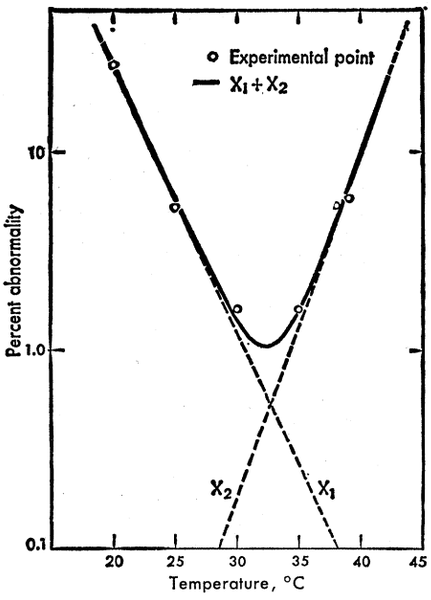


Fig. 1. Abnormalities as a function of temperature in degrees Celsius.

where $T$ is the absolute temperature and $\alpha$ and $b$ are constants.

Also,

$$mC + D \overset{k_2}{\rightleftarrows} C_mD$$

$$k_2 = (C_mD)/(C)^m(D)$$

and

$$(D) = [(C_mD)/(C)^m] \cdot \gamma e^{(+d/T)}$$

where $\gamma$ and $d$ are constants. Therefore

$$(A_nB) = ae^{(-b/T)} \qquad (1)$$

and

$$(D) = ce^{(+d/T)} \qquad (2)$$

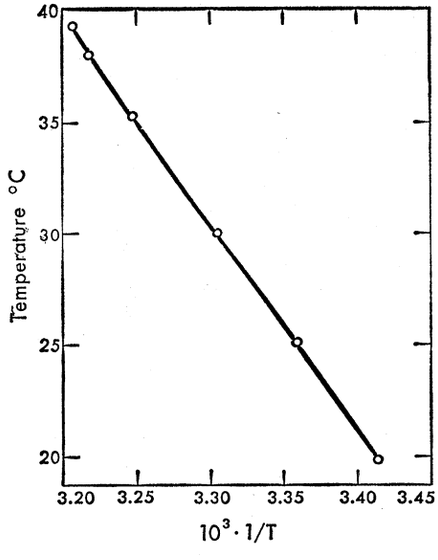


Fig. 2. Correspondence between $t$ (in degrees Celsius) and $1/T$.

where $a$ and $c$ can be considered to be constant if $(A_nB) \ll (B)$ and $(A)$, and if $(D) \ll (C_mD)$ and $(C)$ over the temperature range considered (where $k_1$ is very small and $k_2$ is very large). Thus, $X_1$, the first process, is proportional to Eq. 1, and the second process, $X_2$, is proportional to Eq. 2. From this hypothesis it can be seen that abnormal development may be caused by the presence of either an unusual quantity of some product or by an imbalance in the presence of one of the precursors of a separate set of reactions. From Fig. 2 it is evident that $1/T$ is approximately a linear function of $t$. Furthermore, from Fig. 1 it can be seen that $X_1X_2/100$ is much smaller than $X$. Therefore our experimental data fit both models equally well. The exponential constants of the second model were calculated to be $b = 2.5 \times 10^4$ and $d = 3.2 \times 10^4$. From Fig. 1 it is evident that the values of

$$Q_{10} = (K_{T+10})/K_T$$

for the two separate reactions are approximately 20 and 50, thus being 10 to 20 times greater than one usually expects for simple biochemical reactions (*3*). This consideration suggests that we discard the hypothesis previously indicated where $(A_nB) \ll (A)$ and $(B)$, and $(D) \ll (C_mD)$ and $(C)$. Now by putting

$$n(A_nB) + (A) = (A)_t$$

and

$$(A_nB) + (B) = (B)_t$$

where $(A)_t$ and $(B)_t$ are constant, we arrive at

$$(A_nB) = k_1 [(A)_t - n(A_nB)]^n \cdot [(B)_t - (A_nB)]$$

for the first reaction, and, with similar notation,

$$(D) = \frac{1}{k_2} \frac{(D)_t - (D)}{[(C)_t - m(D)_t + m(D)]^m}$$

for the second reaction. Certain values of the constants $(A)_t$, $(B)_t$, $(C)_t$, $(D)_t$, $m$, and $n$ may explain the high slopes of the two branches of our curve, even with values of $k_1$, $k_2$, $dk_1/dT$, and $dk_2/dT$ that are common in biochemical reactions. The degrees of freedom of this model, however, are too many to calculate any parameter (*4*).

