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Thiobacillus concretivorus Parker 의 呼吸에 미치는 有機物의 影響

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Effects of Organic Compounds on the Respiration of *Thiobacillus concretivorus* Parker.

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ABSTRACT

Effects of 13 organic compounds including glucose, fructose, xylose, glutamate, succinate, malate, glycine, lactate, acetate, pyruvate, citrate, formate and cis-aconitate on the oxidation of thiosulfate and the availability of these compounds as the substrate for the respiration by *Thiobacillus concretivorus*, which is known to be an obligated autotroph, were studied. Malate and glycine at 0.5 per cent concentration nearly doubled the thiosulfate oxidation compared to the control. No other organic substances enhanced the thiosulfate oxidation. Moreover, some 30 to 40 per cent inhibition of thiosulfate oxidation could be observed by glutamate, succinate, lactate and acetate. Pyruvate and citrate inhibited the thiosulfate oxidation by nearly 60 to 70 per cent, and formate and cis-aconitate resulted in the complete inhibition sooner or later. On the other hand, glucose was proved to be of no influence on thiosulfate oxidation. Fructose and xylose injured the activities a little (10 to 20 per cent).

Respiration rates were much lower in the thiosufate-free organic compounds-salts medium, indicating this organism prefers thiosulfate to any other organic compounds tested as the substrate for respiration. Compared to the result obtained from thiosulfate-salts medium, some 30 to 40 per cent decrease was recorded by fructose, citrate, xylose, malate, glucose, glutamate and succinate. No respiration could occur when formate and pyruvate were supplied as the substrate for respiration. But it was obvious that glucose, fructose, xylose, glutamate, malate, citrate and succinate could be used as the substrate for respiration to some extent, regarding the fact that some increase in respiration rates could be recorded compared to the result from the salts medium, where neither thiosulfate nor organic compounds were added.

Thus, it was postulated that this organism could possibly be converted into mixotroph or heterotroph if appropriate conditions could be prepared.

INTRODUCTION

Thiobacillus concretivorus is known to be an obligatory autotrophic bacterium which can utilize thiosulfate, sulfur or H_2S as the sole source of energy, and CO_2 as the sole source of carbon. When Winogradsky first de-

monstrated rigorously the existence of obligate autotrophs through his studies on the nitrifying bacteria (1890), he emphasized that organic compounds are not merely untilizable by these organisms, but are inhibitory at relatively low concentration (Winogradsky and Omeliansky, 1889). Silver *et al.* (1967) demonstrated that 10 per cent glucose inhibited the oxidation of sulfur and iron by *Ferrobacillus ferrooxidans*. By LeJohn *et al.* (1967), several fermentable carbon sources gave rise to catabolic repression of all enzymes implicated in thiosulfate oxidation in the facultative chemoautotroph, *T. novellus*. By Borichewski (1967), a low concentration of keto acids (10^{-4}M), especially pyruvic acid, is a growth-limiting factor in autotrophic growth of *T. thiooxidans*. Similar observations have been made on *Nitrobacter agilis* and some other autotrophic *Thiobacillus* species, but they demonstrated that addition of pyruvic acid inhibited only the heterotrophic growth of *T. denitrificans* (Pan and Umbreit, 1972).

On the other hand, a number of workers have shown that obligatory autotrophs are not, in general, unusually susceptible to growth inhibition by organic substances. As early as 1902, Nathanson observed that *Thiobacillus thioparus* can grow in the presence of some simple organic compounds at concentrations as high as 0.5 per cent (w/v). Later work on *T. thiooxidans* indicated that up to 1 per cent concentration of dextrose did not injure the activities of this organism (Waksman and Starkey, 1923).

Recently, the use of tracer method has shown that some organic compounds can be absorbed by obligate autotrophs and incorporated into cell materials. The assimilation of one or more organic compounds has been demonstrated for thiobacilli (Butler and Umbreit, 1966; Vishniac and Santer, 1957; Smith, London, and Stanier, 1967), *Nitrobacter* (Smith and Hoare, 1968; Ida and Alexander, 1965) and blue-green algae (Smith, London, and Stanier, 1967).

