

Fish Amino Acid Efficiency in the Growth of Spirulina Platensis

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Abstract

This study looked at the efficiency of fish amino acid on the growth of *Spirulina platensis* in two samples of fish amino acid in five concentrations, i.e., 0, 0.5, 1.0, 1.5, and 2.0 %. The first sample was composed of fish and molasses in a 1:1 ratio; the second was 1:0.5. They were both allowed to ferment for 30 days. Acetic acid bacteria were absent in both samples, but lactic acid bacteria and yeast levels were fluctuated. These data were confirmed by gram's staining, visual analysis, and plate counts techniques. The quantity of microorganisms was found to decrease over the course of the study, whereas the second sample found that levels of lactic acid bacteria and yeast were higher than that of the first sample. Observations of optical density using a wavelength at 560 nm every three days for 21 days checked the culture for *S. platensis*. We found that optical density in the first sample increased to various concentrations, i.e., 1.4458, 1.2968, 0.9708, 1.2391 and 1.2507, respectively. The optical density of *S. platensis* in the second sample also increased to different concentrations, i.e., 1.7236, 1.6799, 1.3380, 1.4189 and 1.3335, respectively. Therefore, the best results for the cultivation of *S. platensis* came from a 0 % concentration of fish amino acid. We concluded that fish amino acid did not affect the production of *S. platensis*, whereas the growth of *S. platensis* was related to the concentrations of lactic acid bacteria and yeast.

Keywords: *Spirulina platensis*, fish amino acid, effective microorganisms, yeast, lactic acid,

INTRODUCTION

Spirulina platensis is a multicellular blue-green algae, which is classified as a member of Cyanobacteria. It is characterized by a helical shape of the filaments or trichomes. It is an autotrophic organism and reproduces by binary fission. The cell is consisted of 55-70% dry weight of proteins, essential fatty acids, vitamins, minerals, and carotenoids. It contains several nutrients, i.e., vitamin B complex, β -carotene, vitamin C, vitamin D, vitamin E, manganese, zinc, copper, iron, selenium, and γ -linolenic acid (Habib et al., 2008). *Spirulina* is normally harvested from the surface of lakes and ponds. It is an alternative nutrient source of food in some parts of Africa and Mexico, because it can be eaten in its natural form or added to other foods and beverages (Talaro, Cowan and Chess, 2009). It is a good gradient that used as a supplement of food for human and animals because it has no cellulose in its cell walls so it is very easily digested and absorbed by the alimentary cells (Sundaravalli, 2011). Moreover, it can be mixed and used in cosmetic products for the face and skin because there are vitamins A and D including phycocyanin and β -carotene, which are antioxidants (Tongsiri, 2013).

In Thailand, *Spirulina* was found in the Northeast by Mrs. Jiemjit Boonsom, a retired government officer, who was the first person that was interested in *Spirulina* and had given the Thai name "Klieo Thong" (Boonsom farm, 2013). *Spirulina* is found in fresh water with high pH, especially in wastewater from industry or activated pond (Peerapornpisal, 2006). There are several studies concerning the growth and utilization of *Spirulina*, i.e., using biological-treated swine wastewater for the cultivation of *S. platensis* (Mezzomo et al., 2010), while Andrade and Costa (2007) used molasses as an organic substrate for *S. platensis* cultivation. Pongpera and Boonnak (2012) used *S. platensis* for the treatment of wastewater from fermented rice noodle factory using. The aforementioned studies suggest that we can modify the cultivation of *Spirulina* to obtain the highest quantity. Moreover, the Thailand Institute of Scientific and Technological Research (TISTR) has been cooperated with Malee Sampran Public Company Limited in studying the cultivation of *Spirulina* using wastewater from fruit factories and yield a very good result (Manager Online, 2013). Thus, it is a bright prospect in the industry where *Spirulina* is a good choice to be used as a gradient to mix in human food and animal food. Additionally, it can diminish environmental pollution problems in wastewater and be able to cultivate with shrimp.

In Thailand there are many aquatic animals in the rivers, especially fish, where fishes are an important component for Thai food such as Spicy and Sour Curry or Gang Som, Tom Yum (fish soup flavored with lemongrass and lime), rice congee mixed with fishes, dried fishes on skewers and many different kinds of preserved fishes. Some parts of fish after cooking cannot be consumed. The fermentation of fishes will solve a problem about the pollution from remaining fish.

In this study, fish amino acid is defined as the fluid from the fermentation of fishes via an anaerobic process. The facultative microorganisms transform complex organic molecules into simple organic compounds that can be absorbed directly by plants (Higa and Parr, 1994). Fish amino acid is composed of proteins (amino acid), carbohydrate, fat and minerals, which are an important food for microorganisms in soil. Amino acid in fish will be changed to form as a nitrogen source for plants. Nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, zinc and copper can be found in fish amino acid (Tonsho, 2006). However, fermentation is a metabolic process that produces ethanol, acetic acid or lactic acid by microorganisms such as yeast and bacteria (Wikipedia, 2013).

The main objective of this study was aimed at determining types of microorganisms in fish amino acid and concentrations of fish amino acid that enhance the growth of *S. platensis*.

