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Isolation and Characterization of Endophytic Bacteria Isolated from Legumes and Non-Legumes Plants in Egypt

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Endophytic bacterial isolates were isolated from roots, nodules, leaves and stems of faba bean (*Vicia faba*) , pea (*Pisum sativum*) , fenugreek (*Trigonella foenum-gracum*) , lupine (*Lupinus* spp.), common bean (*Phaseolus vulgaris*) and rice (*Oryza sativa*) at flowering stage. A total of 167 endophytic isolates were screened qualitative and quantitative for cellulase and pectinase activities. Result showed that more than 55 isolates out of 167 were able to produce cellulase and pectinase enzymes. Total of 55 isolates were screened as plant growth-promoters (PGP) traits and root colonization. The highest values of log CFU was $6.4x10^5$ of isolate TN10 inside the roots of faba bean plant. Moreover, 12 endophytic isolates produced IAA more than 25μ/ml in the presence of the precursor tryptophan. Also TN12, HN32 and RN62 isolates recorded the highest values of siderophores production. About 82% of the isolates showed positive results of HCN, 44 isolates were able to produce ammonia. The phosphate solubilization efficiency percentage were detected for endophytic isolates, 19 isolates showed the maximum range of phosphate solubilization efficiency (SE).

Keywords: Root colonization, endophytic bacteria, celullase, pectinase, PGP Traits, IAA, Phosphate solubilizers**.**

Plants are constantly involved in interactions with a wide range of bacteria. These plants-associated bacteria colonize the rhizosphere (rhizobacteria), the phyllosphere (epiphytes) and the inside of plants tissues (endophytic). Endophytes were sheltered from environmental stresses and microbial competition by the host plant and they seem to be ubiquitous in plant tissues, having been isolated from flowers, fruits, leaves, stems. Some endophytic bacteria exert several beneficial effects on host plants, such as stimulation of plant growth[35] , nitrogen fixation[30] and induction of resistance to plant pathogens[34], bacterial genra isolated from legume tissues include *Agrobacterium*, *Bacillus, Curtobacterium, Enterobacter, Erwinia, Mycobacterium, Paenibacillus, Pseudomonas, Phyllobacterium, Ochrobactrium, Sphingomonas* and others. Available reports indicate improved plant yield, plant health and nodulation when co-inculated with nodule endophytes, compared to inoculation with rhizobia alone[5, 35]. Endophytic bacteria can be defined as a group of beneficial free-living soil bacteria that colonize the inside root cells of plant without showing any external sign of infection on their host [3].The use of beneficial bacteria as agricultural inputs for increasing crop production needs the selection of competent rhizobacteria with PGP traits. Nature selects endophytes that are competitively fit to inhibit compatible niches within this nutritionally enriched and protected habitat of the root interior without causing pathological stress on the host plant. However, when screening these bacteria for achieving the most promising isolates having

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suitable colonization and PGP attributes[10].The aim of this study is to isolate and characterize of some endophytic bacteria and study their ability for production of plant growth promoters such as IAA, siderophores, phosphate solubilization, HCN, ammonia, cellulolytic and pectinolytic activity.

MATERIALS AND METHODS

Plant samples collection

About 167 endophytic bacterial isolates were isolated from healthy legumes and non-legumes plants such as faba beans (*Vicia faba*), peas (*Pisum sativum*), fenugreek (*Trigonella foenum-gracum*), lupine (*Lupinus* spp.), common beans (*Phaseolus vulgaris*) and rice (*Oryza sativa*) were obtained from agricultural fields at Qualubia governorate, Egypt .

Isolation of endophytic bacteria

Endophytic bacteria were isolated from roots, nodules, leaves and stem of legumes and non-legumes. The plants were collected at the flowering stage. Stems and roots were cut into sections 2.0-3.0 cm long. The tissue was put in beaker, soaked in distilled water and drained. They were rinsed in 70% ethanol for 30 seconds and then sterilized for 4 minutes in sodium hypochlorite (3%) and then washed ten times with sterile water[12, 10]. Surface-disinfected tissue was aseptically macerated with homogenizers. Macerated tissue was diluted into 10^{-1} dilution by adding 9 volumes of sterile distilled water. Serial dilution was made up to 10^{-6} dilution by taking 1 ml of well-shaken suspension and adding into 9 ml water blank tubes. 1 ml from appropriate dilutions were spread and plated on different media, Pikovskaya medium (PVK)[28], yeast extract mannitol agar medium (YEMA)[39], King's medium[20], and tryptic soya agar (TSA)[9, 4]. The plates were incubated at 28°C for 3 days. The pure colonies were selected according to color and morphological characteristics, picked up and transferred to slant specific media.