Though a variety of researches has been concentrated on the autotrophic metabolism of most species of thiobacilli, there still is little information concerning the nutritional nature of *T. concretivorus*. The experiments reported here, so, were undertaken to determine the effect of various organic compounds on the oxidation of thiosulfate by *T. concretivorus* and the availability of these compounds as the substrate for respiration by this organism.

MATERIALS AND METHODS

Cells of *T. concretivorus* isolated and identified in this laboratory were grown for 7 days at 30°C in a 500 ml flask on a reciprocal shaker (112 rev/min). The medium used contained: 2 g of $(NH_4)_2SO_4$, 0.3 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of $CaCl_2 \cdot 2H_2O$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 4 g of KH_2PO_4 and 10 g of $Na_2S_2O_3 \cdot 5H_2O$ in 1,000 ml of distilled water, with the final *p*H of 4.6. Thiosulfate was sterilized separately with Seitz filter and added aseptically after the medium was autoclaved for 15 min at 121°C. Cells were harvested by centrifugation at 10,000 × G for 10 min and were washed twice with 0.0016 M H_2SO_4 (*p*H 2.4).

These cells were subsequently ino-

culated in the same medium and incubated for 60 hr to get physiologically active cells (late lag phase) and harvested by centrifugation, which again were washed twice and suspended in the medium above ($0.5-1.5 \times 10^8$cells/ml). This suspension was used for the determination of the effects of organic compounds on the oxidation of thiosulfate. For the determination of the availability of organic compounds, exactly the same procedure was adopted except for the omission of thiosulfate in preparing the cell suspension after 60 hr of incubation.

Experiments were carried out by standard manometric method. The main compartment of each Warburg vessel contained 1.8 ml of cell suspension. In the center well was placed 0.2ml of 20 per cent KOH, 0.2 ml of organic compound was placed in the side arm which was tipped into after 2 to 3 hr, yielding the final concentration of 0.5 per cent. Control was prepared by replacing the organic compound with sterilized H_2O, for the determination of the effects of organic compounds on the oxidation of thiosulfate. For the determination of the availability of organic compounds, control was prepared by placing thiosulfate in the side arm. To measure the endogeneous respiration, neither thiosulfate nor organic compounds was added. Instead, H_2O was added.

All the organic compounds were sterilized separately. Initial *p*H of the cell suspension and organic substances was adjusted to 4.8. The gas phase was air, 13 kinds of organic compounds were used as follows; glucose, fructose. glutamate, xylose, acetate, cis-aconitate, lactate, succinate, malate, pyruvate, citrate, formate, and glycine. All the organic acids used were of sodium salts.

Consumption of O_2 was measured at 20 min interval for 8 to 10 hr. Uptake of O_2 during 6.5 hr and 5 hr after tipping was expressed as μl $O_2/10^8$ cells/*ml* for the determination of the effects of organic compounds on the oxidation of thiosulfate and the availability of organic compounds, respectively.

RESULTS AND DISCUSSION

Results of the determination of the effects of organic compounds on thiosulfate oxidation are shown in Figs. 1, 2, 3 and Table 1. As seen in Fig. 1 and Table 1, control (no organic matter added) showed 73.5μl of O_2 uptake per 10^8 cells/*ml* during 6.5hr when fructose and xylose were added, the O_2 uptake-decreased to 89.8 and 81.6 per cent of control, respectively, where no marked inhibition was noted. No inhibition of thiosulfate oxidation was observed by the addition of glucose (Fig. 2, Table 1).

