

## Effect of Plant Growth Hormones on Growth of *Azospirillum* sp. in Media with Different Carbon Sources

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### Abstract

Studies on the effect of IAA, GA<sub>3</sub> and kinetin on the growth of 3 strains of *Azospirillum* have shown that it depended on the strain, the kind of plant growth regulator in the medium, the concentration of a given compound and on the kind of carbon source (malate, succinate or fumarate). IAA and GA<sub>3</sub> in most cases stimulated the growth of azospirilla in the presence of succinate and fumarate; the same hormones have not exerted any noticeable effect in media containing malate. Kinetin inhibited the growth of azospirilla in media with succinate and fumarate. Kinetin in the presence of malate weakly stimulated the proliferation of *Azospirillum* sp., if applied in low concentrations (0.001-0.01 µg/ml). The same hormone exerted inhibitory effect in malate-containing media, when applied in high concentrations (1-10 µg/ml).

### Introduction

Different soil microorganisms were found to produce plant growth regulators (Greene, 1980; Strzelczyk *et al.*, 1987). *Azospirillum* sp., a widespread group of nitrogen fixing and plant growth producing bacteria have received considerable attention in recent years. These bacteria are considered to be of importance for inoculation of plants. Plant growth substances are also believed to affect mycorrhizae formation and functioning (Gogala, 1991). It is likely that rhizosphere bacteria capable of fixing nitrogen may improve plant productivity both by hormonal stimulation and by supplying nitrogen (Tien *et al.*, 1979).

Azospirilla are known to produce plant growth regulators (Tien *et al.*, 1979; Strzelczyk *et al.*, 1993). However, nothing is known about the effect on *Azospirillum* of these substances which may be produced by associated microorganisms or may occur in root exudates.

## Experimental

### Materials and Methods

**Strains.** Three following strains of *Azospirillum* were used in our studies:

1. *Azospirillum* sp. (1) isolated from ectomycorrhizae of Douglas fir formed with *Rhizopogon vinicolor*.
2. *Azospirillum* sp. (2) isolated from within the sporocarp of *Laccaria laccata*.
3. *Azospirillum* sp. (3) isolated from within sporocarp of *Hebeloma crustuliniforme*.

**Culturing.** The bacteria were cultured in triplicate in 100 ml Erlenmeyer flasks with sidearm each containing 25 ml of Döbereiner (1980) medium with appropriate concentrations of plant growth regulators (indoleacetic acid - IAA, gibberellic acid - GA<sub>3</sub>, kinetin). The following concentrations of plant growth regulators were used: 1-0.001 µg/ml, 2-0.01 µg/ml, 3-0.1 µg/ml, 4-1 µg/ml, 5-10 µg/ml.

Three carbon sources were used in our study - malate, succinate and fumarate-Na in amount of 5 g/1000ml. The medium in each flask was inoculated with 0.5 ml of 3-day old culture of *Azospirillum* grown in Döbereiner liquid medium. The inoculated media were incubated at 26°C for 72 hours. Cultures without plant growth regulators were used as controls.

**Growth of *Azospirillum*.** Growth of *Azospirillum* was recorded by optical density measurements with Klett-Summerson (filter 42) photocolormeter during 72 hours of growth after 0, 4, 12, 24, 36, 48 and 72 hours. Corrected values of optical density of hours cultures (as the most representative ones) were statistically analyzed by 1-factor analysis of variance (ANOVA) and the Newman-Keuls multiple range test ( $p \leq 0.05$ ). Calculations were performed using the program CSS: STATISTICA for IBM PC and compatibles (release 3.OE, 1991, StatSoft, Tulsa, Oklahoma, USA).

## Results

The results of our studies are presented in Tables I-III and Fig. 1. It appears from Table I that the effect of IAA on growth of *Azospirillum* depends both on the concentration of IAA and the source of carbon. In media with malate IAA did not affect growth of *Azospirillum* at either concentration used. However in the presence of succinate stimulation of growth of this bacterium was observed and the growth was proportional to the concentration of IAA. Growth stimulation by IAA in succinate-containing media was significant ( $p \leq 0.05$ ) at concentrations  $\geq 0.01$  µg/ml (strains Nos 1 and 3) and  $\geq 0.001$  µg/ml (strain No 2). In media with fumarate IAA significantly ( $p \leq 0.05$ ) stimulated growth of all the strains of *Azospirillum* except strain No 2 at 0.001 µg IAA/ml (Table I, Fig. 1).

