KOR. JOUR. MICROBIOL. September, 1991 p. 263-266 Vol. 29, No. 4

Growth and Physiology of *Thiobacillus novellus* under Autotrophic and Heterotrophic Conditions

Park, In Kook
Department of Agrobiology, Dongguk University, Seoul, Korea

자가영양과 타가영양 조건하에서 *Thiobacillus novellus*의 생리 및 성장

박 인 국
[1]동국대학교 농생물학과

ABSTRACT: The growth of *T. novellus* in autotrophic and heterotrophic media was studied to determine the time required for cells to enter stationary phase and relative percentage of ribosomal proteins. When *T. novellus* was grown autotrophically, growth proceeded at a slow rate characteristic of autotrophs and did not enter log phase until the end of the first day. Logarithmic growth proceeded for 3-4 days at which time the cells entered the stationary phase. In particular, logarithmic growth was accompanied by decreasing pH of culture media and in the stationary phase the pH levelled off at 6.0, a decrease of 1.6 pH value compared to original pH of media. The pH decrease was greatest during log phase when cells oxidized thiosulfate to H_2SO_4. The doubling time was about 26 h. In heterotrophic media growth proceeded at a much faster rate and cells entered stationary phase 20-22 h after inoculation. The doubling time was 3 h. The protein content of the ribosomes in *T. novellus* grown heterotrophically was 4.2% greater than those from the organism grown autotrophically.

KEY WORDS □ ***Thiobacillus novellus*, autotrophy, heterotrophy.**

INTRODUCTION

Facultatively autotrophic thiobacillus, *Thiobacillus novellus*, is capable of growing either as chemolithotrophic autotrophs or as heterotrophs, but their ecological importance and capacities for mixotrophic growth have been recognized recently (Charles and Suzuki, 1966; Perez and Matin, 1982). The organisms is a small and non-motile rod, about 0.5 to 1 μ wide and 1 to 4μ long. They grow best at a pH between 8 and 9 and somewhat resemble the strict autotroph *T. thioparaus* (Matin *et al.*, 1980a).

T. novellus differs from other facultative organisms in this genus in several respects. For instance, *T. intermedius* can grow heterotrophically only in the presence of thiosulfate, indicating that this species still has a requirement for an inorganic energy source although it is able to use an organic carbon source (London and Rittenberg, 1966). Also, *T. novellus* appears to be unique in not producing tetrathionate ($S_4O_6=$) as an end product. This is a characteristic feature of other thiobacilli growing on thiosulfate.

The process of reversible adaptation to autotrophic and heterotrophic modes of growth has been little studied, although the process may be fundamental to understanding evolution and cell differentiation. To adapt *T. novellus* to heterotrophy or autotrophy, the organism was subjected to repeated transfers into mineral salts with increasing or decreasing concentrations of glutamate and decreasing or increasing concentrations of thiosulfate. This paper provides an illustration of how different physiological behavior can be under two different nutritional conditions.

MATERIALS AND METHODS

Organism and growth conditions

The ATCC type strain of *T. novellus* (no. 8093) was used in this investigation. The cells were grown in 500 ml Erlenmeyer flasks on a rotary

shaker. The autotrophic and heterotropic media employed were obtained by supplementing the basal medium (Matin and Rittenberg, 1971) with 1% $Na_2S_2O_3$ or 1% glutamate, respectively. The composition of the basal medium per liter is as follows: 4 g, K_2HPO_4; 1.5 g, KH_2PO_4; 0.02 g, $CaCl_2 \cdot 2H_2O$; 0.1 g, $MaSO_4 \cdot 7H_2O$; 0.3 g, $(NH_4)_2SO_4$; 0.02 g, $MnSO_4 \cdot 2H_2O$ and 0.02 g, $FeCl_3 \cdot 6H_2O$. A small amount of 0.2% phenol red was added to the medium as a pH indicator. The pH was adjusted to 7.8 before sterilization and maintained at this pH during cell growth by the addition of 10% sterilized Na_2CO_3. Afte the culture reached the stationary phase and was harvested by centrifuging then cells stored at −70° until used.

To adapt cells to autotrophic growth conditions the method basically consisted of culturing cells by repeated transfers to medium with progressively decreasing glutamate concentrations (1 to 10%) and increasing $Na_2S_2O_3$ concentrations (0 to 1%). Cells were allowed to grow to late logarithmic phase in each medium then 0.1 volume was inoculated into the succeeding medium.