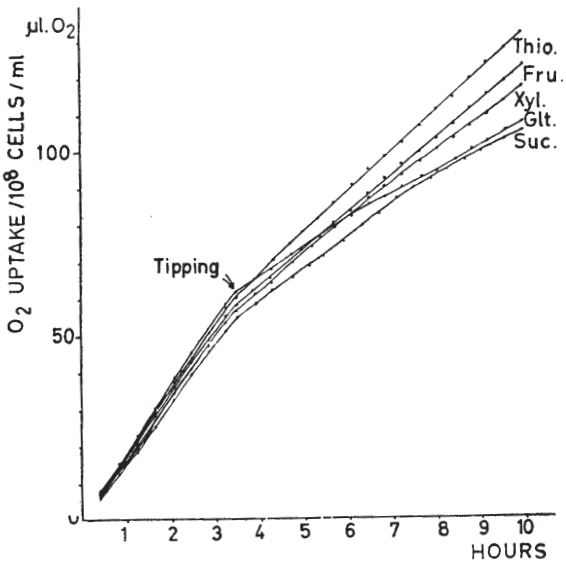

Fig 1. Effects of organic compounds on the oxidation of thiosulfate by *T.concretivorus*. (Thio=thiosulfate; Fru=fructose; Xyl=Xylose; Glt=glutamate; Suc=succinate)

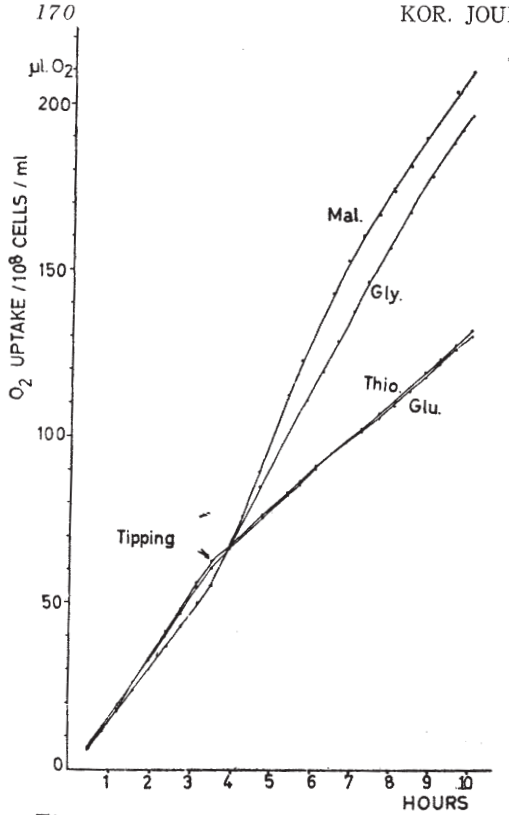

Fig 2. Effects of organic compounds on the oxidation of thiosulfate by *T. concretivorus*. (Mal=malate; Gly=glycine; Thio=thiosulfate; Glu=glucose)

This well coincides with the observations for other autotrophic thiobacilli (Waksman *et al.*, 1923; Silver *et al.*, 1967).

Malate and glycine nearly doubled the O_2 uptake of this organism (Fig. 2, Table 1). Borichewski (1967) reported that keto acids at low concentration (10^{-4}M) inhibited the growth of *T. thiooxidans*. In present study, result is quite different from his observation. On the other hand, Pan and Umbreit (1972) indicated that, though pyruvate inhibited the growth of *Nitrobacter agilis* at 5×10^{-5} M concentration, 10^{-3} M pyruvate did not inhibit the growth of *T. thioparus*, *T. denitrificans*, and *T. neapolitanus* on thiosulfate. But on glucose, addition of 10^{-4} M pyruvate to the culture of *T. denitrificans* growing in dialysis culture immediately stopped the growth, whereas the same addition to the dialysis culture on thiosulfate

Table 1. Effects of organic compounds on the oxidation of thiosulfate by *T. concretivorus*.

Material	O_2 uptake at tipping*	O_2 uptake 6.5 hr after tipping*	Net O_2 uptake after tipping*	Per cent of control
Thiosulfate	60	133.5	73.5	100
Fructose	58	124	66	89.8
Xylose	58	118	60	81.6
Glutamate	62.5	109.5	47	73.9
Succinate	56	106.5	50.5	68.7
Glucose	60	131.5	71.5	97.3
Malate	56	213	157	213.6
Glycine	56	199	143	194.6
Lactate	27	73.5	46.5	63.3
Acetate	30	71.5	41.5	57.8
Pyruvate	60	92	32	43.5
Citrate	56	82	26	35.4
Formate	62	85	23**	—
Cis-aconitate	57.5	57.5	0	0

* : $\mu lO_2/10^8$ cells/*ml*

** : Complete inhibition was recorded 4.5 hours after tipping.

had no significant effect. Though they observed that no significant inhibition on thiosulfate oxidation was occured by pyruvate, there still is no report that keto acids accelerated the oxidation of thiosulfate. Thus, it is very interesting that malate at such a high concentration as 0.5 per cent did really double the O_2 uptake. Further study should be necessary on this problem.