The effect of GA<sub>3</sub> on growth of *Azospirillum* similarly as in case of IAA dependent upon kind of carbon source (Table II). In media with malate, GA<sub>3</sub> affected slightly growth of this bacterium. The stimulating effect was statistically significant at the concentration of 0.1 µg/ml of the above growth substance (*Azospirillum* 1) and at the concentration 0.1 to 10 µg/ml for the remaining strains.

In media with succinate GA<sub>3</sub> exhibited the strongest stimulatory effect in *Azospirillum* No 1 (significant stimulation at  $p \leq 0.05$  for all the concentrations

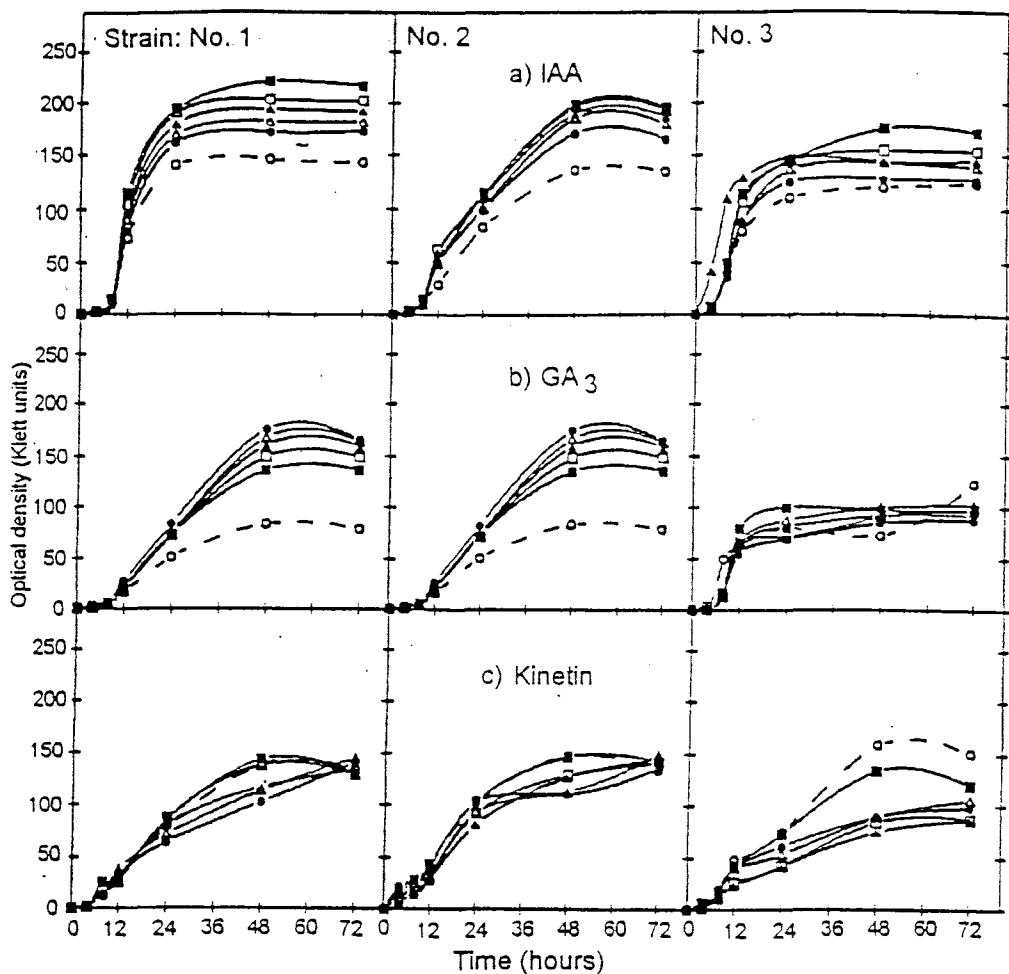


Fig. 1. Effect of different concentrations of plant growth hormones (PGH) on growth kinetics of *Azospirillum* sp.

○—○ Control, ●—● 0.001  $\mu\text{g}$  PGH/ml,  $\Delta$ — $\Delta$  0.01  $\mu\text{g}$ /ml,  $\blacktriangle$ — $\blacktriangle$  0.1  $\mu\text{g}$  PGH/ml,  $\square$ — $\square$  1  $\mu\text{g}$  PGH/ml,  $\blacksquare$ — $\blacksquare$  10  $\mu\text{g}$  PGH/ml.

used). However  $\text{GA}_3$  significantly ( $p \leq 0.05$ ) promoted the growth of the two remaining strains in succinate-containing media (except strain No 3 at the lowest concentration of  $\text{GA}_3$ ). The stimulating effect of this hormone in media with fumarate was significant (except strain No 3 at 0.001  $\mu\text{g}$   $\text{GA}_3$ /ml). With fumarate the most stimulatory were medium concentrations of  $\text{GA}_3$  (0.01–1  $\mu\text{g}$ /ml) for strain No 1; the lowest ones (1–10  $\mu\text{g}$ /ml) for strain No 3 (Table II).

