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# Screening of Antagonistic Bacteria against Bacterial Wilt of Tomato, Eggplant and Potato in Bangladesh

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## Abstract

*Ralstonia solanacearum* (Smith) Yabuuchi et al is the causative agent of bacterial wilt that causes considerable damages in the yield of various crop plants. Five antagonistic bacteria against *R. solanacearum* were identified based on *in vitro* screening of 69 isolates of endophytic and plant pathogenic bacteria isolated from fresh and diseased specimens of various plants. While isolates SS21 and IB55 were effective against all isolates of *R. solanacearum*, isolates ST26, PE31 and AC53 demonstrated antagonistic activity only against tomato and eggplant isolates. Physiological and biochemical tests suggested that isolates SS21, ST26, PE31 and AC53 were Gram negative pseudomonads and isolate IB55 was a Gram positive bacterium. Molecular identification and field performance evaluation of these species are necessary prior to their applications as a biocontrol agent against bacterial wilt. 

**Keywords:** Antagonist; Bacterial wilt; *Ralstonia solanacearum;* Pseudomonads; Gram positive and Gram negative bacteria

## Introduction

*Ralstonia solanacearum* (Smith) Yabuuchi et al is responsible for the bacterial wilt disease that causes appreciable yield loss of various crop plants of different families (Hayward, 1991; Ali, 1993; Chakraborty *et al.*, 1994; Hayward and Hartman, 1994). The crop plants of solanaceae family, such as tomato (*Lycopersicon esculentum* L.), eggplant (*Solanum melongena* L.) and chilli (*Capsicum* spp.) are predominantly affected by *R. solanacearum* in various countries, including China, India, Egypt, Indonesia, Philippines and Brazil (Hayward and Hartman, 1994). In Bangladesh, the cultivations of eggplant, potato, tomato and tobacco are greatly affected by bacterial wilt caused by *R. solanacearum* (Miah and Hoque, 1987; 1991). In the current practices, cultivation of resistant varieties, crop rotation and soil disinfection with chemicals are usually used to control the wilt diseases of various crop plants (Bari *et al.*, 2001). However, these measures are not only unsatisfactory and ineffective in controlling the bacterial wilt but also some of them, such as chemical treatment of soil is hazardous to the environment and its biota. Thus, a bio-friendly green tech approach is extremely desired to control the *R. solanacearum* infection and wilt diseases in various crop plants.

The soil environment houses many species of antagonistic microorganisms that live in close proximity with other microorganisms and higher plants but provide immunity to certain plant species against certain microbes. These antagonistic microorganisms inhibit the growth of other microorganisms including plant pathogens by antibiosis, competition and exploitation. The exploitation of these natural phenomena might be a green tech approach to control the wilt diseases of economically important crop plants for human benefits (Cook and Baker, 1985). Here we report the *in vitro* identification of five antagonist bacteria against *R. solanacearum* from a total screening of 69 isolates of endophytic and plant pathogenic bacteria isolated from tomato, potato and eggplants in Bangladesh.

## Materials and Methods

Isolates of *R. solanacearum were* isolated from wilt infected plant specimens of tomato (*L. esculentum*), eggplant and potato and were used as indicator bacteria.

Sixty nine isolates of probable antagonistic bacteria were isolated from fresh and diseased plant specimens like root, stem and leaves collected from different locations of Bangladesh (Tables 1-5). They were tested against the three isolates of *R. solanacearum*:

Isolates of *R. solanacearum* which was identified according to Rahman (2002) using a series of physiological and biochemical tests. In the pathogenicity test, one isolates from tomato (T01) and one isolate from eggplant (E01) caused typical bacterial wilt symptoms in their respective host plant under inoculated condition. Wilt symptoms in the inoculated plants were confirmed through ooze test following Kelman (1954).

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**Table 1:** List of probable antagonistic bacteria isolated from wild plants

| Isolate no. | Host name | Host organs and Symptoms | Medium used | Colony characteristics | Locations |
|---|---|---|---|---|---|
| SS17 | *Solanum sysmbriifolium* | Fresh leaf | YPDA | Light green | BSMRAU* |
| SS18 | -do- | -do- | -do- | White, small | -do- |
| SS19 | -do- | -do- | -do- | White, big | -do- |
| SS20 | -do- | -do- | -do- | Greenish white small | -do- |
| SS21 | -do- | -do- | -do- | Big colony white, | -do- |
| ST22 | *S. torvum* | -do- | -do- | White, small | -do- |
| ST23 | -do- | Fresh stem | -do- | -do- | -do- |
| ST24 | -do- | -do- | -do- | Big,white, lethery | -do- |
| ST25 | -do- | -do- | -do- | Big watery | -do- |
| ST26 | -do- | -do- | -do- | Small | -do- |
| ST27 | -do- | Fresh root | -do- | Big lethery | -do- |
| ST28 | -do- | -do- | -do- | White lethery | -do- |
| SI41 | *S. indicum* | Fresh fruit | -do- | White, small | -do- |
| SI42 | -do- | Fresh stem | -do- | White, leathery | -do- |