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## Cytochrome in Thiobacillus thiooxidans

Abstract. *Cytochrome is present in the autotrophic sulfur bacterium,* Thiobacillus thiooxidans. *As mediator in the final electron transfer to oxygen, the cytochrome participates in the oxidation of sulfur compounds by extracts of the organism.*

During an extensive study of the Thiobacillus group, Szczepkowski and Skarzynski (*1*) failed to observe cytochrome in *Thiobacillus thiooxidans.* The absence of this important constituent usually present in aerobic cells was explained (*2*) by suggesting that the bulk of energy from sulfur oxidation was derived through substrate phosphorylation by way of an adenosine phosphosulfate complex, whereby oxidative phosphorylation would be made superfluous. Nevertheless, cytochromes are readily detectable in other members of the genus (*3*), which would imply that the metabolic activities of *T. thiooxidans* differ fundamentally from those of other thiobacilli. Because this seemed unlikely, a reexamination of the situation was undertaken.

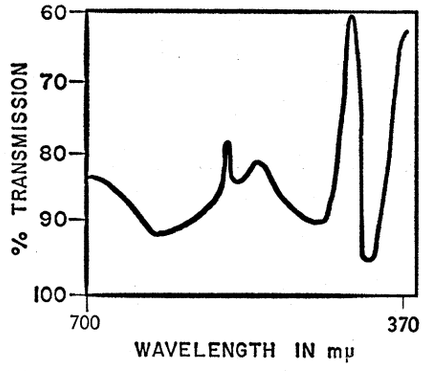


Fig. 1. The reduction of cytochrome from *T. thiooxidans* by sodium dithionite. Cuvette contents: 2 ml of extract (13 mg protein): 1 ml of 0.1*M* phosphate buffer, *p*H 6.9; 0.2 ml of $Na_2S_2O_4$ (20 $\mu M$); distilled water to 4 ml.

Table 1. Oxidation of reduced sulfur compounds by cell-free preparations of *T. thiooxidans*.

| Substrate | Concn. ($\mu M$) | $O_2$ consumed ($\mu$mole) | $SO_4$ ion produced ($\mu$mole) |
|---|---|---|---|
| $Na_2S$ | 10 | 17.2 | |
| $Na_2S_2O_3$* | 10 | 13.0 | 10.5 |
| $K_2S_4O_6$* | 5 | 13.8 | 16.5 |
| $K_2S_3O_6$ | 5 | 10.6 | 15.0 |
| $Na_2SO_3$ | 10 | 3.5 | 10.4 |

* Reactions stopped before the oxidation was complete.

A bacterium corresponding to the original description of *T. thiooxidans* by Waksman and Joffe (*4*) was isolated from a sewage effluent at a spot where sulfur was deposited, by means of enrichment cultures in a mineral salts medium containing sulfur. The isolate is an obligatorily autotrophic, motile rod, 2 to 3 $\mu$ by 0.5 $\mu$; it can oxidize sulfur to sulfuric acid at a *p*H less than 1.

Mass cultures were grown in the medium of the Baalsruds (*5*), supplemented with $FeCl_3$ (2 mg per liter). Frozen cell pastes were processed through a Hughes press, or cell suspensions in 0.1*M* phosphate buffer, *p*H 6.9, were disrupted in an 8-kcy/sec Raytheon supersonic oscillator. Cell-free extracts were prepared by centrifugation at 10,000*g* for 1 hour at 10°C.

The cell-free preparations rapidly and completely oxidized sulfide, thiosulfate, tetra- and trithionate to sulfate in the presence of $O_2$ (Table 1). That they contained cytochrome was shown by spectrophotometric examination of extracts reduced with dithionite (Fig. 1), which revealed absorption bands at 551, 522 and 420 m$\mu$, corresponding to the $\alpha$, $\beta$ and $\gamma$ bands of reduced cytochrome *c*, respectively. The same bands were observed if, instead of dithionite, any one of the above mentioned sulfur compounds was added to the extracts. This unequivocally demonstrates that the oxidation of substrates by *T. thiooxidans* can be coupled with the reduction of cytochrome, and thus supports the inference that the organism can derive its energy from a cytochrome-linked phosphorylation.