Screening of endophytic isolates

Cellulase activity

Qualitative screening of cellulase producers were done on carboxy methyl cellulose (CMC) agar[2].0.5 ml of bacterial suspension isolates were plated on CMC agar. The plates were incubated at 30°C for 5 days. At the end of the incubation, the culture surface was flooded with an aqueous solution of Congo red (1% w/v) for 15 minutes. The Congo red solution was then poured off, and the plates were further treated by flooding with 1M NaCl for 15 minutes. The formation of a clear zone of hydrolysis indicated cellulose degradation. Endo-b-1,4-glucanase activity was quantitatively determined according to the method of [6, 19], 100 µl of culture supernatant was added to 300 µl of 1.0% carboxy methyl cellulose (CMC) in 0.05 M sodium-acetate buffer (pH 7). The reaction mixture was incubated at 50°C for 25 min. Finally, the concentration of reducing sugars was determined and calculated as glucose. Dinitrosalicylic acid reagent (600 µl) was added to the enzyme reaction. The solution was boiled for 5 min and the absorbance was recorded at 540 nm after cooling down for 5 min. After subtraction of enzyme and substrate blank dinitrosalicylic acid reagent was added to the reaction mixture before incubation), the absorbance values were translated using the standard curves into micromoles of produced reducing sugars during the enzyme reaction.

Pectinase activity

Halls of 5mm in diameter were cut in the agar with the help of cork-borer[38] in the petri dishes containing pectin agar medium. Halls were filled with 0.5 ml of the tested isolates then, plates incubated for 4 days at 30°C. Iodine-potassium/iodide solution (1.0g iodine, 5.0g potassium iodide and 330 ml H_2O) added to the plate's surface to detect clearance zones[11].The enzyme activity was qualitatively detected by measuring the diameter of clear zone around the halls in millimeter. Pectinase activity was quantitatively assayed using a method described by[25]. Reaction mixture containing equal amounts of 1% pectin (0.5 mL) prepared in citrate buffer (0.05 M; pH5) and crude enzyme (0.5 mL). Blank solution maintained by using an enzyme (0.5 mL) with buffer (0.5 mL) instead of substrate was incubated at 50 °C in water bath for 30 min. 1.5 ml of. Dinitrosalicylic acid (DNS) reagent was added and the test tubes were shaken to mix the contents. The test tubes were heated to boiling on water bath for 5 min. The absorbance was measured at 540 nm using spectrophotometer. The standard curve was prepared for reducing sugars with glucose

Phosphate solubilization

Phosphate-solubilizing ability of bacteria was determined on Pikovskaya agar medium. The

isolates were spotted onto Pikovskaya agar and incubated at 28 ± 2°C for 3 days[10]. The presence of halo zone around the bacterial colony was considered as indicator for positive ability to inorganic phosphate solubilization. The results are expressed as solubilization efficiency (SE)[24].

$$SE = \frac{\text{Solubilization diameter}}{\text{Growth diameter}} \times 100$$

The ability of endophytic isolates for phosphate solubilization was quantitatively detected according to the method described by[22] as the following: Each isolate was singly cultured in 100 ml Erlenmeyer flask contained 20 ml of Pikovskaya's broth medium and inoculated with 5 ml of cell suspension, then incubated at 30±2 for 7 days. At the end of incubation period, the cultures were centrifuged at 5000 rpm for 20 minutes to remove the cells. One ml of supernatant was taken in test tube and one ml of detection solution (ascorbic acid in potassium molybdate and tartrate) was added and reaches to 10 ml with distilled water. The mixture was allowed to stand for 15 min. Intensity of the produced blue color was measured at 600 nm using spectrophotometer. Similarly, color was also developed in standard solution of potassium di hydrogen phosphate (KH_2PO_4).

