CHEMICAL ENGINEERING TRANSACTIONS
VOL. 113, 2024
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Jeng Shiun Lim, Bohong Wang, Guo Ren Mong, Petar S. Varbanov

ISBN 979-12-81206-14-4; **ISSN** 2283-9216
DOI: 10.3303/CET24113013

Impact of *Acinetobacter soli* on Tomato Plants

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The bacterium *Acinetobacter soli* was initially discovered in the forest soil of Baekwoon Mountain in the Republic of Korea. The strain *A.soli* Y-3 is well-known for its ability to produce L-asparaginase, the broader functionalities of A.soli remain unexplored. This study investigated the effect of *A.soli*, isolated from tomato plants with green wilt symptom, on tomato plants by introducing the bacteria at seed stage, 2-leaf stage and 4-leaf stage. The germination rate, stem height, root length and leaf number were recorded. The introduction of this bacterium into seeds during the seed stage did not affect germination rate but significantly enhanced stem height from the bacterial density of 2.5×10^4 CFU/mL and root length of tomato plants at bacterial densities ranging from 2.5×10^5 CFU/mL to 2.5×10^7 CFU/mL. Conversely, infection at the 2-leaf stage from a density of 2.5×10^4 CFU/mL led to a reduction in root length. Infection at the 4-leaf stage from the same density resulted in decreased stem height and leaf count. *A.soli* exhibited the capacity to induce auxin activity without gibberellic acid activity. This research sheds light on the multifaceted interaction between *A.soli* and tomato plants, paving the way for a deeper understanding of their potential applications in agriculture.

1. Introduction

Facing the challenges of climate change and global food security (Talib et al., 2023), agricultural practices are increasingly focusing on sustainable farming techniques (Hien et al., 2023), among which the use of interactions between bacteria and plants is a growing area of interest (Ramli et al., 2023).
The genus Acinetobacter is a highly diverse group, belonging to γ-Proteobacteria and Pseudomonadales order (Brisou and Prévot, 1954). As of 2021, there are 144 known species within the genus Acinetobacter, with 68 identified species and 76 unnamed taxa (Qin et al., 2021). These bacteria can be found in various environments such as soil and water (Baumann, 1968). One of the remarkable characteristics of Acinetobacter is their versatile metabolic capabilities. They can degrade a wide range of compounds, including long-chain dicarboxylic acids and aromatics. This metabolic diversity allows them to actively participate in the nutrient cycle within ecosystems. In recent years, several emerging topics have gained attention on Acinetobacter. Several research are exploring the potential of Acinetobacter in various biotechnological processes, such as bioremediation (Cai et al., 2021) and biofuel production (Bernadino et al., 2024). Understanding the metabolic capabilities of these bacteria can provide valuable insights for these applications. Advances in sequencing technologies have enabled researchers to explore the genetic makeup of different Acinetobacter species. Comparative genomics studies have shed light on the evolutionary relationships between different strains and provided insights into their adaptation to different environments.
Acinetobacter soli, a bacterium that is non motile and rod shaped, was first isolated in the forest soil of Baekwoon mountain in the Republic of Korea. The bacteria were found to be Gram-negative, catalase-positive, and oxidase-negative (Kim et al., 2008). L-Asparaginases, enzymes with the ability to impede the generation of acrylamide, a hazardous toxin that arises during the high-temperature treatment of food, hold promise as a means of ensuring food safety. Through a screening process involving soil samples, *Acinetobacter soli* Y-3 emerged as a standout candidate due to its remarkable L-asparaginase activity. This finding opens exciting

Paper Received: 25 June 2024; Revised: 4 August 2024; Accepted: 6 November 2024
Please cite this article as: Hoang D.M., Ngo D.T., Huynh O.N., Nguyen T.K., Pham T.T., Le T.T., 2024, Impact of Acinetobacter soli on Tomato Plants, Chemical Engineering Transactions, 113, 73-78 DOI:10.3303/CET24113013

possibilities for leveraging the potential of *A.soli Y-3* in the development of innovative strategies to mitigate acrylamide formation in food processing (Jiao et al., 2020).
The findings of the study on the isolation and determination of bacteria capable of causing disease in tomatoes with green wilt disease have revealed the presence of *Acinetobacter soli*. This strain of bacteria has been identified with different effects on the growth of tomato plants.