Growth was followed by measuring the optical density of cell suspensions at 540 nm in a Klett-Summerson colorimeter. At the end of growth in all cases, the culture purity was checked by microscopic examination and streaking on plates containing autotrophic and heterotrophic media.

Determination of protein

Protein concentration was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Preparation of ribosome

Ribosomes were prepared from 20 g frozen cells by a slight modification of the method of Ramagopal (1989). Cells were disrupted by 1 passage through an Aminco French pressure cells in TKM buffer (0.1 M Tris(pH 7.8)-HCl, 0.05 M KCl, 0.01 M magnesium acetate) containing 6 mM β-mercaptoethanol, 0.1 m/ DNase(1 mg/m/) and 2% macaloid. The cell debris was removed by centrifugation at 15,000 rpm form 30 min in a Sorval RC2-B. The supernatant was subjected to two successive $(NH_4)_2SO_4$ fractionations and the final pellet was dissolved in TM buffer containing 0.6 M $(NH_4)_2SO_4$. The ribosomes were washed twice in this buffer by pelleting at 87,500×g for 3½ h, resuspended in fresh buffer and then stored at −70℃ until analysed.

RESULTS AND DISCUSSION

Growth of organisms

T. novellus is quite unique in the genus *Thiobacillus* in its ability to grow as a facultative autotroph. The growth of *T. novellus* in autotrophic and heterotrophic media was investigated to determine the time required for cells to enter the stationary phase. Figure 1 shows the results obtained when *T. novellus* was grown autotrophically. Growth proceeded at a slow rate characteristic of autotrophs and did not enter logarithmic growth phase until the end of the first day. Logarithmic growth proceeded for 3 to 4 days at which time the culture entered the stationary phase.

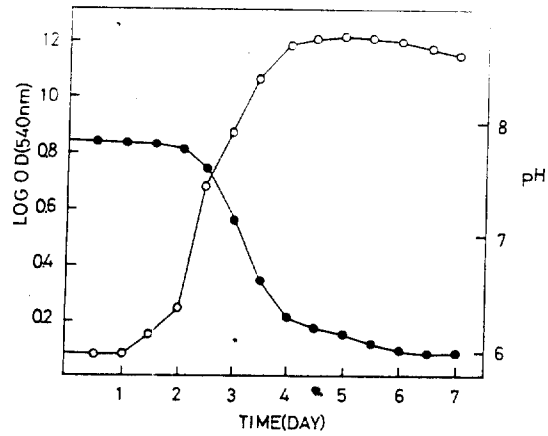

Fig. 1. *Growth of T. novellus in autotrophic medium and concomitant change of pH in cell culture.* Cells were grown in the basal medium with 1% $Na_2S_2O_3$(w/v) at 28°, aeration being provided by shaking. Details of growth conditions are given in methods. ○—○, optical density; ●—●, pH.

As demonstrated in Figure 1, logarithmic growth was accompanied by decreasing pH of the culture medium and in the stationary phase the pH levelled off at 6.0, a decrease of 1.6 pH units compared to the original pH of the medium. As would be expected, the rate of decrease in pH is greatest during logarithmic growth when cells actively oxidize thiosulfate to H_2SO_4(London and Rittenberg, 1967; Oh and Suzuki, 1977). During this phase the doubling time was calculated to be approximately 26 h.

The growth in the heterotrophic medium, *i.e.*, with sodium glutamate as carbon and energy source, proceeded at a much faster rate(Figure 2) and the culture entered into the stationary phase 20-22 h after inoculation. The doubling time was 3 h during the logarithmic phase. The growth in the heterotrophic medium was much faster than that in the autotrophic medium. Judging by the optical density at the stationary phase in autotrophic and heterotrophic media, the cell density was approximately 3 fold greater in the heterotrophic medium. The increased growth and growth rates indicated that glutamate was utilized more readily and efficiently than were CO_2 and $Na_2S_2O_3$ as carbon and energy sources, respecti-

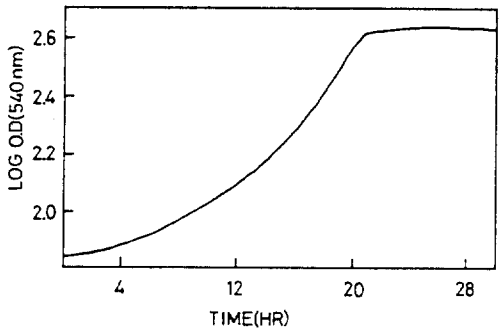

Fig. 2. *Growth of T. novellus in heterotrophic medium.* Cells were grown in the basal medium with 1% glutamate (w/w) at 28° , aeration being provided by shaking.

vely, under autotrophic conditions. Similar growth pattern was also observed when glucose replaced glutamate as an organic source(Matin, 1978; Matin *et al.*, 1980b).