Siderophores production

The ability of siderophores production by endophytic isolates was qualitatively detected according to[1]. 8-hydroxyquinoline (50 mg/L medium) was added to tryptic soya agar (10%) and inoculated with tested isolates. Ability of isolates to grow on this medium was considered as a positive result for siderophores production. Method described by[8] was used for detection of catecholate-type siderophores quantitatively. This method was performed by mixing 4.0 ml of culture supernatant with 0.25 ml 2 molar HCL, then 0.5 ml nitrite-molybdate reagent (sodium nitrite 20g/100 ml + sodium molybdate 20g/100 ml) was added. The identification of this type is detected by the formation of a yellow color.

IAA production

The obtained isolates were grown in 100 ml flasks containing 50 ml of specific medium supplemented with L-tryptophan (100 ¼g ml^{-1}). Flasks were inoculated with 2.5 ml of cell suspension 3-5-days old then, incubated at 30°C for 7 days. At the end of incubation period, cultures were centrifuged at 10,000 g for 15 min and the supernatants were collected. Two ml of Salkowsky reagent (12g $FeCl_3$ per liter in 7.9 M H2SO4) with two ml of the supernatant was allowed to react with at 28 ± 2°C for 30 min. Pink color developed indicating the presence of IAA was determined by measuring the absorbance by spectrophotometer at 535 nm[27].

HCN production

Production of HCN was estimated qualitatively according to the methodology described by[21]. The endophytic isolates were grown in nutrient agar supplemented with glycine (4.4 g L^{-1}). One sheet of the sterilized whatman filter paper was immersed in 1% picric acid in 10% sodium carbonate for 1 min then placed on the surface of the plate. The plates were sealed with parafilm and incubated at 28 ± 2°C for 2 days. Development of reddish brown color on the Whatman filter paper indicated production of HCN.

Production of ammonia

Indophytic bacterial isolates were tested for the ability to produce ammonia in nutrient broth. Freshly grown bacterial cultures were inoculated in 10ml nutrient broth in and incubated at 30°C for 48 hours in a rotator shaker at 200 rpm. After incubation period, 0.5 ml of Nessler's reagent was added and thoroughly mixed in each tube. The development of a yellow to brown color indicated a positive reaction for ammonia production[7].

Root Colonization

Seeds of faba bean, peas, fenugreek, lupine, common beans, and rice were surface sterilized with 70% ethanol for 2 min and with 5% sodium hypochlorite for 30 min then, seeds were washed three times with sterile distilled water and kept for germination on 1% agar for 3 days in the dark at 30°C. Seedlings were transferred to flasks (100 ml) containing 30 ml of MS medium. Each flask was inoculated with 1 mL of isolates suspension growth sterilized conditions and incubated under for 10 days. For colonization study roots from each plant were removed from the flasks after incubation period and sterilized using 95% ethyl alcohol and $HgCl_2$ and crushed aseptically. Contents were transferred to sterilized distilled water and after appropriate dilutions log CFU g^{-1} of fresh root weight was determined by dipped in 1 mL of sterilized distilled water and vortexes

vigorously. Appropriate dilutions were plated on tryptic soya (TSA) plates and incubated at 28±2°C and observed for microbial growth.

Statistical Analysis

Data were subjected to the statistical analysis according to[33] and the means were compared using L.S.D test at 5% significance level.

RESULTS AND DISCUSSION

Endophytic bacteria were isolated from sterilized root, nodules, leaves and stems of plants.

Isolation and screening of endophytics

A Total 167 bacterial isolates were isolated and performed to determine the clearing zone diameter of endophytic isolates grown on CMC agar medium as an indicator of endophytes isolates ability to produce cellulase through the halo zone observation[37].

Data in Table 1 show the clearing zone diameter of endophytic isolates, the isolates were categorized to four categories on the basis of clear zone size. Out of 167 isolates 12 isolates by 7.2%

Table 1. The clearing zone diameter of endophytic isolates grown on cellulose agar medium

