# NUTRITIONAL CHARACTERISTICS OF MOON DUST FOR SOIL MICROORGANISMS

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ABSTRACT

Approximately 46% of the lunar sample (10084,151), 125.42 mg, was solubilized in 680 ml 0.01 M salicylic acid. Atomic absorption spectroscopic analysis of the solubilized lunar sample showed the following amount of metal ions: Ca, 3.1; Mg, 4.0; K, 0.09; Na, 0.67; Fe, 7.3; Mn, 1.6; Cu, Ni, Cr, less than 0.1 each. All are in ppm.

Salicylic acid used to solubilize the lunar sample was highly inhibitory to the growth of mixed soil microbes. However, the mineral part of the lunar extract stimulated the growth. For optimal growth of the soil microbes the following nutrients must be added to the moon extract: sources of carbon, nitrogen, sulfur, phosphorus, and magnesium in addition to water.

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## INTRODUCTION

Although there are many studies on chemical composition of lunar rocks and dust (Laul and Papike, 1980; Morris et al., 1983; Abelson, 1970), relatively little work has been reported on effects of the lunar dust on growth of living organisms. Taylor et al. (1971) observed a toxic effect of an Apollo 11 lunar core sample on growth of Pseudomonas aeruginosa (ATCC 15442). Later, Taylor and his associates (1973) described failure to observe toxicity under a somewhat different condition. Silverman et al. (1971) found no inhibition of the growth of any of the organisms tested in growth media containing Apollo 11 samples.

Examination of available chemical data on the moon dust makes it clear that the moon dust is nutritionally deficient in C, H, O, N, P, S, and perhaps Mg as well for the normal growth of living organisms. Other micronutrients required for the organisms, such as Fe, Cu, Zn, Co, Mn, Mo, are probably sufficient in the moon dust provided they become available for the microorganisms during an extraction process of the moon dust. The purpose of the present study is to find what nutrients are available (and unavailable) and whether toxins are present in the moon dust for the optimal growth of soil microorganisms.

## MATERIALS AND METHODS

### Moon Extract in 0.01 M Salicylic Acid

The lunar sample (10084,151) weighing 0.12542 g was stirred in 680 ml of 0.01 M salicylic acid at near boiling temperature for about five hours. The mixture was filtered through Whatman No. 42 filter paper (ashless). The final

volume of the filtrate was adjusted to 680 ml with 0.01 M salicylic acid. A portion of this extract was neutralized with a sodium hydroxide solution to pH near 7.0, and used as needed.

Composition of Growth Media

Growth medium I consisted of 4.5 ml moon extract, 0.1 ml 0.8 M sodium glutamate, 0.1 ml 0.04 M magnesium sulfate, and 0.1 ml 0.4 M sodium potassium phosphate buffer pH 7.0.

Growth medium II consisted of 4.5 ml moon extract, 0.1 ml 0.6 M sodium nitrate, 0.1 ml 0.1 M magnesium chloride, 0.1 ml 0.07 M sodium sulfate, 0.1 ml 0.4 M sodium potassium phosphate buffer pH 7.0, and 0.1 ml 0.56 M D-glucose.

Other growth media included those in which one or more of the constituents of the above media was omitted and replaced by water and those in which the moon extract was replaced by 4.5 ml 0.01 M salicylate in distilled water pH 7.0, or replaced by 4.5 ml 0.01 M salicylate in tap water pH 7.0. Nutrient broth was used as a positive control to test whether an inoculum contained any living cells. In some experiments, nutrient broth was prepared in 0.01 M salicylate pH 7.0 instead of in water.

The growth media were dispensed to culture tubes (12x100mm) with screw caps and not sterilized since the inoculum was a mixed population of soil microorganisms. Since they were not sterilized, they were inoculated immediately after the preparation.

Inoculum

The inoculum was prepared by filtering a mixture of 10 g wet garden soil and 50 ml 0.85 percent sodium chloride (isotonic saline), centrifuging the filtrate at full speed (2500 RPM) with a HN-S International centrifuge for 15 minutes,

washing twice with isotonic saline, and suspending the sediment in a 50 ml isotonic saline. One drop of the inoculum was added to each culture tube containing a culture medium.