Kinetin affected distinctly the growth of *Azospirillum* with malate as carbon source. However the higher concentration of this plant growth regulator inhibited the growth of this organism (Table III).

Table I

Growth yield (optical density after 48 h of culturing) of *Azospirillum* in the presence of different concentrations of IAA

| Strain No. | Carbon source | Experimental combinations (concentrations of IAA) |  |                                       |                                      |                                      |                                     |
|------------|---------------|---|--|---------------------------------------|--------------------------------------|--------------------------------------|-------------------------------------|
|            |               | 0 (control)                                       | 0.001 $\mu\text{g}\cdot\text{ml}^{-1}$ | 0.01 $\mu\text{g}\cdot\text{ml}^{-1}$ | 0.1 $\mu\text{g}\cdot\text{ml}^{-1}$ | 1.0 $\mu\text{g}\cdot\text{ml}^{-1}$ | 10 $\mu\text{g}\cdot\text{ml}^{-1}$ |
| 1          | Malate        | 166.0 a<br>$\pm 11.53$                            | 155.0 a<br>$\pm 20.66$                 | 160.7 a<br>$\pm 26.31$                | 137.0 a<br>$\pm 26.06$               | 175.3 a<br>$\pm 25.95$               | 178.3 a<br>$\pm 18.77$              |
|            | Succinate     | 145.3 a<br>$\pm 2.33$                             | 173.3 a<br>$\pm 3.85$                  | 182.3 a<br>$\pm 1.87$                 | 194.0 d<br>$\pm 2.52$                | 203.7 e<br>$\pm 3.18$                | 221.0 f<br>$\pm 4.36$               |
|            | Fumarate      | 100.0 a<br>$\pm 1.00$                             | 116.7 b<br>$\pm 5.86$                  | 130.7 c<br>$\pm 8.39$                 | 140.0 d<br>$\pm 3.18$                | 151.7 e<br>$\pm 4.51$                | 158.3e<br>$\pm 3.51$                |
| 2          | Malate        | 97.0 a<br>$\pm 32.34$                             | 69.3 a<br>$\pm 2.31$                   | 82.0 a<br>$\pm 23.26$                 | 99.3 a<br>$\pm 25.15$                | 76.0 a<br>$\pm 20.07$                | 102.0 a<br>$\pm 17.52$              |
|            | Succinate     | 136.7 a<br>$\pm 3.35$                             | 169.7 b<br>$\pm 6.01$                  | 185.7 c<br>$\pm 2.40$                 | 190.3 cd<br>$\pm 0.88$               | 196.3 cd<br>$\pm 1.45$               | 199.7 d<br>$\pm 0.88$               |
|            | Fumarate      | 83.7 a<br>$\pm 1.53$                              | 91.7 ab<br>$\pm 1.53$                  | 95.3 b<br>$\pm 3.06$                  | 105.0 bc<br>$\pm 5.29$               | 123.7 c<br>$\pm 10.21$               | 135.7 d<br>$\pm 6.81$               |
| 3          | Malate        | 173.7 a<br>$\pm 5.56$                             | 162.3 a<br>$\pm 3.67$                  | 170.0 a<br>$\pm 9.50$                 | 190.0 a<br>$\pm 4.36$                | 158.0 a<br>$\pm 2.65$                | 167.0 a<br>$\pm 15.72$              |
|            | Succinate     | 120.0 a<br>$\pm 3.61$                             | 127.7 a<br>$\pm 5.71$                  | 143.0 b<br>$\pm 2.52$                 | 148.0 b<br>$\pm 0.58$                | 154.0 b<br>$\pm 6.24$                | 175.3 c<br>$\pm 3.01$               |
|            | Fumarate      | 95.0 a<br>$\pm 3.61$                              | 117.0 b<br>$\pm 7.81$                  | 121.7 b<br>$\pm 7.57$                 | 129.3 b<br>$\pm 1.53$                | 140.7 c<br>$\pm 4.57$                | 151.0 d<br>$\pm 3.61$               |

Explanations: - mean values marked with the same letter in a given line do not differ significantly ( $p \leq 0.05$ );  
- standard errors are given in lines below of those containing the mean values.