Isolate No.	Clearing zone diameter [cm]	Isolate No.	Clearing zone diameter [cm]	Isolate No.	Clearing zone diameter [cm]	Isolate No.	Clearing zone diameter [cm]	Isolate No.	Clearing zone diameter [cm]
TN1	2.40	HN36	2.40	RN71	ND	HHN106	1.50	RRN141	2.83
TN2	ND	HN37	2.90	TTN72	ND	HHN107	1.25	RRN142	2.33
TN3	ND	HN38	2.16	TTN73	ND	HHN108	ND	RRN143	ND
TN4	0.82	HN39	1.70	TTN74	ND	HHN109	2.75	RRN144	0.20
TN5	0.50	HN40	1.53	TTN75	0.50	HHN110	0.20	RRN145	0.70
TN6	0.60	HN41	2.16	TTN76	ND	HHN111	3.50	RRN146	ND
TN7	0.80	HN42	1.50	TTN77	2.60	HHN112	1.20	RRN147	ND
TN8	ND	HN43	2.00	TTN78	2.73	HHN113	ND	RRN148	0.35
TN9	0.90	HN44	2.50	TTN79	3.00	HHN114	3.20	RRN149	2.92
TN10	ND	HN45	ND	TTN80	1.00	HHN115	3.00	RRN150	2.42
TN11	1.95	HN46	0.95	TTN81	ND	HHN116	1.85	RRN151	2.00
TN12	0.35	HN47	1.57	TTN82	ND	HHN117	0.57	RRN152	ND
TN13	0.55	HN48	2.06	TTN83	0.60	HHN118	1.50	RRN153	1.25
TN14	ND	HN49	ND	TTN84	ND	HHN119	3.00	RRN154	ND
TN15	3.25	HN50	2.75	TTN85	3.00	HHN120	1.50	RRN155	1.97
TN16	0.60	HN51	1.30	TTN86	ND	HHN121	1.40	RRN156	ND
TN17	1.85	HN52	ND	TTN87	ND	HHN122	1.70	RRN157	ND
TN18	1.00	HN53	ND	TTN88	ND	HHN123	1.10	RRN158	1.40
TN19	ND	HN54	ND	TTN89	2.23	HHN124	ND	RRN159	1.75
TN20	1.00	RN55	3.17	TTN90	ND	HHN125	1.57	RRN160	1.90
TN21	ND	RN56	2.90	TTN91	ND	HHN126	ND	RRN161	ND
TN22	ND	RN57	ND	TTN92	ND	HHN127	2.40	RRN162	1.75
HN23	1.00	RN58	2.85	TTN93	3.00	RRN128	1.58	RRN163	1.00
HN24	0.20	RN59	ND	TTN94	2.00	RRN129	1.25	RRN164	2.50
HN25	0.60	RN60	2.67	TTN95	2.50	RRN130	2.25	RRN165	1.97
HN26	1.20	RN61	ND	TTN96	1.03	RRN131	1.85	RRN166	1.55
HN27	0.90	RN62	1.60	HHN97	2.25	RRN132	1.62	RRN167	ND
HN28	2.05	RN63	0.55	HHN98	2.37	RRN133	1.00		
HN29	0.70	RN64	3.30	HHN99	2.20	RRN134	0.50		
HN30	0.60	RN65	ND	HHN100	ND	RRN135	0.75		
HN31	1.70	RN66	1.50	HHN101	1.00	RRN136	0.75		
HN32	1.95	RN67	1.93	HHN102	ND	RRN137	ND		
HN33	0.95	RN68	3.30	HHN103	2.95	RRN138	2.43		
HN34	1.60	RN69	0.60	HHN104	ND	RRN139	0.50		
HN35	2.35	RN70	ND	HHN105	2.1	RRN140	3.00		