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**Table 2:** List of probable antagonistic bacteria isolated from fruits and forest tree plants

| Isolate no. | Host name | Host organs and symptoms | Medium used | Colony characteristics | Locations |
|---|---|---|---|---|---|
| CL07 | Citrus limon | Canker on leaf | YPDA | Yellow | BSMRAU |
| VV10 | *Vitis vinifera* | Vine canker | -do- | Yellow | Kashimpur |
| PE31 | *Phyllanthus emblica* | Browny rot | 523 | White, small | Dhaka |
| PE32 | -do- | -do- | -do- | White, big | -do- |
| MS60 | *Musa* spp | -do- | -do- | Yellowish small | BSMRAU |
| AM33 | *Acacia moniliformis* | Fresh leaf | YPDA | Small colony white, | -do- |
| AM34 | -do- | Fresh leaf | -do- | White, big | -do- |
| AM35 | -do- | Fresh stem | -do- | Yellow, small | -do- |
| AM36 | -do- | -do- | -do- | Big, white | -do- |
| AI43 | *Azadiracta indica* | -do- | -do- | White colony | -do- |

**Table 3:** List of probable antagonistic bacteria isolated from vegetables and spices

| Isolate no. | Host name | Host organs and Symptoms | Medium used | Colony characteristics | Locations |
|---|---|---|---|---|---|
| PS08 | *Solanum tuberosum* | Soft rot | PSA | White | BSMRAU |
| BC09 | *Brassica chinensis* | Leaf spot | -do- | -do- | Kashim-pur |
| VV10 | *Vitis vinifera* | Canker of Vine | YPDA | Yellowish | -do- |
| CS11 | *Coriandrum sativum* | Soft rot on leaf | PSA | White | -do- |
| ST12 | *Solanum tuberosum* | Scab | YPDA | ,, | -do- |
| LN13 | *Lablab niger* | Leaf spot | -do- | Yellowish | -do- |
| BB14 | *Beta bengalensis* | Soft rot on leaf | -do- | White | -do- |
| IA15 | *Ipomoea aquatica* | -do- | -do- | Yellow | -do- |
| BO16 | *Brassica oleracea.var. botrytis* | Wilting | 523 | Whitish | -do- |
| ZO39 | *Zinziber officinail* | Fresh leaf | -do- | White, big | BSMRAU |
| ZO61 | -do- | Leaf spot | -do- | White, small | -do- |
| AS48 | *Allium sativum* | Fresh root | -do- | Small colony | -do- |
| AS49 | -do- | -do- | YPDA | Big colony | -do- |
| AS50 | -do- | Spot on leaf | -do- | ,, | -do- |
| AC51 | *Allium cepa* | Fresh root | -do- | Small colony | -do- |
| AC52 | -do- | -do- | -do- | Big colony | -do- |
| AC53 | -do- | -do- | -do- | White | -do- |
| OS59 | *Oryza sativa* | Grain rot | -do- | White Small | -do- |
| AC62 | *Amorphallus campanulatus* | Leaf spot | -do- | White, big | -do- |
| AC 68 | -do- | Stem canker | -do- | ” | -do- |
| CE63 | *Colocasia esculenta* | Leaf sheath rot | -do- | Very small, Yellowish | -do- |
| VS71 | *Vigna sesquipedalis* | Pod rot | -do- | White | -do- |

### *In vitro* Test to Screen Antagonistic Bacteria

Antibacterial activity of the probable antagonistic bacteria (producer bacteria) was tested *in vitro*. A fresh culture of the producer bacteria were grown on potato semi-synthetic agar (PSA) medium (5 g peptone, 15 g sucrose, 2 g $Na_2HPO_4.12H_2O$, 0.5 g Ca $(NO_3)_2$. $4H_2O$ and 15 g agar/L and potato decoction 300 g in 1 L water and pH 7). One loopful of 24 h old bacterial culture of producer bacteria was transferred to the center of Petri dishes containing 20 mL of yeast peptone dextrose agar (YPDA) medium. The plates were incubated at 30°C for 2 to 3 days. When the