Glutamate, succinate, lactate and acetate inhibited the thiosulfate oxidation by some 30 to 40 per cent (Figs. 1, 3 and Table 1). Though pyruvate inhibited by nearly 60 per cent, this still did not coincide with the results of Borichewski who observed the complete inhibition of sulfur oxidation by *T. thiooxidans* by 2×10^{-5} M pyruvate (1967).

Formate completely inhibited the thiosulfate oxidation 4.5 hr after addition, and no thiosulfate oxidation could take place when cis-aconitate was added

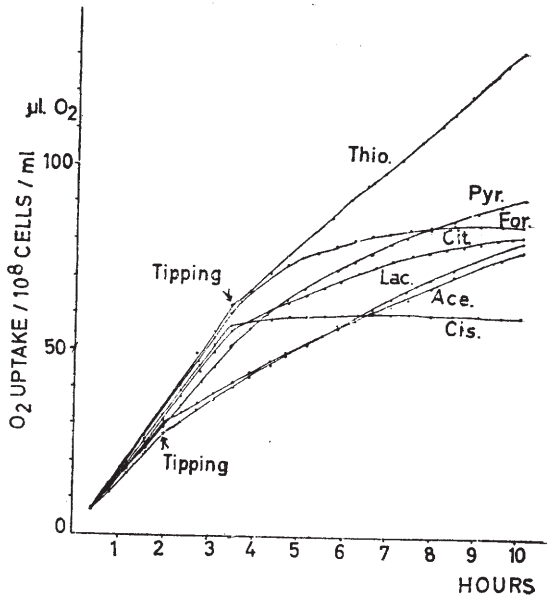

Fig. 3. Effects of organic compounds on the oxidation of thiosulfate by *T. concretivorus*. Thio=thiosulfate; Pyr=pyruvate; For=formate; Cit=citrate; Lac=lactate; Ace=acetate; Cis=cis-aconitate.

Table 2. Effects of organic compounds on the respiration of *T. concretivorus* in the absence of thiosulfate.

Material	O_2 uptake at tipping*	O_2 uptake 5 hr after tipping*	Net O_2 uptake after tipping*	Per cent of control
Thiosulfate	45	128	83	100
Fructose	51	109	58	69.9
Citrate	47	102	55	66.3
Xylose	47	100	53	63.9
Malate	49	98	49	59
Glucose	51	97	46	55.4
Glutamate	46	94	48	57.8
H_2O	48	92	4	53
Succinate	40	90	50	60.2
Cis-aconitate	48	78	30	36.1
Glycine	47	79	32	38.6
Acetate	50	70	20	24
Lactate	32	60	28	33.7
Formate	45	45	0	0
Pyruvate	32	32	0	0

*: μl $O_2/10^8$ cells/ml.

(Figs. 1, 3 and Table 1). By Smith and Hoare(1968), 1 to 10 mM concentration of acetate did not affect the rate of nitrite oxidation by *N. agilis*.Moreover, they reported that *N. agilis* could assimilate acetate even in the absence of nitrite. In present study, on the contrary, a marked decrease in thiosulfate oxidation was resulted by the addition of acetate (42.2 per cent). Result was the the same when the respiration was measured in the absence of thiosulfate (Fig.5, Table 2).