Table 2. Cellulase activity of endophytic isolates

Isolate No.	Cellulase activity [U/ml]	Isolate No.	Cellulase activity [U/ml]	Isolate No.	Cellulase activity [U/ml]	Isolate No.	Cellulase activity [U/ml]	Isolate No.	Cellulase activity [U/ml]	Isolate No.	Cellulase activity [U/ml]
TN1	0.746746	HN32	0.227	RN63	0.305	TTN94	0.786	HHN125	0.128	RRN156	0.039
TN2	0.029	HN33	0.138	RN64	0.530	TTN95	0.437	HHN126	0.039	RRN157	0. 019
TN3	0.022	HN34	0.144	RN65	0.026	TTN96	0.369	HHN127	0.617	RRN158	0.254
TN4	0.128	HN35	0.959	RN66	0.487	HHN97	0.913	RRN128	0.241	RRN159	0.161
TN5	0.189	HN36	0.706	RN67	0.227	HHN98	0.469	RRN129	0.148	RRN160	0.507
TN6	0.558	HN37	0.424	RN68	0.750	HHN99	0.522	RRN130	0.683	RRN161	ND
TN7	0.883	HN38	0.390	RN69	0.185	HHN100	ND	RRN131	0.159	RRN162	0.120
TN8	0.032	HN39	0.149	RN70	ND	HHN101	0.143	RRN132	0.171	RRN163	0.099
TN9	0.588	HN40	0.141	RN71	0.031	HHN102	ND	RRN133	0.114	RRN164	0.683
TN10	0.028	HN41	0.650	TTN72	ND	HHN103	0.720	RRN134	0.054	RRN165	0.100
TN11	0.803	HN42	0.409	TTN73	0.048	HHN104	0.039	RRN135	0.633	RRN166	0.143
TN12	0.153	HN43	0.799	TTN74	ND	HHN105	0.650	RRN136	0.189	RRN167	ND
TN13	0.159	HN44	0.683	TTN75	0.158	HHN106	0.111	RRN137	0.029		
TN14	0.038	HN45	ND	TTN76	0.037	HHN107	0.329	RRN138	0.503		
TN15	0.853	HN46	0.123	TTN77	0.450	HHN108	0.028	RRN139	0.274		
TN16	0.035	HN47	0.460	TTN78	0.460	HHN109	0.587	RRN140	0.836		
TN17	0.608	HN48	0.723	TTN79	0.372	HHN110	0.023	RRN141	0.605		
TN18	0.142	HN49	0.022	TTN80	0.286	HHN111	0.434	RRN142	0.510		
TN19	ND	HN50	0.646	TTN81	ND	HHN112	0.256	RRN143	0.029		
TN20	0. 304	HN51	0.518	TTN82	0.031	HHN113	0.025	RRN144	0.024		
TN21	0.038	HN52	ND	TTN83	0.034	HHN114	0.656	RRN145	0.051		
TN22	0.023	HN53	0.074	TTN84	ND	HHN115	0.457	RRN146	0.026		
HN23	0.031	HN54	0.064	TTN85	0.786	HHN116	0.374	RRN147	ND		
HN24	ND	RN55	0.982	TTN86	ND	HHN117	0.138	RRN148	ND		
HN25	0.064	RN56	0.673	TTN87	0.024	HHN118	0.139	RRN149	0.733		
HN26	0.417	RN57	ND	TTN88	ND	HHN119	0.829	RRN150	0.620		
HN27	0.187	RN58	0.482	TTN89	0.675	HHN120	0.726	RRN151	0.350		
HN28	0.883	RN59	0.053	TTN90	0.039	HHN121	0.331	RRN152	ND		
HN29	0.131	RN60	0.743	TTN91	0.021	HHN122	0.198	RRN153	0.267		
HN30	0.101	RN61	0.019	TTN92	ND	HHN123	0.133	RRN154	0.060		
HN31	0.254	RN62	0.355	TTN93	0.816	HHN124	ND	RRN155	0.196		

ND. Not detected

has clear zone over 3.0 cm, 40 isolates by 24% has clear zone between 2.0-3.0 cm, 43 isolates by 25.7% recorded clear zone between 1.0-2.0 cm and 62 isolates by 37.1% has clear zone less than 1.0 cm . Clearly, the ability of endophytic bacteria to grow on cellulose proof that the cellulase play a role in the mechanisms by which endophytic bacteria penetrate into and persist in the host plant[14, 30].

Cellulase activity of endophitic isolates: Data in Table 2 show the cellulase activity of isolates on CMC medium. Various amounts of cellulase activity of the endophytic isolates were

Table 3. The clearing zone diameter of endophytic isolates grown on pectin agar medium

