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藍藻類(*Nostoc pruniforme*)의 窒素固定能에 關한 硏究

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Nitrogen Fixation of Blue Green Algae(*Nostoc pruniforme*)

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ABSTRACT

Three kinds of organic matter such as glucose, oxalic acid, and ethanol were added to the media(N-free or NO_3-riched) and their effects on the nitrogen fixation of *Nostoc pruniforme* were measured by manometric technique through the experiments *in vivo*.

1) The organic matters used in this experiments showed effective results as a role of substrate for the fixation of atmospheric nitrogen.
2) In the nitrogen-free medium treated with the both of glucose and ethanol, the highest nitrogen uptakes were detected in the treated of low concentrations (glucose; 0.1%, 0.5%, ethanol; 0.1%, 0.5%). On the contrary, the highest nitrogen uptakes in NO_3-riched medium were measured at the treated of high concentrations (glucose; 2%, 1%, ethanol; 1.5%, 1.0%).
3) The highest nitrogen uptakes in N-free medium treated with oxalic acid were measured at the concentration of 2% and 1%, respectively. In the medium of NO_3-riched, the nitrogen uptakes were in the opposite directions.

INTRODUCTION

It is known to that molecular N_2 in the air is utilized directly by the following organisms; the first groups are photosynthetic bacteria such as *Rhodospirillium*, the second groups are *Azotobacter*, *Rhizobium*, and *Clostridium*, the third groups are *Rhodotorula* species, and the fourth groups are some of the blue green algae (Webster, 1965).

The blue green algae, which are similar to bacteria, are belonged to the Cyanophyta and generally known to that the number of species are about 2,000(Patrick, 1966). Of the species, only 34 species are reported having an ability of nitrogen fixation (Watanabe, 1966). The capacity of these algae for nitrogen fixation is generally lower than in bacteria such as *Azotobacter*, *Clostridium* and others. The amount of total nitrogen fixed by blue green algae *Tolypothrix tenui*, *Calothrix brevissima*

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and *Anabaenopsis circularis* per 100m*l* of nitrogen-free medium in 2 months were 9.6mg, 5.2mg, and 3.4mg, respectively (Watanabe, 1966).

On the contrary, *Azotobacter* are able to fix molecular N_2 as amount of 10mg, whenever they consume 1g of available carbohydrate (Norris, 1968). Because of the lower capacity for nitrogen fixation, these blue green algae were not concentrated in studying the mechanism of nitrogen fixation. However, since the practical use of these algae for biological improvement of reclaimed soil in India and for increase of crops yield in Japan, the algae have been concentrated on using the materials for nitrogen metabolism.

Schneider *et al.* (1960) took an experiment for nitrogen fixation with cell free extract from some blue green algae and Fogg *et al.*(1964) found that the photosynthetic portions of blue green algae contain chlorophyll and carotenoid. In 1964, Howell *et al.* carried out an experiment on the comparative studies of nitrogen fixation and photosynthesis with the materials of *Anabaena cylindrica* through the method of manometric technique.

The authors are intended to detect the effects of some organic materials on the nitrogen fixation of *Nostoc pruniforme* which are being cultured in our laboratory. Finally, this experiment is designed to elucidate some factors affecting to the distribution of this species in case of their practical uses.

MATERIALS AND METHODS

1) Cultures:

Blue green algae, *Nostoc pruniforme* used in this experiment were collected at a waterway of the reclaimed area, Chozi-ri, Kangwha-Is. The algae were cultured in the modified Chu's No. 10 medium at 28±1°C and identified on the basis of the references (Geitler, 1925., Prescott, 1944., Desikachery, 1959., Smith, 1950.). The components of the medium are the followings;

$CaCO_3$ ·····0.04g
K_2HPO_4·····0.01g
$MgSO_4 \cdot 7H_2O$ ···0.025g
Na_2SiO_3·····0.025g
Na_2CO_3 ·····0.02g
Ferric citrate···0.003g
Citric acid ·····0.003g
Dist. water·····1*l*

The blue green algae has been cultured for 2 months in the above medium added the microelement solution 1m*l* (Arnon, 1938), then harvested for this experiment. The cultures were put into Sorenson's phosphate buffer(1/10M, *p*H6.5) at dark condition for 4—7 days, therefore starved to exhaust its energy source in cells. And then the cultures were exposed under ultra-violet light for 5 minutes to sterilize some of the symbiont bacteria in which is slime layer of the algae (Watanabe) and weighed about 100mg in fresh weight. The cultures were put into media(N-free or NO_3-riched) 200 m*l*, ground to appear 1 or 2 cell state under low temperature. Number of cells in the suspension were counted with hemacytometer.