2. Materials and methods

2.1 Isolation of *A.soli* from tomato plants exhibiting green wilt symptoms

Tomato leaf samples showing symptoms of green wilt were collected and rinsed under running water. The samples (Dong Nai, Vietnam) were then cut into small pieces of 3-4 mm using a sterilized knife, rinsed again with sterile distilled water, and dried. The cut diseased plant samples were placed into a mortar and ground with 0.85 % saline solution. The resulting sample solution was subjected to serial decimal dilutions with 0.85 % saline solution and spread onto Tetrazolium Chloride (Sigma, Germany) Agar (TZCA) medium for isolation. The Petri dishes with the spread sample solution were incubated at 37°C for 48 h to obtain separate bacterial colonies, which were then Gram stained. Each isolated bacterium was tested for pathogenicity by inoculating them onto tomato plants at the four true leaf stage. The development status of the tomato plants was recorded 14 d after infection.

2.2 Effect of *A.soli* on germination capacity of tomato seeds

Bacteria were cultured in LB medium (Trypton, yeast extract – Himedia- India, NaCl-Xialong-China) at room temperature with a shaking speed of 150 rpm. Tomato seeds (TN 448, Vietnam) were sterilized with 4 % H_2O_2 for 4 min, followed by rinsing with sterile distilled water 3 times. The tomato seeds were then immersed in a bacterial inoculum at various densities for 6 h. After soaking, the seeds were sown on Tribat soil (Vietnam). After sowing the seeds, germination of tomato seeds was recorded when the root tips emerged from the seed. Results were collected after 7 d from the sowing date.

2.3 Experiment on tomato plant infection

Tomato seeds were sterilized and placed on moist blotting paper until the radicle emerged. The seeds were then sown in clean Tribat soil (Viet Nam). When the plants began to produce true leaves, Howard Resh hydroponic solution (Pinho et al., 2024) was used to water the plants. At the 2-leaf and 4-leaf stages, 40 plants per treatment were randomly selected. The plants were wounded and sprayed with bacterial suspensions at different density from 2.5 x 10^4 CFU to 2.5 x 10^8 CFU/mL. The development of the plants in terms of height, root length, and number of leaves was observed. The results were collected by measuring root length and stem height on the 7^{th} day post-infection for the 2-leaf stage and by counting the number of tomato leaves and measuring stem height on the 30^{th} day post-infection for the 4-leaf stage.

2.4 Determination of auxin (IAA) and gibberellic acid (GA_3) activity in *A.soli* culture

Determination of IAA concentration in the bacterial culture: The amount of IAA in the microbial culture was determined using the optical density (OD) measurement method. The absorbance is measured at a wavelength of 530 nm using a spectrophotometer with Salkowski reagent (Gang et al., 2019).
Determination of GA_3 activity in the culture: Seeds are sown in Petri dishes with moist blotting paper and kept in the dark. After 24 h, when the radicles emerge from the seed coat, 30 germinated seeds are placed into beakers containing distilled water, *A.soli* culture, and the standard GA_3 solution. The beakers are covered with plastic wrap and placed under continuous light at an intensity of 3,000 lux, at a temperature of 28 ± 2°C. After 72 h, the hypocotyl height of seedlings is measured using a millimetre ruler. Differences in hypocotyl height indicate the equivalent activity of endogenous GA_3. GA_3 activity is directly proportional to the difference in hypocotyl length of the seedlings compared to the control (distilled water) and is calculated by comparison with a standard solution of GA_3 at 10 mg/L.