**Table 4:** List of probable antagonistic bacteria isolated from ornamental plants

| Isolate no. | Host name | Host organs and Symptoms | Medium used | Colony characteristics | Locations |
|---|---|---|---|---|---|
| AV29 | *Aloe vera* | Blacksopt on leaf | 523 | White small | Dhaka |
| AV30 | -do- | -do- | -do- | White big | -do- |
| ST40 | *Sanseviera trifasciata* | Fresh leaf | YPDA | White, big | BSMRAU |
| TO64 | *Thuja orientalis* | Stem gall | -do- | Yellowish, big | -do- |
| TO65 | -do- | -do- | -do- | Big white, | -do- |
| DF66 | *Dracaena fragrans* | Blight on leaf | -do- | White, big | -do- |
| DF67 | -do- | -do- | -do- | small | -do- |
| DF69 | -do- | -do- | -do- | Yellow | -do- |
| DF70 | -do- | Leaf blight | -do- | White small | -do- |
| DF73 | -do- | -do- | -do- | White | -do- |

**Table 5:** List of probable antagonistic bacteria isolated from flowering plants

| Isolate no. | Host name | Host organs and Symptoms | Medium used | Colony characteristics | Locations |
|---|---|---|---|---|---|
| NI37 | *Nerium indicum* | Fresh leaf | YPDA | Light yellow, small | BSMRAU |
| NI38 | -do- | Fresh stem | -do- | White, big | -do- |
| TE44 | *Tagetis erecta* | Fresh root | -do- | White, big | -do- |
| TE45 | -do- | -do- | -do- | Greenish white small | -do- |
| DV46 | *Dahlia variabilis* | -do- | -do- | Big colony white, | -do- |
| DV47 | -do- | Fresh stem | -do- | White, small | -do- |
| IB54 | *Impatiens balsamina* | Fresh root | -do- | -do- | -do- |
| IB55 | -do- | Black rot on stem | -do- | White small | -do- |
| ZE56 | *Zenia elegans* | Fresh root | -do- | White | -do- |
| ZE57 | -do- | Fresh stem | -do- | White Small | -do- |
| ZE58 | -do- | Spotted leaf | -do- | Yellowish small | -do- |
| PT72 | *Polianthus tuberosa* | Leaf blight | -do- | White | -do- |
| PT74 | -do- | -do- | -do- | Yellow | -do- |

bacteria formed colonies of several millimeters in diameter, the plate was turned upside down. A sheet of filter paper was placed in the Petri dish lid with 0.5 mL chloroform. The dish was kept at room temperature (25±2°C) for 2 h. After complete exclusion of chloroform vapor, the bacterial colonies were overlaid with 5 mL melted water agar at 50°C containing a suspension of ca. $10^8$ cfu/mL of indicator bacteria (*R. solanacearum*). The plate thus prepared was incubated at 30°C for 2 days. If an inhibition zone appeared around any test bacteria, its semidiameter was measured to evaluate the antibacterial activity of the producer bacteria following a standard method (Furuya *et al.,* 1997).

### Characterization of Antagonistic Bacteria

Preliminary characterization of the producer bacteria that showed recognizable antagonistic effect against isolates of *R. solanacearum* was performed by physiological and biochemical tests. A series of physiological and biochemical tests such as gram reaction, potato soft rot, catalase production, oxidase reaction, arginine utilization, nitrate reduction and gelatin liquefaction test were done following standard methods described elsewhere (Schaad, 1988).

## Results

### Antagonistic Activity of Producer Bacteria

Among 69 isolates of producer bacteria, five isolates viz. SS21, ST26, PE31, AC53 and IB55 showed antagonistic effect against three isolates of *R. solanacearum*. Distinct inhibition zones were developed around the colonies of antagonistic bacteria (Fig. 1). Isolates SS21 (from *S, sysmbriifolium*) and isolate IB55 (from *Impatiens balsamina*) were antagonistic against all of the three isolates of *R. solanacearum.* Isolates ST26 (from *S. torvum*), PE31 (from *Phyllanthus emblica*) and AC53 (from *Allium cepa*) were antagonistic against the isolates from tomato (T01) and eggplant (E01) of *R. solanacearum* but ineffective against potato isolates (P01) of the wilt causing bacteria (Table 6).

### Characterization of Five Antagonistic Bacterial Isolates

**Gram-reaction, catalase, oxidase and potato soft rot test:** Among the five antagonistic isolates of bacteria, four were Gram-negative and isolate IB55 was Gram positive. In case of potato soft rot test all the isolates showed negative reaction. All of the isolates produced gas bubbles on glass slide after addition of $H_2O_2$ solution to the culture, which indicated positive reaction to catalase test. A deep purple color was developed by isolates SS21 and PE31, and was oxidase positive, ST26 showed delay positive and AC53 and IB55 showed negative results (Table 7).