Determination of Growth

The culture tubes to which the growth media were dispensed were directly used as cuvettes to determine the absorbance at 700 nm. The selection of this particular wavelength was based on a finding that the moon extract especially at pH near 7 was highly colored (wine color) with an absorption peak at 470 nm. At 700 nm absorption was negligible.

Determination of Extracted Matters from the Lunar Sample

A 100 ml of the moon extract (unneutralized) was evaporated to dryness in a platinum crucible on a hotplate and placed in a 600°C oven for three days, and the weight of the dry matter was determined.

Determination of Undissolved Matters from the Lunar Sample after Extraction in the Salicylic Acid Solution

The undissolved materials filtered on the filter paper in the process of the moon extract preparation were added in a platinum crucible and placed in a 600°C oven for three days, and the weight of the residue was determined.

Analysis of the Moon Extract

The following metal ions in the moon extract (unneutralized) were analyzed by atomic absorption spectroscopy: Ca, Mg, K, Na, Fe, Mn, Cu, Ni, Cr.

## RESULTS

### Extent of Extraction of the Lunar Sample by Salicylic Acid

The treatment of 125.42 mg of the lunar sample (10084,151) in 680 ml aqueous salicylic acid solution resulted in 58.14 mg into the solution and 59.96 mg undissolved. The correction due to the oxygen addition to the minerals during the combustion at 600°C was not made.

### Chemical Analysis of the Moon Extract

The moon extract in 0.01 M salicylic acid contained the following amount of metal ions: Ca, 3.1; Mg, 4.0; K, 0.09; Na, 0.67; Fe, 7.3; Mn, 1.6; Cu, Ni, Cr, less than 0.1 each. All are in ppm (part per million).

### Effect of the Moon Extract on the Growth of Soil Microorganisms

Removal of either phosphate or magnesium sulfate from the complete growth medium that contained the moon extract, glutamate, magnesium sulfate, and phosphate buffer decreased the growth of the soil microbes. The removal of the moon extract greatly increased the growth. The replacement of the moon extract with unboiled tap water completely inhibited the growth (Fig. 1).

In order to determine whether the inhibitory effect of the moon extract was due to a possible toxic effect of the moon minerals or to an effect of the salicylic acid, 0.01 M salicylate in distilled water pH 7.0 was used in place of the moon extract in a growth medium. The salicylate decreased the microbial growth considerably, and the moon minerals stimulated the growth (Fig. 2). The replacement of the distilled water with boiled tap water slightly inhibited the growth (Fig. 2), although not as much as found in the previous experiment (Fig. 1).

In order to determine what nutrients must be added to the moon extract for optimal growth of the soil microbes, various growth media were tested. As can be seen in Fig. 3, the removal of the moon extract (i.e., salicylate) again showed a dramatic increase in growth. The considerable inhibition of the microbial growth by salicylate was quite evident. The addition of salicylate in the nutrient broth (Fig. 3) increased the lag period considerably over the nutrient broth alone (Fig. 1).

Although not shown in the graph, in the absence of any one of the nutrients sulfate, magnesium, nitrate, and phosphate buffer, the highest absorbance reading of the triplicate samples of each growth medium lacking one of these nutrients was lower than the lowest absorbance reading of the triplicate samples of the complete growth medium (i.e., growth medium II). The average growth of the triplicate samples containing no glucose as a carbon source was lower than the one containing the complete medium. However, two of the absorbance readings were higher than the lowest reading of the triplicate samples of the complete medium.

## CONCLUSIONS AND DISCUSSION

One can conclude that the moon would form excellent trace mineral nutrients for soil microbes, since nearly one-half of the lunar sample used went into the salicylic acid solution and since the moon extract prepared in this study was probably even a saturated solution of the lunar sample according to Keller and Huang (1971), whose data on an Apollo 12 sample indicated that twice as much solids could go into the salicylic acid solution. Another reason for this conclusion is that the moon minerals did stimulate the growth of the soil microbes (Figs. 2, 3).