Isolate No.	Clearing zone diameter [cm]	Isolate No	Clearing zone diameter [cm].	Isolate No.	Clearing zone diameter [cm]	Isolate No.	Clearing zone diameter [cm]	Isolate No.	Clearing zone diameter [cm]
TN1	ND	HN40	2.70	TTN79	2.84	HHN118	ND	RRN157	ND
TN2	3.10	HN41	ND	TTN80	ND	HHN119	ND	RRN158	ND
TN3	ND	HN42	2.90	TTN81	ND	HHN120	ND	RRN159	1.83
TN4	1.00	HN43	ND	TTN82	ND	HHN121	ND	RRN160	2.37
TN5	1.10	HN44	2.30	TTN83	ND	HHN122	ND	RRN161	ND
TN6	1.07	HN45	1.50	TTN84	ND	HHN123	ND	RRN162	ND
TN7	1.97	HN46	ND	TTN85	1.50	HHN124	ND	RRN163	ND
TN8	ND	HN47	ND	TTN86	2.33	HHN125	ND	RRN164	ND
TN9	ND	HN48	ND	TTN87	1.73	HHN126	ND	RRN165	ND
TN10	2.50	HN49	ND	TTN88	1.90	HHN127	3.00	RRN166	ND
TN11	3.00	HN50	ND	TTN89	1.20	RRN128	2.50	RRN167	ND
TN12	1.95	HN51	ND	TTN90	2.50	RRN129	ND		
TN13	ND	HN52	ND	TTN91	ND	RRN130	ND		
TN14	ND	HN53	ND	TTN92	ND	RRN131	ND		
TN15	1.35	HN54	ND	TTN93	ND	RRN132	ND		
TN16	1.57	RN55	ND	TTN94	ND	RRN133	1.55		
TN17	ND	RN56	ND	TTN95	ND	RRN134	ND		
TN18	ND	RN57	ND	TTN96	3.25	RRN135	1.2		
TN19	ND	RN58	ND	HHN97	2.93	RRN136	1.35		
TN20	ND	RN59	ND	HHN98	2.00	RRN137	1.37		
TN21	ND	RN60	ND	HHN99	1.37	RRN138	ND		
TN22	ND	RN61	ND	HHN100	ND	RRN139	ND		
HN23	2.00	RN62	ND	HHN101	ND	RRN140	1.50		
HN24	1.25	RN63	1.1	HHN102	2.15	RRN141	2.00		
HN25	1.75	RN64	ND	HHN103	3.00	RRN142	3.13		
HN26	1.40	RN65	ND	HHN104	ND	RRN143	2.70		
HN27	1.20	RN66	ND	HHN105	ND	RRN144	ND		
HN28	2.10	RN67	ND	HHN106	2.17	RRN145	ND		
HN29	ND	RN68	ND	HHN107	ND	RRN146	2.50		
HN30	1.73	RN69	ND	HHN108	1.00	RRN147	ND		
HN31	2.57	RN70	ND	HHN109	ND	RRN148	3.00		
HN32	2.60	RN71	ND	HHN110	ND	RRN149	ND		
HN33	2.06	TTN72	1.00	HHN111	ND	RRN150	ND		
HN34	1.52	TTN73	ND	HHN112	1.00	RRN151	ND		
HN35	ND	TTN74	ND	HHN113	1.60	RRN152	ND		
HN36	ND	TTN75	ND	HHN114	ND	RRN153	2.67		
HN37	2.30	TTN76	ND	HHN115	ND	RRN154	1.73		
HN38	1.85	TTN77	ND	HHN116	ND	RRN155	2.73		
HN39	ND	TTN78	1.15	HHN117	1.6	RRN156	ND		

ND. Not detected

observed. The maximum value of cellulase activity was 0.982 U/ml which obtained by isolate RN55 , 44 isolates of total 167 by 26.3% gives cellulase amounts ranged from 0.50-0.98 U/ml.

Pectinase activity: Data in Table 3 display the ability of endophytic isolates to grow on pectin agar medium and gave a clear zone of hydrolysis. Total of 31 isolates out of 167 isolates by 18.6% were able to do a clear zone with 2.0-3.0 cm more in diameter. The maximum clear zone 3.25 cm was recorded by TTN96 isolate while as, the lowest range of clear zone was 1.0 cm obtained by HHN112, HHN108 and TN4 isolates. Results showed the highly pectin hydrolysis isolates were TTH96, RRN142, TN2, TN11, HHN127and RRN148.The clear zone of endophytic isolates on pectin medium proof the isolates ability to secreted pectinases[16].Table 4 displays the Pectinase activity and production of pectinase on pectin medium by 167 isolates of endophytic bacteria. The results