**Arginine utilization, nitrate reduction and gelatin liquefaction test:** Arginine utilization test gave change in color in case of the isolates SS21 and ST26 indicating positive reaction, while no color change was observed for isolates PE31, AC53 and IB55 indicating negative reaction

**Table 6:** Antagonistic activity of five antagonistic bacterial isolates against three isolates of *R. solanacearum*

| Antagonistic bacteria | Width of the inhibition zone[a] | | |
|---|---|---|---|
| | T01[b] | E01[b] | P01[b] |
| SS21 | ++ | +++ | +++ |
| ST26 | ++ | ++ | - |
| PE31 | ++ | ++ | - |
| AC53 | ++ | ++ | - |
| IB55 | +++ | +++ | + |

[a]Activity index (diameter of inhibition zone mm): -, not detected; +, less than 5 mm; ++, 5-10 mm; +++, greater than 10 mm
[b]Abbreviations for indicator bacteria: T01, tomato isolate; E01, eggplant isolate; P01, potato isolate

**Table 7:** Physiological and biochemical characteristics of antagonistic isolates

| Tests | Antagonistic bacteria | | | | |
|---|---|---|---|---|---|
| | SS21 | ST26 | PE31 | AC53 | IB55 |
| Gram reaction test | - | - | - | - | + |
| Potato soft rot | - | - | - | - | - |
| Catalase | + | + | + | + | + |
| Oxidase | + | d+ | + | - | - |
| Arginine utilization test | + | + | - | - | - |
| Nitrate reduction test | + | - | + | + | + |
| Gelatin liquefaction | + | + | + | + | + |

+, = Positive,
-, = Negative
d+,= delay positive

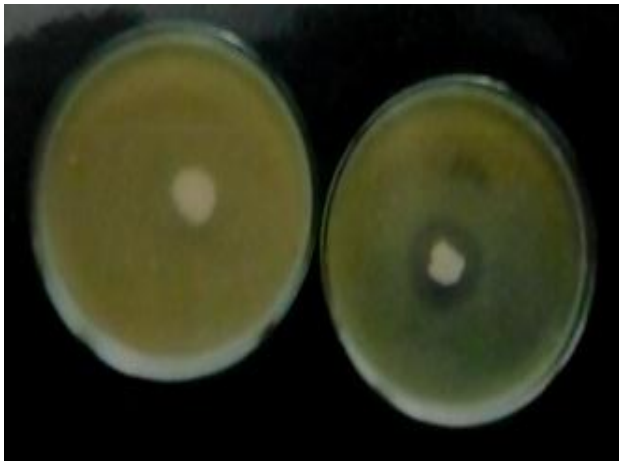


**Fig. 1:** Antibacterial activity showing inhibition zones of antagonistic bacterial isolates against *R. solanacearum* isolated from tomato (A) negative reaction and (B) positive reaction

to arginine utilization. In nitrate reduction test, all the isolates showed positive reaction with the development of orange brown color except ST26 isolate, which indicated the presence of nitrite. All of the isolates showed positive reaction in gelatin liquefaction test (Table 7).

The performed physiological and biochemical tests of the antagonist bacteria suggested that isolates SS21, ST26, PE31 and AC53 were the member of pseudomonads and Gram negative. However, isolates IB55 was a Gram positive pseudomonads.

## Discussion

Results of the present experiment for searching antagonistic bacteria against bacteria wilt pathogen (*R. solanacearum*) of tomato and eggplant clearly demonstrated that many isolates of various antagonistic bacteria possess the ability to inhibit the growth of plant pathogenic bacteria. The antagonistic activity varied greatly, depending upon the type of producer bacteria. Attempts made by different workers to control *R. solanacearum* with beneficial microorganism reveal that certain fungal and bacterial species are able to reduce the growth of *R. solanacearum* (Chen and Echandi, 1984; Furuya *et al.,* 1992; Phae *et al.,* 1992; Ariyanto *et al.,* 1994). Masanori *et al.* (1997) reported that *Pseudomonas aeruginosa* (ATCC 7700) strongly suppressed the growth of *R. solanacearum*. An antibiotic was extracted from the culture filtrate of this strain and identified as pyocyanine. Approximately 100 µg/mL of this extracted pyocyanine completely inhibited the growth of *R. solanacearum.* By soaking the roots of tomato seedlings in suspension of *P. aeruginoa* ATCC 7700 for 24 h before inoculation with *R. solanacearum* resulted in marked disease suppression under greenhouse conditions. Thus, the detected antagonistic bacteria in the present experiment possess antibacterial effect against *R. solanacearum.*

In conclusion, five isolates of bacteria, namely SS21, ST26, PE31, AC53 and IB55 were antagonistic against *R. solanacearum* isolated from various plant species. Antagonistic isolates SS21 and IB55 showed the strongest antibacterial activity against *R. solanacearum* isolated from all plant species, suggesting their probable applications as a bio-control agent for wilt disease. However, isolates ST26, PE31 and AC53 demonstrated antagonistic activity only against tomato and eggplant isolates. Further comprehensive study involving molecular identification and field trial is necessary for the applications of these antagonistic bacteria as a bio-control agent to bacterial wilt of various crop plants.

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