2) Treatment:

Three kinds of organic matter were added into cell suspension to the following concentrations;

Glucose·····2% 1% 0.5% 0.1%

Oxalic acid··· 2% 1% 0.5% 0.1%
Ethanol·····1.5% 1% 0.5% 0.1%
Control·····untreated

Among the component of the modified Chu's No. 10 media, a medium contained $CaCO_3$ was used for N-free medium and another medium substituted $Ca(NO_3)_2$ for $CaCO_3$ was used for NO_3-riched medium.

3) Measurement of Nitrogen Fixation:

Amount of nitrogen fixation was measured by the method of manometric technique which Howell *et al.*(1964) attempted. Manometer flask contained; cell suspension, 2.8m*l*; 40% KOH, 0.2 m*l*; and 25% pyrogallic acid, 0.2m*l*; the total volume was 3.2m*l*. Alkaline pyrogaroll in flask was intercepted from light and the temperature of water bath was maintained at 25°C. Manometer flask was illuminated through water by tungsten lamp provided with about 4000 lux/m^2.

RESULTS AND DISCUSSION

Nitrogen fixing algae grow well in the medium of carbohydrate depleted, because they are autotrophic organism. And in case of mass culture under dark conditions, they can grow heterotrophically, if available carbohydrates are supplied as energy sources(Watanabe, 1967).

Watanabe *et al.* found that nitogen fixing algae showed best growth and the greatest nitrogen fixation in the dark when they were supplied glucose in the absence of combined nitrogen. Similarly, there is a report that the more available carbon source soil be contained, the more nitrogen *Azotobacter* can fix. The authors were intended to pursue the effects of organic matter on the nitrogen fixation of *Nostoc pruniforme.*

Table 1 showed N_2-uptakes in the N-free medium treated with glucose. Concentration of glucose was in order of 2 %, 1%, 0.5%, and 0.1%.

Table 1. N_2-uptakes by algal cells in N-depleted media treated with glucose

Time (Min.) / Conc. (%)	5	10	15	20	25	30
2	0.00273	0.00428	0.00507	0.00583	0.00583	0.00662
1	0.01076	0.01076	0.01230	0.01384	0.01384	0.01384
0.5	0.01408	0.02117	0.02683	0.03388	0.03671	0.03954
0.1	0.01608	0.02486	0.02632	0.03363	0.03436	0.03951
Control	0.00878	0.01617	0.01927	0.02297	0.02422	0.02514

$\mu l/10^4$cells

Table 2. N_2-uptakes by algal cells in NO_3-riched media treated with glucose.

Time (Min.) / Conc. (%)	5	10	15	20	25	30
2	0.00805	0.01646	0.02066	0.02811	0.02881	0.02986
1	0.01164	0.02011	0.02513	0.02772	0.02913	0.03054
0.5	0.00453	0.00648	0.00874	0.00971	0.01003	0.01036
0.1	0.01164	0.01795	0.02228	0.02328	0.02395	0.02429
Control	0.01205	0.01846	0.02229	0.02543	0.02704	0.02803

$\mu l/10^4$ cells

The amount of N_2-uptakes were measured by Warburg-manometer at the intervals of 5 minutes and meant cumulative values.

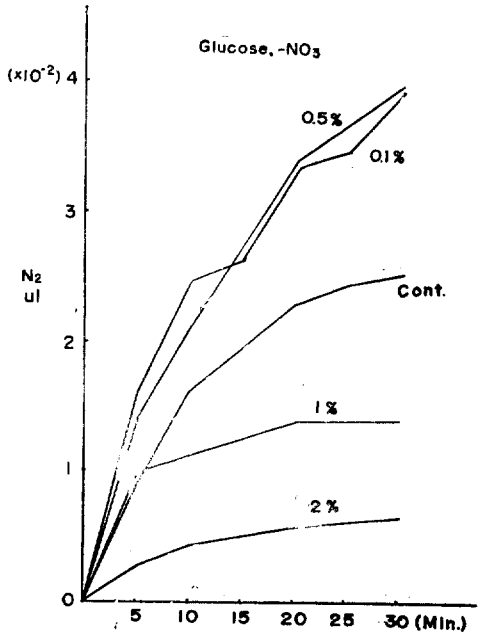

Fig. 1. N_2-uptakes by the algal cells in N-free medium. The medium was treated with glucose. The unit of N_2-uptakes are equivalent to $\mu l/10^4$ algal cells.

