-1} and 3.00 for *K. fragilis* and 0.59 hr^{-1} and 2.80 for *C. utilis*. It was also found that the lag phase of the growth curve incresed with increasing initial zinc and NaCl concentrations and decreased with increasing inoculum dosage. Both yeasts could survive relatively well at 3.5% NaCl concentration, and zinc as a cofactor was utilized well only with *C. utilis* yeast.

From the all above results, the two yeasts, *K. fragilis* and *C. utilis*, seem to be good as algal substitutes, even though they have not proven to be of consistently high nutritional value.

Acknowledgement

This work was supported by '95 research grant to Research Center for Ocean Industrial Development from Korea Science and Engineering Foundation.

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초록 : 양식을 위한 먹이사료로서의 Yeast 균주의 개발 : 가능성 있는 효모 균주

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양식을 위한 초기먹이사료로서 가능성 있는 효모균주를 연구하였다. *Kluyveromyces fragilis* 효모와 *Candida utilis* 효모의 최대 비증식 속도와 바이오매스 효율은 *Saccharomyces cerevisiae* 효모에 비하여 굉장히 높은 값을 나타내었다. 따라서, 이 연구는 대량생산을 위하여, 플라스크 배양을 통한 이들 두 효모균들의 성장특성에 그 촛점을 맞추었다.

5% 접종량을 가지고 실험한 결과, 가장좋은 μ_{max}와 OD_{max} 값들은 *K. fragilis*는 2.5% fructose 배지에서, *C. utilis*는 2% YE배지에서 각각 얻어졌으며, 이때의 이 값들은 *K. fragilis*는 0.73 hr^{-1}와 3.00을 *C. utilis*는 0.59 hr^{-1}와 2.80으로 밝혀졌다. 또한, 아연과 염의 초기농도가 증가할수록 균성장에 있어서의 유도기는 증가하였으며, 접종량이 증가할수록 유도기는 감소하였다. 두 효모균은 3.5% 염농도에서 비교적 잘 자랄 수 있었으며, *C. utilis* 효모균만이 아연을 이용할 수 있었다.