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6. Supported by a grant of the National Science Foundation (G-6436) to C. B. van Niel. I thank Dr. van Niel and Dr. S. C. Rittenberg for encouragement and advice.
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## Phytotoxic Substance from a Species of Penicillium

Abstract. *A* Penicillium urticae *Bainer, isolated from subsurface-tilled plots showing reduced wheat growth, produced a phytotoxic substance. The melting point and infrared and ultraviolet spectra of the crystalline material showed this substance to be patulin. Wheat shoot growth inhibition of 50 percent required 20, 20, and 75 parts per million of patulin in solution, sand, and soil culture, respectively.*

Subsurface tillage is a farming method to keep stubble mulch on the soil surface to control water and wind erosion. During a 23-year period at Lincoln, Nebraska, subsurface-tilled, as compared with plowed plots, on a corn, oats, and wheat rotation have produced, in the years of normal to above-normal rainfall, decreased yields and abnormal appearance of crops (*1*).

Fungi, isolated from subsurface-tilled soil showing reduced plant growth, were grown in potato dextrose broth at 28°C. Corn seeds were soaked in this liquid for 6 hours and placed in petri dishes between double layers of germination paper moistened with the liquid. The corn seeds were measured for percent of germination and growth lengths of shoot and root after germinating 3 days at 25°C. In a group of 91 isolates, 14 reduced germination to 50 percent or less.

One fungus, isolated from plots at Alliance, Nebraska, was identified as *Penicillium urticae* Bainer by the Northern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, U.S. Department of Agriculture, Peoria, Ill. This fungus, when grown in potato dextrose broth, produced a toxic material which caused severe stunting of germinating corn.

To obtain concentrated toxic material, the isolate was grown in 200 ml of potato dextrose broth in 1-liter erlenmeyer flasks for 14 days. The filtered culture fluid was flash-evaporated at 40°C and 2 cm-Hg pressure to 1/20 of its volume and extracted with ethyl acetate. Bioassay tests of the culture medium, culture fluid, condensed culture fluid, and ethyl acetate extract revealed the toxic principle to be generated by the fungus and to be readily extracted from the condensed culture fluid. The ethyl acetate extract was dried over anhydrous $MgSO_4$ and evaporated in a flash evaporator to a heavy, brown syrup which crystallized after standing for a few minutes.

The crude crystals were dissolved in ethyl ether and the solution was filtered to separate a small amount of dark-brown, insoluble material. Slow evaporation or chilling of the saturated ether solution produced colorless crystals with a melting point of 109° to 111°C uncorrected. In one experiment, 9 liters of original culture medium yielded 15.1 g of crystals.

Paper chromatography, using a developer of ethanol and water (4 : 1), separated the ethyl acetate extract into several components. Some of the components were fluorescent under an ultraviolet lamp with maximum intensity near 3600 Å. A complete chromatogram, cut into 1-cm sections, was tested for toxicity by placing separate sections between double layers of germination paper moistened with 6 ml of distilled water in 9-cm petri dishes. Five wheat seeds were germinated directly on each chromatogram section for 3 days at 25°C. A control consisted of a similar chromatogram of an ethyl acetate extract of the sterile potato dextrose broth. Only the chromatogram section with an $R_F$ value of 0.76 proved to be toxic to the germinating wheat seeds. Chromatographing the crystalline material showed it to be toxic and to have an $R_F$ value of 0.76. The physiologically active section of the chromatograms reacted with ammonia vapor at room temperature to give a visible, pale yellow spot fluorescing a bright tan color under the ultraviolet lamp.

Comparison of the melting point and infrared and ultraviolet spectra of the crystalline material with the corresponding characteristics of compounds known to be produced by *Penicillium urticae* Bainer showed the substance to be patulin (*2*).

Cheyenne wheat in bioassay tests was used to compare the effects of patulin on germinating seeds with 2,4-dichloro-