MATERIALS AND METHODS

Fish amino acid

Two samples of fish amino acid were used for the cultivation of *S. platensis*. The first sample was a mixture of fish and molasses in a 1:1 ratio. The second sample was a mixture of fish and molasses in a 1:0.5 ratio. They were allowed to ferment for thirty days, and samples were used to determine for the type of microorganisms at every ten days interval. The medium that was used for culturing the microorganisms was MRS (de Man, Rogosa and Sharpe) supplemented with 0.5% CaCO₃, GEAM (Glucose Ethanol Acetic Medium) and PDA (Potato Dextrose Agar). The samples were determined for the type of microorganisms by using gram's staining, visual analysis, and plate counts techniques.

Culture medium of *S. platensis*

The culture medium for cultivation of *S. platensis* was composed of NaHCO₃, K₂PO₄, NaNO₃ and NPK 16:16:16 in distilled water. NaOH was subsequently used to adjust the pH of the medium to the level of 9 - 11. Two samples of fish amino acid were used to mix with the culture medium. At the beginning of the cultivation, fish amino acid were diluted 1:1000 and used in five consecutive concentrations, i.e., 0, 0.5, 1.0, 1.5, and 2.0 %.

Analysis the growth of *S. platensis*

S. platensis was cultivated for twenty-one days in the laboratory. The growth of *S. platensis* was subsequently checked by using the optical density of the culture at 560 nm wavelength every three days.

RESULTS

Lactic acid bacteria were found in fish amino acid in MRS medium supplemented with 0.5% CaCO₃, which was stained as a gram-positive rod. There was a clear zone around the colony. Yeast was found in PDA medium. There was no growth of acetic acid bacteria on the GEAM medium. Acetic acid bacteria were not detected in GEAM for both samples (Table 1). From the results, the quantity of microorganisms was found to decrease over the course of the study and it was found that levels of lactic acid bacteria and yeast in the second sample were higher than that of the first sample.

Table 1. Bacteria and yeast that found in fish amino acid from culture mediums at 37 °C.

Time (Days)	Type of microorganisms	Fish amino acid I			Fish amino acid II		
		MRS	GEA M	PDA	MRS	GEA M	PDA
10	Bacteria	9.2x10 ⁷	-	-	2.28x10 ⁸	-	-
	Yeast	-	-	3.2x10 ⁴	-	-	1.51x10 ⁵
20	Bacteria	1.22x10 ⁶	-	-	2.73x10 ⁶	-	-
	Yeast	-	-	2.9x10 ⁴	-	-	1.02x10 ⁵
30	Bacteria	6x10 ³	-	-	9.9x10 ³	-	-
	Yeast	-	-	6.15x10 ³	-	-	7.9x10 ⁴

Table 2. An optical density of *S. platensis* using a wavelength at 560 nm every three days in fish amino acid I.

Time (Days)	Concentrations of fish amino acid I (%)				
	0	0.5	1.0	1.5	2.0
0	0.0470±0.0023	0.0401±0.0081	0.0420±0.0095	0.0478±0.0030	0.0465±0.0024
3	0.0753±0.0082	0.0581±0.0037	0.0525±0.0027	0.0538±0.0030	0.0600±0.0037
6	0.2478±0.0082	0.2388±0.0239	0.1730±0.0192	0.1888±0.0056	0.1960±0.0060
9	0.3333±0.0095	0.3271±0.0410	0.2343±0.0263	0.2542±0.0073	0.2656±0.0140
12	0.5122±0.0296	0.4698±0.0594	0.3638±0.0424	0.3961±0.0373	0.3641±0.0099
15	0.7455±0.0355	0.6969±0.1004	0.4981±0.0683	0.5562±0.0338	0.5230±0.0235
18	0.8639±0.0358	0.7955±0.1250	0.5773±0.0720	0.6590±0.0348	0.6402±0.0215
21	1.1106±0.0516	1.0399±0.1771	0.7661±0.0950	0.9083±0.0451	0.9206±0.0054

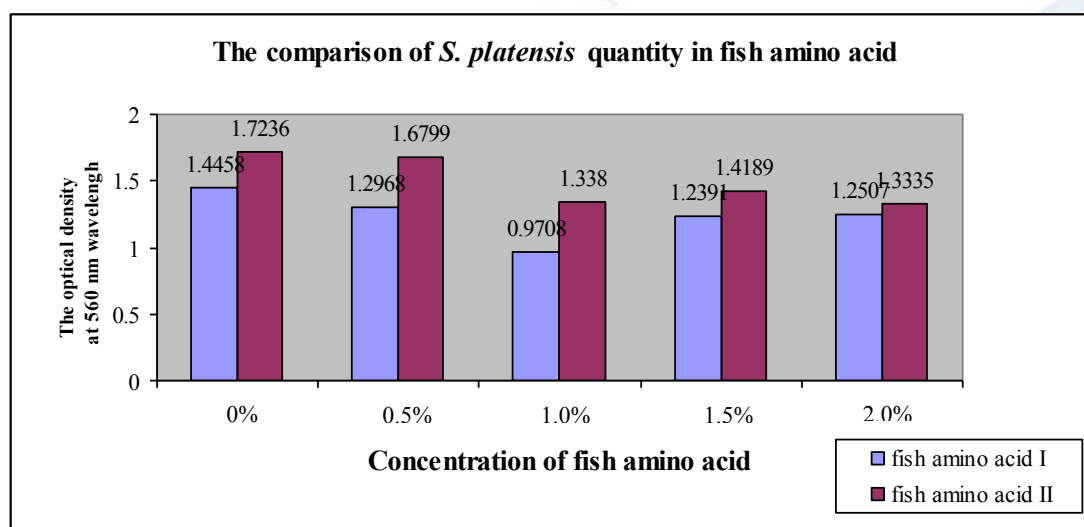
The optical density of *S. platensis* in the first sample was increased to various concentrations, i.e., 1.4458, 1.2968, 0.9708, 1.2391 and 1.2507, respectively (Table 2). The optical density of *S. platensis* in the second sample also increased to various concentrations, i.e., 1.7236, 1.6799, 1.3380, 1.4189 and 1.3335, respectively (Table 3)