Fig. 1 which was based on table 1 showed that the treated of glucose 0.5% and 0.1% fixed N_2 more than control, and that treated of glucose 1% and 2% uptook N_2 less than control. Watanabe (1967) reported that *Anabaenopsis circularis* showed greatest nitrogen fixation in the dark occurred on glucose 0.5%, and that optimal concentration of glucose was 0.5%. Though this experiment was carried out under the illumination of light, the results of Fig. 1 were similar to the mentioned above.

Supplying carbon sources in nitrogen fixation means two points, one is utilized as energy sources, another is a role of substrate which accept the fixed-NH_3 and transformed into amino acid. Since the algae have been starved in phophate buffer, it was believed that the concentration of glucose 0.5% and 0.1% were optimal conditions for nitrogen fixation, especially in early stages.

Table 2 represents the values of nitrogen fixed by algal cells in NO_3-riched medium. Medium was treated with glucose same as N-free medium.

The highest N_2-uptakes were measured at the treated of glucose 2% and 1%, and in case of glucose 0.1% and 0.5%, the N_2-uptakes are lower than in control. These phenomena were opposite to those of Fig. 1.

According to Virtanen *et al.* (1952), perhaps such phenomena were due to the addition of NO_3. For examples, it is reported that *Azotobacter* could fix the

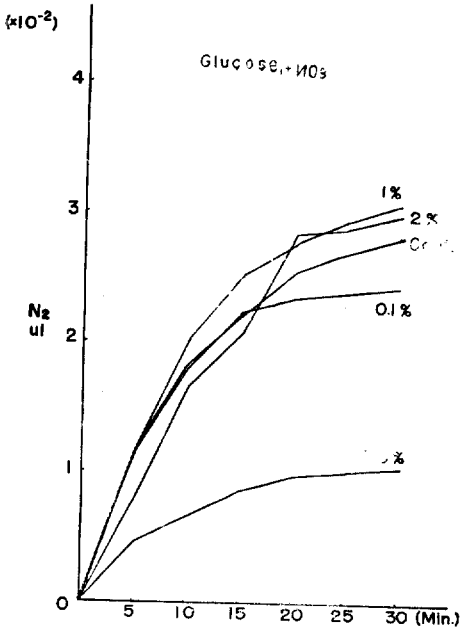

Fig. 2. N_2-uptakes by algal cells in NO_3-riched medium. The medium was treated with glucose same as in N-free medium. The unit of N_2-uptakes are equivalent to $\mu l/10^4$ algal cells.

Table 3. N_2-uptakes by algal cells in N-depleted media treated with oxalic acid.

Time (Min.) / Conc. (%)	5	10	15	20	25	30
2	0.01643	0.02352	0.02560	0.03193	0.03447	0.03489
1	0.00680	0.01661	0.02429	0.02770	0.03197	0.03578
0.5	0.01352	0.02118	0.02552	0.02600	0.02929	0.02929
0.1	0.00721	0.01141	0.01421	0.01742	0.01772	0.01852
Control	0.00878	0.01617	0.01927	0.02297	0.02422	0.02514

$\mu l/10^4$ cells

Table 4. N_2-uptakes by algal cells in NO_3-riched media treated with oxalic acid.

Time (Min.) / Conc. (%)	5	10	15	20	25	30
2	0.00466	0.00505	0.00622	0.00777	0.00971	0.01148
1	0.00450	0.00638	0.00676	0.00939	0.01052	0.01202
0.5	0.00317	0.00554	0.00910	0.01187	0.01345	0.01543
0.1	0.00909	0.01541	0.01897	0.02134	0.02332	0.02530
Control	0.01205	0.01846	0.02229	0.02543	0.02704	0.02803

$\mu l/10^4$ cells

atmospheric nitrogen in nitrate-riched medium within a certain period. This fact may be interpreted that the supplied NO_3 is easily reduced by nitrate reductase. and that reduced nitrate is transformed into amino acid by carbohydrate. Therefore, it is believed that glucose treatment may be utilized as a substrate rather than in energy sources.