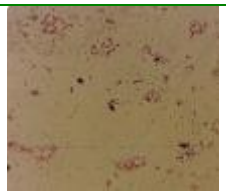
3. Results and discussion

3.1 Isolation of *A. soli* from tomato plants exhibiting green wilt symtoms

In a study focusing on the isolation of bacteria responsible for causing disease in tomato plants expressing symptoms of green wilt, among the eight bacterial strains exhibiting characteristics consistent with those of wilt-inducing bacteria on TZCA, *A. soli* showed a noticeable reduction in plant height. Specifically, the plants infected with *A. soli* at the 4-leaf stage and possessing infection density 2.5 x 10^8 experienced a significant decrease in height when measured 16 d post-infection in comparison to the non-infected control group. The plant height of the infected group was recorded at 17.89 cm, while that of the non-infected control group stood at 22.33 cm

(Table 1). The findings indicated that not only does the *A.soli* strain possess the capacity to synthesize L-asparaginase (*Jiao et al.*, 2020), but it was also discovered within tomato plants in Vietnam and has the unique ability to stunt their growth. This discovery sheds light on the multifaceted nature of this strain and underscores the importance of further research to better understand its effects on plant physiology.

*Table 1: A. soli shorten stem height. * indicated the significant value (p<0.05).*

A.soli on TZCA	Gram stain	Control (cm)	*A.soli* treatment (cm)
		22.33 ± 3.36	17.89 ± 3.08*

The influence of A. soli on plant growth may exhibit variability based on factors such as the density of infection and the plant growth stage. Research conducted on the timing and concentration of *A. soli* infection in tomatoes has been conducted in enhancing our comprehension of the properties and behaviours of the bacteria.

3.2 Effect of *A.soli* on germination period

Following the sowing of tomato seeds, the germination process is monitored by observing the emergence of root tips from the seed coat. Table 2 displays the germination rates of tomato seeds ranging from 87.5 % to 97. 5%. Interestingly, there was no notable difference in the germination rates of tomato seeds treated with *A.soli* at densities ranging from 2.5 x 10^4 to 2.5 x 10^8 CFU/mL. In conclusion, the treatment with *A.soli* at different densities did not have a significant impact on the germination rate of tomato seed.

Table 2: Effect of A.soli on germination capacity of tomato seeds

CFU/mL	0	2.5 x 10^4	2.5 x 10^5	2.5 x 10^6	2.5 x 10^7	2.5 x 10^8
Germination rate (%)	92.5	87.5	97.5	95	95	87.5

After the seeds germinated, stem colour, leaf colour, and leaf shape showed no differences between the treated groups and the control group. However, *A. soli* impacts the stem height of tomato plants 7 d after germination from seeds treated with these microorganisms. The results of the study showed a statistically significant difference in stem height between the control group and the treated groups, with *A. soli* increasing stem height in infected seeds. However, the different density of the infection did not have a significant effect on stem height. The findings of this study shed light on the potential role of *A. soli* in influencing the growth of tomato plants. The increase in stem height observed in infected seeds suggests that this particular microorganism may have a stimulatory effect on plant growth. This is consistent with previous research that has shown the beneficial effects of certain soil microorganisms on plant growth and development (Gómez-Godínez et al., 2023). Another explanation for this result could be that *A. soli* exerts its growth-promoting effects on plants through a mechanism that is not dependent on the density of the infection. It is also possible that there is a threshold level of infection beyond which further increases in density do not produce additional benefits in terms of plant growth. The study on the infection of *A. soli* at the seed stage and its impact on root length revealed intriguing results. It was observed that the presence of the bacterium initially led to an extension of root length in the plant. This phenomenon indicated a potential positive influence of *A. soli* on root development. However, the study also highlighted a crucial finding regarding the role of bacterial density in this process. In fact, when seeds were treated with varying bacterial densities ranging from 2.5 x 10^4 to 2.5 x 10^7 CFU/mL, a clear trend emerged. Root length showed a significant increase compared to the control group. However, as the density approached the upper limit of the range (2.5 x 10^8 CFU/mL), the elongation effect on roots was noticeably diminished. At the maximum density value, the root length did not show any significant difference from the untreated control group. This finding suggests that high bacterial densities may have a limiting effect on the ability of *A. soli* to promote root elongation (Table 3). These results were similar to a previous study, where the introduction of interacting bacteria to seeds increased stem and root length of pepper plant under drought stress conditions (Admassie et al., 2022). In addition, the concept of seed priming includes seeds treated with biological agents such as *Trichoderma* and *Azospirillum*, which help plants resist stress factors and support stem and root elongation (Devika et al., 2021).