The weights of the soluble and the insoluble materials, 58.14 mg and 59.96 mg respectively from the total of the 125.42 mg lunar sample, were obviously a little overestimated since the combusion at 600°C would oxidize some mineral components of the lunar materials, particularly ferrous oxide. Assuming that all ferrous oxide was converted to ferric oxide during the combustion and that 16 percent of the total lunar sample used was ferrous oxide (Laul and Papike, 1980), the correction would be approximately 0.9 mg. From the atomic absorption data given in Results, it can be calculated that the moon extract in the salicylic acid solution contained 11 percent (16.2 percent) ferrous oxide, 9.8 percent (9.2 percent) magnesium oxide, 6.4 percent (12.4 percent) calcium oxide, and 1.8 percent (0.38 percent) sodium oxide in the total solid dissolved. The percentage in the parentheses was from the data of Laul and Papike (1980), indicating the content in this particular lunar sample. Compared with their data, one can see that magnesium oxide and ferrous oxide but not calcium oxide went proportionately into he salicylic acid solution. The high concentration of sodium may have been due to a possible presence of sodium in the salicylic acid used. This could not be ascertained, since the salicylic acid control was not

analyzed.

The complete inhibition of the microbial growth (Fig. 1) in the medium containing unboiled tap water in the place of the moon extract was probably in major part due to the chlorine in the tap water. However, even when boiled tap water containing salcylate replaced the moon extract, there was a slight inhibition of growth compared with a similar growth medium in which distilled water instead of the tap water was used. It is possible that some minerals in the tap water were inhibitory to the growth of some microbes but perhaps not of others in the mixed population of soil organisms. This was probably why the absorbance readings of the triplicate samples were widely different. (Individual readings were not shown.) It can be speculated that calcium ions, a component of hard water, in the tap water may be inhibitory to the growth of some microbes since it is not required for microorganisms in as large a quantity as vertebrates.

The considerable inhibition of the growth of the soil microbes by salicylate was quite evident in this study (Figs. 1, 2, 3). The use of pure culture of a salicylate-utilizing soil Pseudomonad instead of the mixed soil microbes as an inoculum would remove the complication created by the inhibititon of the microbial growth by salicylate.

The finding that even without glucose two of the three culture tubes gave higher absorbance readings than with glucose needs explanation. Without glucose, there was still salicylate as a carbon source in the growth medium and perhaps the rate of the growth of salicylate-utilizing bacteria, which were obviously expected in the inoculum, in these two tubes was high. This result was another example of the complications created by the use of the mixed soil microbes as an inoculum in this study. In any case, a carbon source must be added to a growth medium for optimal growth of the microbes because available

chemical data show that very little carbon is present in the lunar sample (Laul and Papike).

It appeared that sulfate, nitrate, magnesium, and phosphate, in addition to a source of carbon, were required for the growth of the soil microbes.

The finding that without the moon minerals there was an optimal growth (Figs. 1, 2, 3) of the soil microbes indicated that the lunar sample was a poor source of nutrients and that the mineral requirements for the optimal growth were evidently met by contaminants coming from the chemicals and the laboratory wares used in the experiments. The lunar dust, however, did obviously supply the trace nutrients required for the growth, for the growth was stimulated by the presence of the moon extract (Fig. 2).

In conclusion, the lunar dust was a poor source of nutrients for the soil microbes to grow, although it appeared that it contained enough trace mineral nutrients and could be solubilized to support the growth of the soil microbes provided other macronutrients, including sources of carbon, nitrogen, sulfur, magnesium, and phosphate in addition to water, were available to them. It showed no toxicity for the microbial growth. The ready solubilization into the salcylic acid solution, which is a normal component of humus in earth soil, the growth stimulation by the solubilized moon minerals, and the lack of toxicity obviously mean that the lunar dust (although it is a poor source of nutrients) will certainly become a productive soil to support growth of microbes and higher plants if supplemented with organic, inorganic macronutrients and an atmosphere similar to air.

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Fig. 1 Effects of Moon Extract and Other Nutrients on Growth of Soil Microbes.

● : Growth medium I minus moon extract.