Comparing Fig. 1 with Fig. 2, the

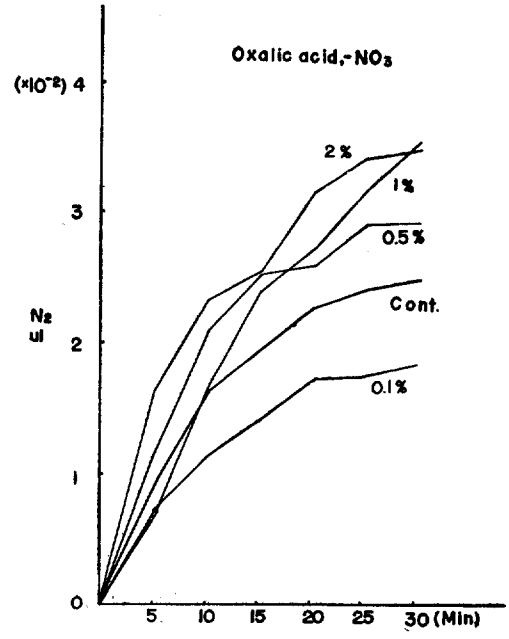

Fig. 3. N_2-uptakes by algal cells in N-free medium treated with oxalic acid. The unit of N_2-uptakes are equivalent to $\mu l/10^4$ cells.

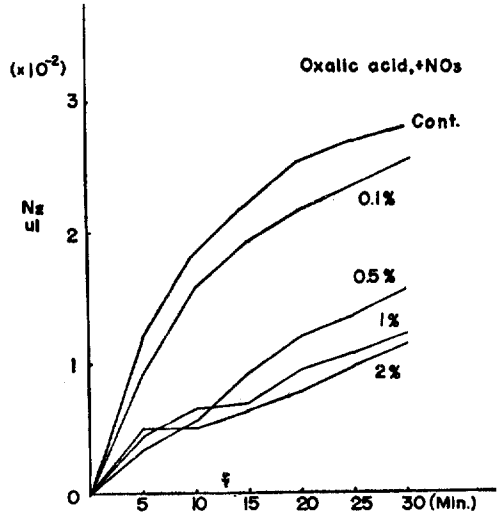

Fig. 4. N_2-uptakes in NO_3-riched medium treated with oxalic acid.

amount of N_2-uptakes in N-free medium was increased more than that of NO_3-riched medium. Table. 3 and Fig. 3 represent N_2-uptakes in the N-free medium treated with oxalic acid. Concentration of oxalic acid are same as in the glucose treatment.

Referring to the Fig. 3 and Fig. 4, results showed that the amount of N_2-uptakes appeared in order of 2%, 1%, 0.5%, and 0.1%, and that fixed N_2 in the N-free medium treated with oxalic acid (2% and 1%) were measured more than those of NO_3-riched medium. These facts could be explained on the basis of that oxalic acid is effective in energy utilization through the process of respiration. It seems the oxalic acid is of more importance in energy source than in substrate. N_2-uptakes in both of the media were opposed to each other, however, the amount of fixed nitrogen in NO_3-riched medium was lower than that of N-free medium.

Table 5 and Fig. 5 represent N_2-uptakes when ethanol-1.5%, 1.0%, 0.5%, and 0.1%-was added into N-free medium.

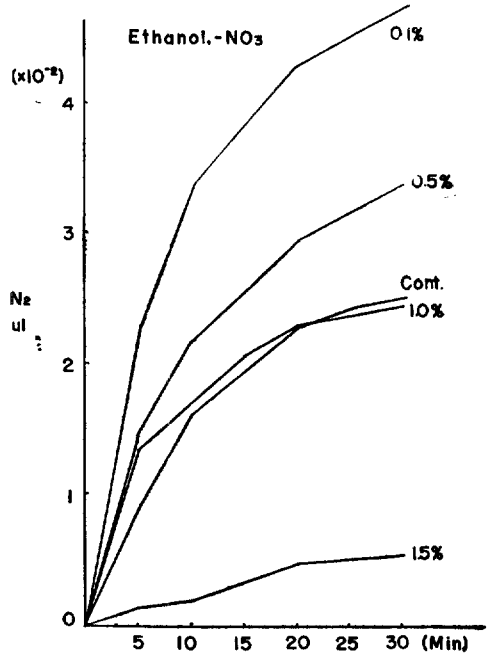

Fig. 5. N_2-uptakes of algal cell in N-free medium. The medium was treated with ethanol. The unit of N_2-uptakes are equivalent to $\mu l/10^4$ algal cells.