Table 3: Effect of A.soli on stem height and root length at day 7th after germination. a, b in the same column showed significant difference value (p<0.05).

CFU/mL	Stem height	Root length
0	4.92 ± 0.45[a]	3.18 ± 0.51[a]
2.5 x 10^4	5.49 ± 0.65[b]	3.89 ± 0.69[a]
2.5 x 10^5	5.33 ± 0.39[b]	5.0 ± 0.97[b]
2.5 x 10^6	5.35 ± 0.43[b]	5.27 ± 0.99[b]
2.5 x 10^7	5.55 ± 0.4[b]	5.56 ± 0.89[b]
2.5 x 10^8	5.53 ± 0.57[b]	4.16 ± 0.88[a]

3.3 Effect of *A.soli* infection in 2-leaf stage

At the 2-leaf stage of tomato plants, infection of *A. soli* was conducted, resulting in no discernible impact on stem height. A notable reduction in root length was observed as a consequence of the infection. This decrease in root length exhibited a pattern influenced by the density of the infection. Within the infection density spectrum ranging from 2.5 x 10^4 to 2.5 x 10^8 CFU/mL, the root length displayed distinctive changes falling into two distinct intervals: declining from 7.13 cm to 7.83 cm and further from 5.18 cm to 5.63 cm (Table 4, Figure 1). Remarkably, these variations in root length were deemed to be statistically significant, underscoring the direct correlation between *A. soli* infection density and its impact on the root system of the tomato plants.

Table 4: Effect of A.soli infection on 2-leaf stage on stem height and root length. a, b, c in the same column showed significant difference value (p<0.05).

CFU/mL	Stem height	Root length
0	7.28 ± 1.05	9.71 ± 1.31[a]
2.5 x 10^4	6.8 ± 0.9	7.20 ± 1.25[b]
2.5 x 10^5	7.6 ± 0.87	7.83 ± 1.26[b]
2.5 x 10^6	7.67 ± 0.98	7.13 ± 1.41[b]
2.5 x 10^7	7.16 ± 1.09	5.18 ± 1.19[c]
2.5 x 10^8	7.98 ± 0.92	5.63 ± 1.34[c]

Figure 1: Tomato plant in 2-leaf stage experiment. (a) control- uninfected tomato plants, (b) tomato plant infected with 2.5 x 10^8 CFU/mL of A.soli.

3.4 Effect of *A.soli* infection in 4-leaf stage

When *A. soli* infect tomato plants at the 4-leaf stage, significant alterations in plant morphology are observed. One notable effect of this infection is the reduction in stem height across all infection densities, with infected plants exhibiting shorter stem lengths ranging from 61.23 cm to 65.93 cm as compared to the uninfected control plants, which maintained a stem height of 71.7 cm. The presence of *A. soli* resulted in a decrease in the number of leaves, with all infection treatments showing statistically significant differences when compared to the non-infected control group (Table 5).

Table 5: Effect of A.soli infection on 4-leaf stage on stem height and number of leaf. a, b, c in the same column showed significant difference value (p<0.05).