Table II

Growth yield (optical density after 48 h of culturing) of *Azospirillum* in the presence of different concentrations of  $\text{GA}_3$

| Strain No. | Carbon source | Experimental combinations (concentrations of $\text{GA}_3$ ) |  |                                       |                                      |                                      |                                     |
|------------|---------------|--|--|---------------------------------------|--------------------------------------|--------------------------------------|-------------------------------------|
|            |               | 0 (control)  | 0.001 $\mu\text{g}\cdot\text{ml}^{-1}$ | 0.01 $\mu\text{g}\cdot\text{ml}^{-1}$ | 0.1 $\mu\text{g}\cdot\text{ml}^{-1}$ | 1.0 $\mu\text{g}\cdot\text{ml}^{-1}$ | 10 $\mu\text{g}\cdot\text{ml}^{-1}$ |
| 1          | 2             | 3  | 4                                      | 5                                     | 6                                    | 7                                    | 8                                   |
| 1          | Malate        | 251.0 a<br>$\pm 14.19$                                       | 228.0 a<br>$\pm 4.00$                  | 223.7 a<br>$\pm 19.00$                | 272.7 b<br>$\pm 22.75$               | 239.0 a<br>$\pm 34.83$               | 225.0a<br>$\pm 30.45$               |
|            | Succinate     | 84.0 a<br>$\pm 34.39$  | 135.7 b<br>$\pm 10.60$                 | 148.7 bc<br>$\pm 4.42$                | 161.0 c<br>$\pm 1.00$                | 167.0 c<br>$\pm 8.89$                | 174.7 cd<br>$\pm 12.01$             |
|            | Fumarate      | 101.3 a<br>$\pm 2.52$  | 115.0 b<br>$\pm 1.00$                  | 131.7 c<br>$\pm 9.07$                 | 149.0 e<br>$\pm 9.29$                | 150.7 e<br>$\pm 1.53$                | 140.3 d<br>$\pm 2.53$               |

Table II - continued

| 1 | 2         | 3                 | 4                   | 5                   | 6                   | 7                  | 8                  |
|---|-----------|-------------------|---------------------|---------------------|---------------------|--------------------|--------------------|
| 2 | Malate    | 132.7 a<br>± 2.08 | 138.7 a<br>± 12.34  | 142.0 ab<br>± 13.00 | 182.7 c<br>± 7.77   | 153.0 b<br>± 5.29  | 149.7 b<br>± 7.02  |
|   | Succinate | 91.3 a<br>± 9.50  | 106.7 b<br>± 6.81   | 112.0 bc<br>± 3.00  | 109.7 b<br>± 3.78   | 109.3 b<br>± 7.37  | 118.7 c<br>± 4.62  |
|   | Fumarate  | 103.7 a<br>± 3.21 | 125.3 bc<br>± 14.29 | 118.3 b<br>± 19.73  | 124.3 bc<br>± 5.86  | 116.7 b<br>± 21.08 | 101.3 a<br>± 1.53  |
| 3 | Malate    | 161.3 a<br>± 6.03 | 156.0 a<br>± 45.71  | 188.0 b<br>± 21.28  | 201.7 c<br>± 2.08   | 187.0 b<br>± 18.74 | 185.0 b<br>± 20.82 |
|   | Succinate | 80.3 a<br>± 1.53  | 85.7 ab<br>± 7.23   | 100.3 c<br>± 2.52   | 93.3 b<br>± 4.36    | 91.3 b<br>± 5.51   | 98.0 bc<br>± 8.89  |
|   | Fumarate  | 95.7 a<br>± 1.53  | 121.0 ab<br>± 3.61  | 126.0 b<br>± 3.46   | 127.7 bc<br>± 12.22 | 139.3 c<br>± 3.06  | 135.0 c<br>± 5.00  |

Explanations: - see Table I

Table III

Growth yield (optical density after 48 h of culturing) of *Azospirillum* in the presence of different concentrations of kinetin