Adaptive growth

The time taken for autotrophically-grown *T. novellus* to adapt to heterotrophic medium and *vice versa* was studied. The adaptation to heterotrophic medium took place readily, requiring little time for cells to adapt. The growth response of organisms to glutamate as an organic carbon source is illustrated in Figure 3. In this experiment, cells were in the basal medium with 1% $Na_2S_2O_3$ until cells entered the stationary phase, at which time the sterile 10% glutamate was added to the culture medium to a final concentration of 1%. As demonstrated in Figure 3, growth resumed immediately and continued for another 20 h. There is only a short lag before cells begin to adapt and grow. It is therefore likely that the organism has or can readily elaborate components necessary for heterotrophic growth (Lejohn *et al.*, 1967).

On the other hand, *T. novellus* cells that had been grown under heterotrophic conditions with glutamate did not adapt readily to growth in autotrophic medium. In order to adapt cells to autotrophic growth conditions, cells were cultured by repeated transfers to medium with progressively decreasing glutamate concentrations and increasing $Na_2S_2O_3$ concentrations. It took 4 to 5 weeks for cells to adapt to a completly autotrophic environment.

The results showed a strong indication that whereas adaptation to heterotrophy proceeds readily, that to autotrophy was a slow process, implying that time may be required to acquire functions for CO_2 fixation and $Na_2S_2O_3$ oxidation. The fact that profound changes take place in the cell during adaptation was demonstrated by the differences between organisms grown under the

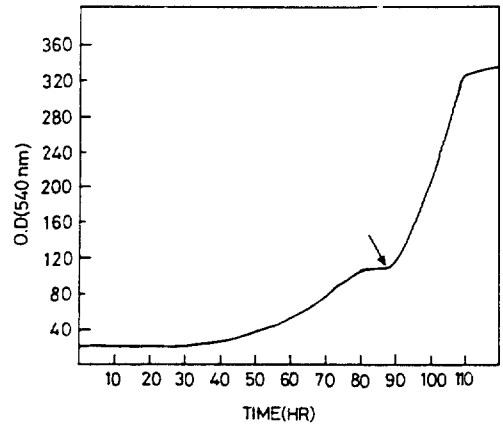

Fig. 3. *Adaptation of T. novellus from autotrophic to heterotrophic growth.* Cells were grown at 28° in the basal medium with 1% $Na_2S_2O_3$ After reaching the stationary phase, the medium of culture was made to 1% glutamate (arrow).

Table 1. *Yield and protein content of ribosomes in T. novellus.*

Growth condition	mg ribosome/g cell	% protein/mg ribosome
Autotrophy	1.6±0.05	42.2±0.5
Heterotrophy	7.3±0.2	46.4±1.0

Values are expressed as mean±S.D.

2 conditions. For instance, cells grown heterotrophically could be suspended to a smooth homogeneous suspension in buffers, whereas autotrophically grown cells formed aggregates and clumps, implying changes in cell surface structures. Possibly these cellular alterations may allow the cell to tolerate acidic conditions during growth on $Na_2S_2O_3$. Similar morphological and physiological alterations were also observed during the transition from $Na_2S_2O_3$-limited heterotrophic growth (Smith *et al.*, 1980; Leefeldt and Matin, 1980). In autotrophic growth, ribulosebisphophate carboxylase, pyruvate carboxylase and fructose-bisphosphatase activities declined along with the reduction of total cell numbers and biomass. Furthermore, Martin *et al.* (1980a) reported that the activity of key pentose shunt and citric acid cycle enzymes was reduced in cells grown autotrophically which can subsequently lead to the repression of ATP and NADH levels. In interpreting these results, however, it can be assumed that $Na_2S_2O_3$ and glucose or glutamate influence the synthesis or regulation of enzymes of each other's metabolism

from the physiologicl point of view although the molecular basis of the nature of this interaction is still unclear.

Protein content of ribosomes

The yield and protein content of ribosomes purified autotrophically- and heterotrophically-grown *T. novellus* were presented in Table 1. It was predetermined that the presence of RNA did not interfere with the colorimetric assay for protein. The results showed a 4.6 fold difference in ribosome content between cells grown autotrophically and heterotrophically. This difference may account for the slow growth of *T. novellus* in $Na_2S_2O_3$.