The data on the determination of the availability of organic compounds as the substrate for respiration are seen in Figs. 4, 5 and Table 2, 3. 83 μl O_2 was consumed in control during 5 hr after tipping by 10^8 cells/ml. In general, respiration rates were much lower when fructose, citrate, xylose, malate, glucose, glutamate and succinate were added, compared to the values obtained

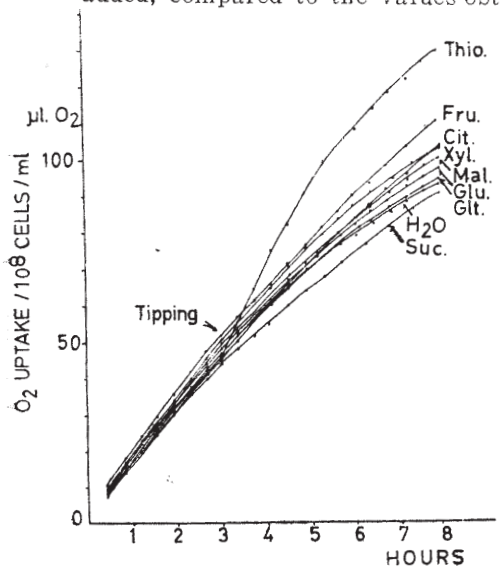

Fig. 4. Availability of organic compounds as the substrate for the respiration of *T. concretivorus*. Thio=thiosulfate; Fru=fructose; Cit=citrate; Xyl=xylose; Mal=malate; Glu=glucose; Glt=glutamate; Suc=succinate

by the addition of thiosulfate. Some 30 to 40 per cent decrease was recorded (Fig. 4, Table 2). Among them, O_2 uptake was highest when fructose was supplied as substrate. Unexpectedly, citrate showed relatively high availability regarding the fact that only 35 per cent of thiosulfate oxidation could occur when it was added to the thiosulfate-salts medium.

One of the results most difficult to explain was the effect of malate on the oxidation of thiosulfate and its availability as the substrate for respiration. When it was added to thiosulfate-salts medium, it nearly doubled the oxidation rate as stated above. But only 60 per cent of respiration could take place when no thiosulfate was present. The reason was not studied yet, remaining to be investigated in the future.

When only H_2O was added instead of other organic substances, the O_2 uptake was recorded to be 53 per cent of the control. With this result, it was supposed that, though thiosulfate proved to be the best substrate for respiration, other organic compounds such as fructose, citrate, malate, glucose, glutamate, xylose and succinate, to some extent, also were able to be utilized for respiration. This relation is well seen in Table 3.

Cis-aconitate, glycine, acetate and lactate all inhibited the respiration markedly. Glycine, which conspicuously enhanced thiosulfate oxidation, showed extremely low respiration rate in the absence of thiosulfate, as was the same in the case of malate. The reason was not followed in detail. Formate and pyruvate completely inhibited the

Table 3. Availability of organic compounds as the substrate for respiration of *T. concretivorus*.

Substrate	O_2 uptake during 5 hr*	Per cent of control
H_2O	44	100
Glucose	46	104.5
Fructose	58	131.8
Glutamate	48	109.1
Malate	49	111.4
Citrate	55	125
Succinate	50	113.5
Thiosulfate	83	180.5

*: μl $O_2/10^8$ cells/ml.

Six other organic compounds were not included in this table since they showed much lower respiration rates compared to H_2O.

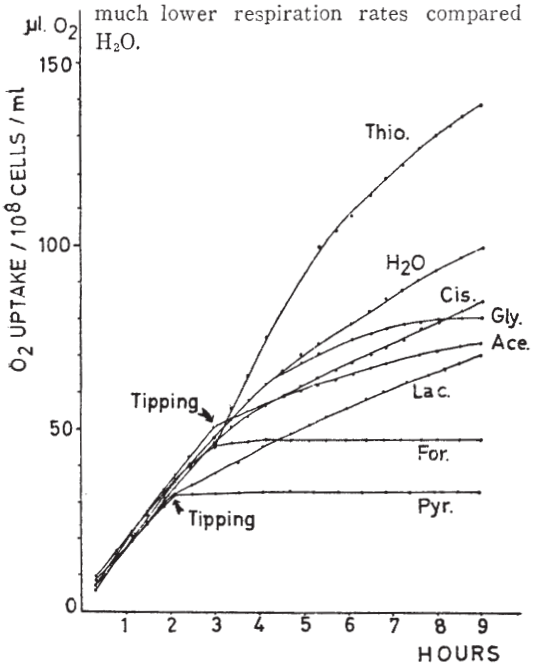

Fig. 5. Availability of organic compounds as the substrate for the respiration of *T. concretivorus*. Thio=thiosulfate; Cis=cis-aconitate; Gly=glycine; Ace=acetate; Lac=lactate; For=formate; Pyr=pyruvate.