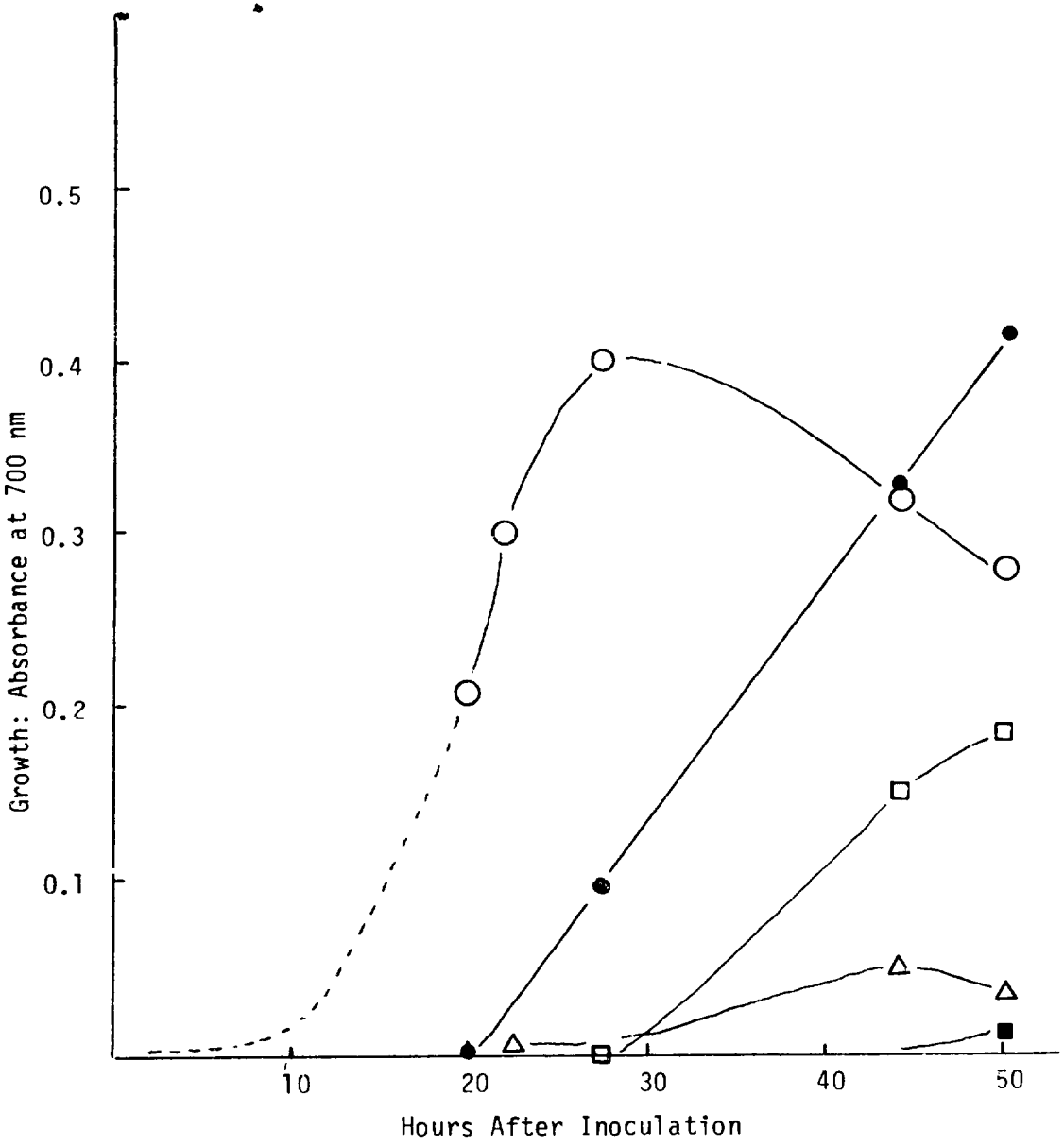
□ : Growth medium I.

■ : Growth medium I minus phosphate buffer.

△ : Growth medium I minus magnesium sulfate.

○ : Nutrient broth.

See the text for the composition of the growth medium I.
Each point represents the average of three absorbance readings.


0.5
0.4
0.3
0.2
0.1
Growth: Absorbance at 700 nm
10
20
30
40
50
Hours After Inoculation

(Fig. 1)

Fig. 2 Effects of Salicylate and Moon Minerals on Growth of Soil Microbes.