Table 5. N_2-uptakes by algal cells in N-depleted media treated with ethanol.

Time (Min.) / Conc. (%)	5	10	15	20	25	30
1.5	0.00148	0.00197	0.00346	0.00497	0.00497	0.00548
1.0	0.01349	0.01649	0.02069	0.02296	0.02395	0.02445
0.5	0.01467	0.02198	0.02518	0.02970	0.03154	0.03382
0.1	0.02265	0.03350	0.04578	0.04625	0.04814	0.04861
Cont.	0.00878	0.01617	0.01927	0.02297	0.02422	0.02514

$\mu l/10^4$cells.

Table. 6 N_2-uptakes by algal cells in NO_3-riched media treated with ethanol.

Time (Min.) / Conc. (%)	5	10	15	20	25	30
1.5	0.01317	0.02894	0.03176	0.03675	0.03842	0.03899
1.0	0.01006	0.02180	0.02892	0.03416	0.03605	0.03680
0.5	0.01544	0.02025	0.02624	0.02672	0.02942	0.03174
0.1	0.01778	0.02508	0.02824	0.03002	0.03061	0.03120
Cont.	0.01205	0.01846	0.02229	0.02543	0.02704	0.02803

$\mu l/10^4$ cells.

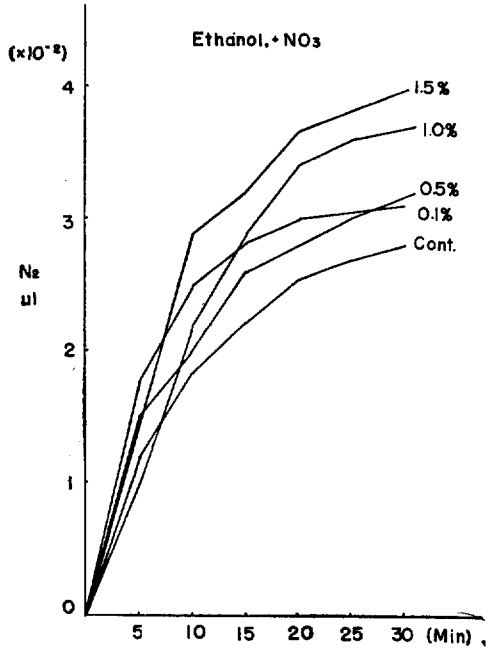

Fig. 6. N_2-uptakes of algal cells in NO_3-riched medium. The medium was treated with ethanol same as in case of N-free medium. The unit of N_2-uptakes are equivalent to $\mu l/10^4$ algal cells.

According to the report of Virtanen *et al.*, *Azotobacter* use alcohol, sugar and organic acid as a carbon source. Since the blue green algae are more similar to bacteria in many respects than the other algae, ethanol was treated as a substrate for the nitrogen fixation.

The amount of N_2-uptakes, as a whole, is more or less better in the treated of ethanol than in that of oxalic acid and similar to that of glucose treatment. Referring to Fig. 5, N_2-uptakes are higher in the treated of low concentrations than in high concentrations. Compared with Fig. 6, the results are opposed to those of NO_3-riched medium. These facts could be appreciated that glucose and ethanol might accelerated the formation of oxime, providing the addition of nitrogen compounds, such as nitrate or nitrite. Oxime is generally found in NOH state as a intermediate and recognized its origination from nitrate rather than from molecular N_2.

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摘 要

질소 고정능이 있는 *Nostoc pruniforme*를 재료로, 3種의 有機物, glucose, oxalic acid, 및 ethanol을 N-free medium과 NO_3-riched medium에 처리해 주었을 때 다음과 같은 효과를 얻었다.

1) N-free medium과 NO_3-riched medium에서 위의 有機物 모두가 질소 고정과정의 energy源으로서 또는 substrate로서의 有効한 결과를 얻었다.

2) Glucose와 ethanol을 처리해 준 실험에서 N-free medium의 경우는 저농도 처리구에 질소고정의 효과가 좋았으며, NO_3-riched medium에서는 그 반대의 경향을 나타냈다.

3) Oxalic acid를 처리한 실험에서는 NO_3-riched medium의 경우 고농도 처리구에서 질소고정량이 많았고, N-free medium은 그 반대의 결과였다.

4) 비교구인 control의 질소 고정량은 一例(oxalic acid+NO_3)를 제외하고는 전반적으로 고농도 처리구와 저농도 처리구의 중간에 위치했다.

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