CFU/mL	Stem height	Number of leaves
0	71.7 ± 5.26[a]	10.39 ± 1.27[a]
2.5 x 10^4	64.34 ± 8.01[b]	9.32 ± 1.25[b]
2.5 x 10^5	61.31 ± 7.38[b]	9.34 ± 1.39[b]
2.5 x 10^6	65.93 ± 8.69[b]	9.36 ± 1.03[b]
2.5 x 10^7	63.61 ± 7.58[b]	9.4 ± 1.07[b]
2.5 x 10^8	61.23 ± 7.03[b]	8.51 ± 1.27[c]

Particularly noteworthy is the distinct impact of infection density on the number of leaves, with plants infected at density 2.5 x 10^8 CFU/mL displaying a pronounced reduction in leaf count (8.51 leaves) in comparison to plants infected at other densities, which maintained leaf numbers ranging from 9.32 to 9.4.These findings underscore the detrimental effects of *A. soli* infection on tomato plant growth and emphasize the importance of understanding pathogen behaviour for effective plant disease management strategies.

3.5 *A.soli* produced auxin (IAA) without synthesis of gibberellic acid (GA_3)

The examination of the influence of *A. soli* on the development of tomato plants at varying stages of infection, with a specific focus on stem length, root length, and the capacity to synthesize plant hormones like IAA and GA_3 was focused. A set of standardized concentrations of IAA was prepared in order to construct a correlation graph delineating the relationship between the concentration of IAA and the OD value at 530 nm. The resultant graph y = 0.0207 x + 0.0083 exhibits a notably high correlation coefficient with an R^2-value of 0.9817 (Figure 2). The cultivation of *A. soli* for a period of 36 h has been demonstrated to result in an indole-3-acetic acid (IAA) concentration of 1.48 µg/mL.

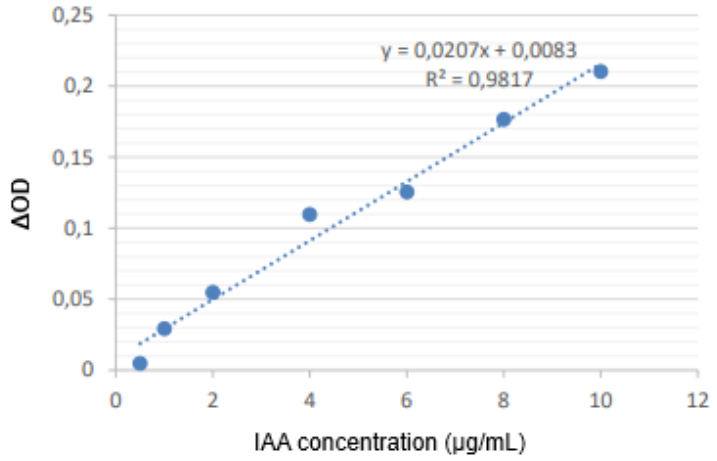


Figure 2: The relationship between the concentration of IAA and the OD value at 530 nm.

The efficacy of the bacterial culture as a potential source of secondary metabolites with a hypocotyl elongation effect analogous to that of GA_3 was investigated through direct application on seeds. The lengths of hypocotyls were meticulously measured to gauge the impact of the culture on seed development. To mitigate the potential interference of salts present in the bacterial culture on the germination process and subsequent hypocotyl elongation, the culture broth was diluted to a ratio of 1/8 and utilized as the soaking medium for seeds. The findings of the research clearly demonstrate that the 1/8 diluted bacterial culture significantly suppresses hypocotyl elongation in comparison to the control groups treated with GA_3 and water (Table 6).

Table 6: Testing for GA_3 activity. a, b, c showed significant difference value (p<0.05).

Effector	GA_3	*A.soli* culture	H_2O
Hypocotyl length	3.38 ± 0.15[c]	1.93 ± 0.11[a]	2.41 ± 0.12[b]

The *A.soli* culture has the effect of promoting root elongation (through the IAA contained in the culture medium) but inhibits hypocotyl elongation. When the bacteria infect at the 4-leaf stage, stem height was reduced. This is entirely consistent with the bacteria inhibiting stem elongation. Furthermore, these bacteria may compete with