respiration on addition. The inhibitory effect of pyruvate in the absence of thiosulfate is well compared with previous report(Pan and Umbreit, 1972).

As a whole, the inhibitory effects of keto acids(Eorichewski, 1967; Pan and Umbreit, 1972) could not be rerecognized clearly in this study. Though only malate and glycine enhanced the oxidation of thiosulfate and no other substrates accelerated thiosulfate oxidation or were used preferably to thiosulfate, glucose and some others were proved to give rise to little or no injuries on the activities of *T. concretivorus*. Moreover, 6 of 13 organic compounds really increased the respiration more or less, indicating that they could be used as the substrate for respiration, although no growth could be confirmed when cultures were undertaken using the organic compounds-salts media in the absence of thiosulfate. But the growth-accelerating effect could clearly be recognized by the addition of some organic compounds to thiosulfate-salts medium at 0.5 per cent concentration. Effects of these organic compounds on the growth of *T. concretivorus* will be included elsewhere.

Regarding all the data above, it is believed that *T.concretivorus*, though known to be an obligate autotroph, could possibly be converted into mixotroph or heterotroph, provided appropriate conditions were to be prepared.

摘　　要

Glucose, fructose, xylose, glutamate, succinate, malate, glycine, lactate, acetate, pyruvate, citrate, formate, 및 cis-aconitate等의 13種의 有機物이 obligate autotroph인 *Thiobacillus concretivorus*의 thiosulfate 酸化에 미치는 影響 및 이 菌에 依한 呼吸基質로서의 適合性 與否에 對하여 考察

하였다.

0.5%의 *malate*와 *glycine*을 添加하면 thiosulfate의 酸化量은 對照區의 約 二倍에 達하도록 增加하였다. 다른 有機物에 依한 促進效果는 나타나지 않았다. 한편 glutamate, succinate, lactate 및 acetate를 加하면 30~40%의 減少가 일어났고 pyruvate와 citrate는 約 60~70%의 抑制效果를 보였으며, formate와 cis-aconitate를 加하면 時間的 差異는 있으나 完全한 沮害를 일으킴을 알 수 있었다. 反面 glucose는 이 菌의 thiosulfate 酸化에 아무런 影響을 미치지 않았고, fructose와 xylose는 10~20%의 輕微한 沮害를 일으켰다.

Thiosulfate를 加하지 않고 無機鹽과 有機物만을 營養源으로 提供하였을 때의 呼吸量은 全般的으로 無機培地에 thiosulfate만을 加했을 때 보다 呼吸量이 낮아 thiosulfate가 呼吸基質로서 가장 優秀함을 알 수 있었다. 前者의 境遇에 fructose, citrate, xylose, malate, glucose, glutamate 및 succinate를 加하면 各各 對照區의 30~40%의 呼吸沮害가 나타났으며, formate와 pyruvate를 加하면 呼吸이 전혀 일어나지 않았다. 그러나 無機鹽의 培地에 thiosulfate나 有機物을 전혀 添加하지 않고 呼吸量을 測定했을 때와 比較하여 보면 glucose, fructose, xylose, glutamate, malate, citrate 및 succinate를 加했을 때 多少間 呼吸量이 增加함을 觀察할 수 있었고 따라서 이들이 어느 정도는 呼吸基質로서 使用될 수 있음을 알았다. 이러한 諸 觀察의 結果, 本菌은 條件如何에 따라서는 mixotroph나 heterotroph로의 轉換도 可能하리라고 思料된다.

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