An analysis of ribosomes from *T. novellus* grown as autotrophs and heterotrophs showed that the protein contents were 42.2 and 46.4%, respectively. This agrees fairly well with 40% reported for Halobacterium (Bayley and Kushner, 1964). The difference of 4.2% between ribosomes of autotrophically- and heterotrophiclly-grown cells strongly suggested that not only does ribosome content of two types of cell differ, but also the ribosome structure itself (Amemiya and Umbreit, 1974). Overall it can be postulated that adaptive processes may require more than simple regulation of enzyme synthesis and involve additional changes in protein synthesizing components.

적 요

*T. novellus*를 자가영양과 타가영양 조건하 배양하였을때 성장속도와 r-protein의 함량을 조사 하였다. 자가영양 조건하 유도기에 이르는데 약 1일이 소요 되었으며 3-4일간의 유도성장 후 정지기에 진입하였다. 특히 유도성장 중 pH가 급속히 감소하고 정지기에 도달하였을 때는 원래 pH 보다 1.6 더 감소한 pH 6을 보였다. 그리고 doubling time은 약 26시간 이었다. 타가영양 조건하 배양하였을 때 *T. novellus*는 빠른 속도로 성장하였고 정지기에 이르는데 약 20-22 시간이 걸렸다. 그리고 배가 시간은 3시간 이었으며 ribosome 함량과 r-protein 함량 모두 자가영양 세포 보다 높게 나타났다.

ACKNOWLEDGEMENT

This work was supported by 1990-92 year grant from Korean Science and Engineering Foundation.

REFERENCES

1. **Amemiya, K. and W.W. Umbreit,** 1974. Heterotrophic nature of the cell-free protein-synthesizing system from the strict chemolithotroph, Thiobacillus thiooxidans. *J. Bacteriol.*, **117**, 834-839.
2. **Bayley, S.T. and D.J. Kushner,** 1964. The ribosomes of the extremely halophilic bacterium, *Halobacterium cutirbruum*. *J. Mol. Biol.*, **9**, 654-659.
3. **Charles, A.M. and I. Suzuki,** 1966. Mechanism of thiosulfate oxidation by *T. novellus*. *Biochim. Biophys. Acta.*, **125**, 510-519.
4. **Leefeldt, R.H. and A. Matin,** 1980. Growth and physiology of *Thiobacillus novellus* under nutrient-limited mixotrophic condition. *J. bacteriol.*, **142**, 645-650.
5. **Lejohn, H.B. L. Van Caeseele and H. Lees,** 1967. Catabolite repression in the facultative chemoautotroph *Thiobacillus*. *J. Bacteriol.*, **94**, 14 84-1491.
6. **London, J. and S.C. Rittenberg,** 1969. Thiobacillus perometabolis nov. sp., a non-autotrohic Thiobacillus. *Arch. Mikrobiol.*, **59**, 218-225.
7. **Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall,** 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265-275.
8. **Matin, A.,** 1978. Organic nutrition of chemolithotrophic bacteria. *Ann. Rev. Microbiol.*, **32**, 433-468.
9. **Matin, A., F.J. Kahan and R.H. Leefeldt,** 1980a. Growth factor requirement of *Thiobacillus novellus*. *Arch. Microbiol.*, **124**, 91-95.
10. **Matin, A. and S.C. Rittenberg,** 1971. Enzymes of carbohydrate metabolism in Thiobacillus species. *J. Bacteriol.*, **107**, 170-186.
11. **Matin, A., M. Schleiss and R.C. Perez,** 1980b. Regulation of glucose transport and metabolism in *Thiobacillus novellus*. *J. Bacteriol.*, **142**, 639-644.
12. **Oh, J.K. and I. Suzuki,** 1977. Isolation and characterization of a membrane-associated thiosulfate-oxidizing system of *T. novellus*. *J. Gen. Microbiol.*, **99**, 397-412.
13. **Perez, R.C. and A. Matin,** 1982. Carbon dioxide assimilation by *Thiobacillus novellus* under nutrient-limited mixotrophic condition. *J. Bacteriol.*, **150**, 46-51.
14. **Ramagopal, S.,** 1989. Acidic γ-protein of *Dictyostelium disoideum* that show electrophoretic similarity to *Escherichia coli* protein. *Biochem. Cell Biol.*, **67**, 712-718.
15. **Smith, A.L., D.P. Kelly and A.P. Wood,** 1980. Metabolism of Thiobacillus A2 grown under autotrophic, mixotrophic and heterotrophic conditions in chemostat culture. *J. Gen. Microbiol.*, **121**, 127-138.

(Received August 15, 1991)
(Accepted September 5, 1991)