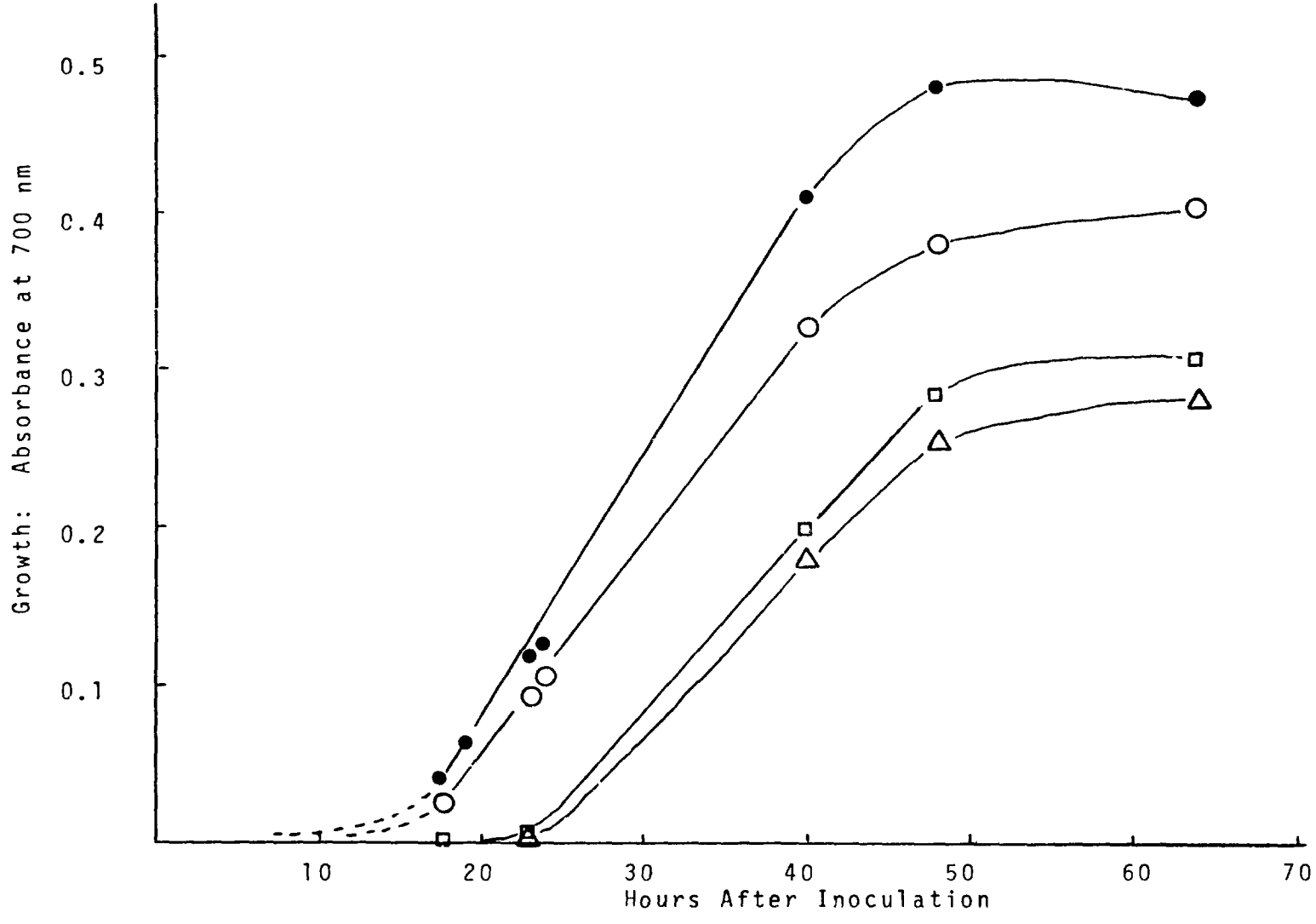
● : Growth medium I minus moon extract.

○ : Growth medium I.

□ : Growth medium I minus moon extract plus salicylate in distilled water pH 7.0.

△ : Growth medium I minus moon extract plus salicylate in tap water pH 7.0.

See the text for the composition of the growth medium I.
Each point represents the average of three absorbance readings.


Growth: Absorbance at 700 nm
0.5
0.4
0.3
0.2
0.1
10
20
30
40
50
60
70
Hours After Inoculation

(Fig. 2)

Fig. 3 Effects of Salicylate, Moon Minerals, and Other Nutrients on Growth of Soil Microbes.

● : Growth medium II.

○ : Growth medium II minus moon extract.

△ : Growth medium II minus glucose.

■ : Growth medium II minus sodidum sulfate.

○ : Growth medium II minus magnesium chloride.