Table 3. Showing the optical density of *S. platensis* using a wavelength at 560 nm every three days in fish amino acid II.

Time (Days)	Concentrations of fish amino acid II (%)				
	0	0.5	1.0	1.5	2.0
0	0.0722±0.001 3	0.1032±0.006 7	0.0736±0.002 2	0.1050±0.006 0	0.1056±0.000 5
3	0.1836±0.001 2	0.1385±0.008 5	0.1648±0.003 2	0.1809±0.004 8	0.1659±0.001 6
6	0.3728±0.034 8	0.3374±0.023 3	0.2946±0.015 9	0.3286±0.014 8	0.3008±0.000 5
9	0.4097±0.001 1	0.4106±0.016 1	0.3390±0.011 5	0.3814±0.025 9	0.3489±0.021 1
12	0.6544±0.007 7	0.6858±0.016 4	0.5903±0.019 0	0.6646±0.058 5	0.5696±0.026 5
15	0.8915±0.148 6	0.7967±0.028 1	0.6997±0.023 4	0.8047±0.107 9	0.6580±0.023 0
18	1.4305±0.388 2	1.3749±0.204 6	1.1804±0.048 5	1.2125±0.187 6	1.0983±0.099 5
21	1.7958±0.297 3	1.7831±0.267 7	1.4116±0.044 7	1.5239±0.259 6	1.4391±0.107 1

Results of the quantity of *S. platensis* in the second sample of fish amino acids I, and II are summarized in Fig. 1. *S. platensis* grew in the fish amino acid II sample better than the first sample. The best results for the cultivation of *S. platensis* came from a 0 % concentration of fish amino acid.

Fig. 1. The comparison of *S. platensis* quantity in fish amino acids I, and II during 21 days interval.



DISCUSSION AND CONCLUSION

The study on fish amino acid found that lactic acid bacteria and yeast but acetic acid bacteria were absent because both lactic acid bacteria and yeast were able to use carbohydrates, molasses, in the process and converted to their products. Lactic acid bacteria converted carbohydrate to lactic acid, while yeast converted carbohydrate to alcohol, whereas acetic acid bacteria convert alcohol to acetic acid (Wikipedia, 2013). Thus, the quantity of alcohol from yeast might be less than that converted to acetic acid. In this study, lactic acid bacteria and yeast were found in the second sample more than the first sample similarly with cultivation of *S. platensis* that grew in the second sample more than the first sample.

From the results of the cultivation of *S. platensis*, we observed that a 0 % concentration of fish amino acid was better than others on account of the fact that there were no fish amino acids in the culture medium. Therefore, fish amino acid decreases the growth rate of *S. platensis* because there is the reduction in pH ranges of culture medium and turbidity. However, *S. platensis* could grow in fish amino acid eventhough it did not get the best results. Results are agreed with the study of Cheunbarn and Peerapornpisal (2010), which shows that *S. platensis* is able to grow in wastewater derived from anaerobically treated-swine wastewater, but only with low dilution. This is probably because the algae is unable to adapt itself due to high substances, with high color and turbidity resulting in affecting the rate of photosynthesis in algae. Our results are opposed to those of Rangel et al. (2004), which is found that the use of urea as a nitrogen source that utilizing fed-batch process can be considered as a promising alternative biomass and chlorophyll production. Furthermore, Ungsethaphand et al. (2009) were able to use dry chicken manure supplemented with urea and sodium bicarbonate to cultivate *S. platensis* for reduces cost of medium, while Mezzomo et al. (2010) found that the cultivation of microalgae, *S. platensis* in biological treated swine wastewater demonstrated the capability of biomass production, which suggested that it could use microalgae as an alternative way to assist in the swine culture wastewater treatment resulting in reducing the environmental impact caused by their pollutants.

Although, fish amino acid does not rapidly enhance the growth of *S. platensis*, it does not make *S. platensis* immediately drop in biomass production. The amount of lactic acid bacteria and yeast are related to the growth of *S. platensis*. However, using fish amino acid does not attain suitable conditions for increasing the population of *S. platensis*.

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