| Strain No. | Carbon source | Experimental combinations (concentrations of kinetin) |  |                                       |                                      |                                      |                                     |
|------------|---------------|---|--|---------------------------------------|--------------------------------------|--------------------------------------|-------------------------------------|
|            |               | 0 (control)   | 0.001 $\mu\text{g}\cdot\text{ml}^{-1}$ | 0.01 $\mu\text{g}\cdot\text{ml}^{-1}$ | 0.1 $\mu\text{g}\cdot\text{ml}^{-1}$ | 1.0 $\mu\text{g}\cdot\text{ml}^{-1}$ | 10 $\mu\text{g}\cdot\text{ml}^{-1}$ |
| 1          | Malate        | 114.0 ab<br>± 4.19                                    | 144.7 c<br>± 12.37                     | 129.3 b<br>± 9.70                     | 104.7 a<br>± 0.67                    | 104.3 a<br>± 1.15                    | 118.8 ab<br>± 5.04                  |
|            | Succinate     | 138.0 c<br>± 3.06                                     | 143.7 c<br>± 3.21                      | 136.0 c<br>± 5.20                     | 124.3 bc<br>± 6.74                   | 114.0 b<br>± 2.65                    | 103.0 a<br>± 6.08                   |
|            | Fumarate      | 165.3 d<br>± 7.77                                     | 138.7 c<br>± 7.09                      | 120.7 b<br>± 2.52                     | 113.7 b<br>± 5.69                    | 108.3 ab<br>± 6.43                   | 99.0a<br>± 1.00                     |
| 2          | Malate        | 124.3 ab<br>± 4.98                                    | 148.7 b<br>± 9.33                      | 138.3 b<br>± 10.53                    | 129.0 ab<br>± 8.14                   | 114.7 a<br>± 6.77                    | 118.3 a<br>± 2.04                   |
|            | Succinate     | 148.3 c<br>± 5.03                                     | 147.3 c<br>± 7.51                      | 133.3 bc<br>± 5.69                    | 128.3 b<br>± 5.51                    | 114.0 a<br>± 5.29                    | 111.3 a<br>± 3.79                   |
|            | Fumarate      | 147.0 d<br>± 3.61                                     | 135.7 c<br>± 9.51                      | 113.3 b<br>± 4.16                     | 103.3 ab<br>± 8.62                   | 97.7 ab<br>± 4.93                    | 87.3 a<br>± 5.03                    |
| 3          | Malate        | 130.0 b<br>± 5.51                                     | 145.7 c<br>± 2.03                      | 139.3 bc<br>± 4.18                    | 135.3 bc<br>± 3.18                   | 117.7 ab<br>± 3.84                   | 109.7 a<br>± 9.54                   |
|            | Succinate     | 159.0 d<br>± 2.65                                     | 137.0 c<br>± 4.58                      | 103.7 b<br>± 18.72                    | 71.7 a<br>± 5.13                     | 89.0 ab<br>± 18.74                   | 89.7 ab<br>± 21.26                  |
|            | Fumarate      | 142.7 d<br>± 7.02                                     | 143.7 d<br>± 3.79                      | 132.3 c<br>± 2.08                     | 119.7 b<br>± 1.53                    | 112.7 ab<br>± 8.62                   | 105.0 a<br>± 5.57                   |

Explanations: - see Table I

In the presence of succinate and fumarate even the lowest concentrations of kinetin inhibited distinctly the growth of *Azospirillum*: for these carbon sources inhibitory effects of kinetin were in most cases statistically significant  $p \leq 0.05$  (Table III).

### Discussion

*Azospirillum* is probably the most studied non-symbiotic  $N_2$ -fixer because of its well known properties demonstrated on broad range of plant species (Fages, 1992).

A considerable interest in *Azospirillum* persists world-wide despite its controversial nature (Bashan and Levanony, 1989). Studies have suggested  $N_2$ -fixation (Kapulnik et al., 1981; Mertens and Hess, 1984; Li and Hung, 1987) hormonal effects (Sarig et al., 1984; Kucey, 1988) improvement of root development, mineral and water uptake (Bashan, 1986; Okon and Kapulnik, 1986). However it was demonstrated that contribution of *Azospirillum brasilense* Cd in growth of tomato seedlings is not through nitrogen fixation (Bashan et al., 1989). Production of B-group vitamins and plant growth regulators has been demonstrated (Dahm et al., 1993; Strzelczyk et al., 1993).

Indole acetic acid, gibberellins and cytokinins were found in culture media of *Azospirillum* by Tien et al. (1979).

Despite the production of plant growth regulators the effect of IAA and  $GA_3$  on growth of *Azospirillum* depend upon the concentration of these substances and the kind of carbon source. This observation seems to be important for a better understanding of the plant *Azospirillum* relations but may be also of practical importance in the technology of inoculants preparation.

According to Bashan and Levanony (1988) inoculation of plants with *Azospirillum* on a large scale should not be yet recommended. Further studies on *Azospirillum* are essential before inoculation technology can start.

*Azospirillum* seems to be for us a promising organism for inoculations of plants (as a PGPR) or as a helper for mycorrhizal inoculation. Therefore it deserves further consideration.

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