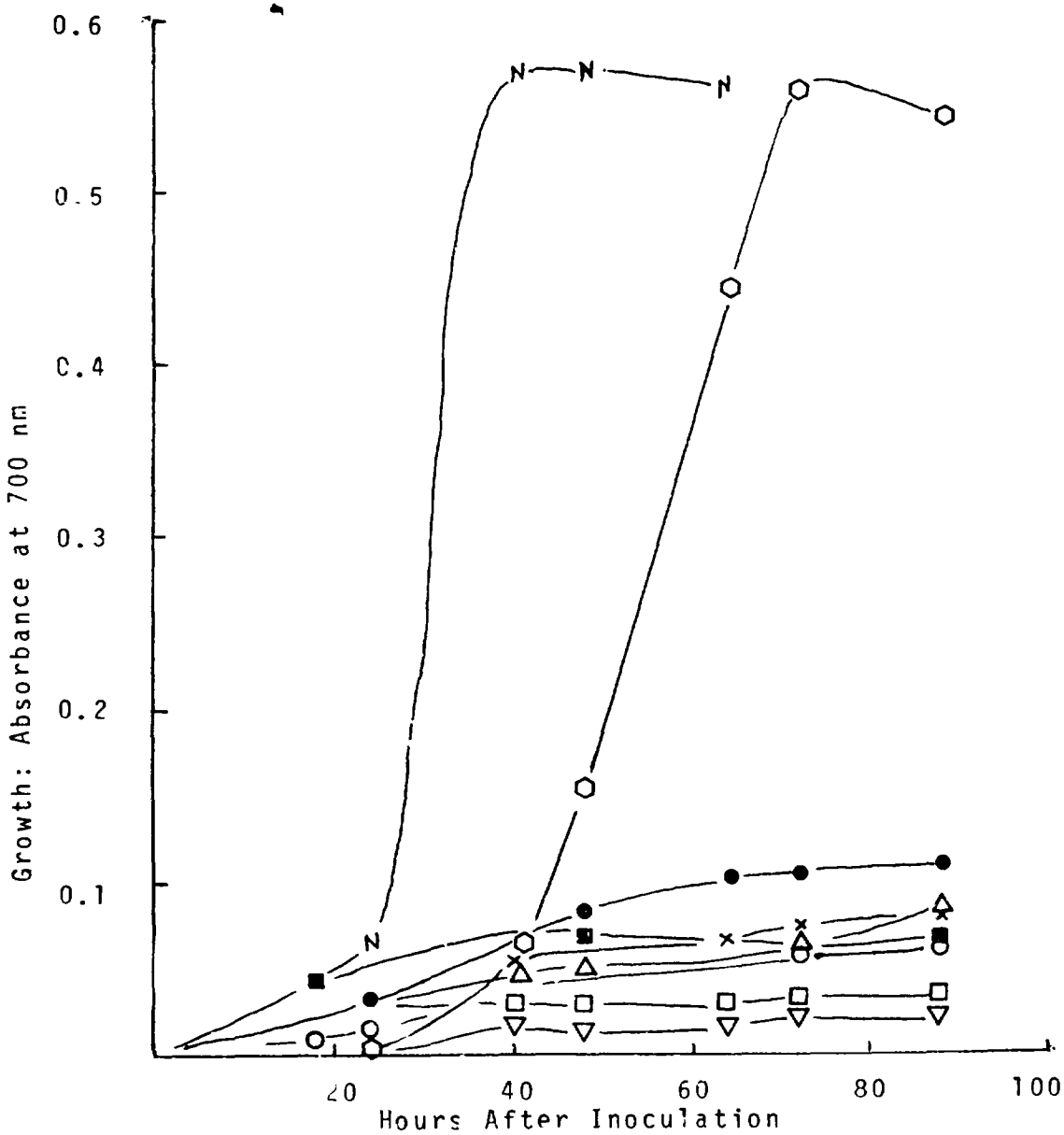
□ : Growth medium II minus sodium nitrate.

▽ : Growth medium II minus phosphate buffer.

X : Growth medium II minus moon extract plus 0.01 M salicylate pH 7.0.

N : Nutrient broth in 0.01 M salicylate pH 7.0.

See the text for the composition of growth medium II.
Each point represents the average of three absorbance readings.


0.6
0.5
0.4
0.3
0.2
0.1
Growth: Absorbance at 700 nm
N
N
N
N
20
40
60
80
100
Hours After Inoculation

(Fig. 3)