Table 4. Pectinase activity of endophytic isolates on pectin production medium

Isolate No.	Pectinase activity [U/ml]	Isolate No.	Pectinase activity [U/ml]	Isolate No.	Pectinase activity [U/ml]	Isolate No.	Pectinase activity [U/ml]	Isolate No.	Pectinase activity [U/ml]
TN1	0.179	HN36	ND	RN71	0.185	HHN106	1.920	RRN141	1.750
TN2	2.420	HN37	1.211	TTN72	0.389	HHN107	ND	RRN142	2.420
TN3	ND	HN38	1.00	TTN73	ND	HHN108	0.666	RRN143	2.260
TN4	0.498	HN39	ND	TTN74	ND	HHN109	ND	RRN144	ND
TN5	0.582	HN40	1.200	TTN75	ND	HHN110	ND	RRN145	0.195
TN6	0.275	HN41	ND	TTN76	0.179	HHN111	0.116	RRN146	1.400
TN7	1.043	HN42	1.090	TTN77	0.195	HHN112	1.08	RRN147	ND
TN8	0.163	HN43	ND	TTN78	0.758	HHN113	1.00	RRN148	2.790
TN9	0.170	HN44	2.00	TTN79	2.240	HHN114	ND	RRN149	ND
TN10	1.840	HN45	1.850	TTN80	0.179	HHN115	0.200	RRN150	ND
TN11	2.250	HN46	0.030	TTN81	ND	HHN116	0.179	RRN151	0.179
TN12	1.080	HN47	ND	TTN82	ND	HHN117	1.195	RRN152	0.208
TN13	0.179	HN48	ND	TTN83	ND	HHN118	ND	RRN153	2.790
TN14	0.112	HN49	ND	TTN84	ND	HHN119	0.222	RRN154	1.840
TN15	1.372	HN50	0.179	TTN85	1.080	HHN120	ND	RRN155	2.590
TN16	0.580	HN51	0.135	TTN86	2.090	HHN121	0.213	RRN156	ND
TN17	ND	HN52	ND	TTN87	1.580	HHN122	ND	RRN157	0.179
TN18	ND	HN53	ND	TTN88	1.550	HHN123	0.166	RRN158	ND
TN19	ND	HN54	ND	TTN89	1.010	HHN124	ND	RRN159	1.920
TN20	ND	RN55	0.167	TTN90	1.840	HHN125	ND	RRN160	1.090
TN21	0.176	RN56	0.175	TTN91	ND	HHN126	ND	RRN161	ND
TN22	0.178	RN57	0.112	TTN92	ND	HHN127	2.420	RRN162	ND
HN23	1.180	RN58	ND	TTN93	ND	RRN128	1.920	RRN163	ND
HN24	0.808	RN59	0.170	TTN94	ND	RRN129	ND	RRN164	ND
HN25	1.00	RN60	ND	TTN95	0.179	RRN130	ND	RRN165	ND
HN26	0.834	RN61	ND	TTN96	1.920	RRN131	0.179	RRN166	ND
HN27	1.095	RN62	ND	HHN97	2.00	RRN132	ND	RRN167	0.195
HN28	1.824	RN63	0.590	HHN98	1.824	RRN133	1.270		
HN29	ND	RN64	ND	HHN99	1.250	RRN134	0.190		
HN30	1.069	RN65	0.179	HHN100	0.108	RRN135	1.830		
HN31	1.841	RN66	ND	HHN101	ND	RRN136	1.080		
HN32	1.178	RN67	ND	HHN102	1.920	RRN137	1.080		
HN33	1.860	RN68	ND	HHN103	2.680	RRN138	ND		
HN34	1.252	RN69	ND	HHN104	ND	RRN139	ND		
HN35	0.120	RN70	ND	HHN105	0.183	RRN140	1.040		

ND: not detected

show that 54 isolates produced 1.0-2.0 U/ml or more of pectinase which detected spectrophotometricall. The isolate RRN148 recorded 2.790 U/ml as a maximum value of pectinase production, while, the isolate HN46 gave the minimum value of pectinase production. The pectinolytic activity may confirm an advantage for intercellular ingress spreading for endophytic bacteria into the host plant whereas the middle lamella between cell walls contains mainly pectin[14].

Phosphate solubilization detection

Results in Table 5 showed that out of 55 isolated endophytic bacteria, 19 isolates showed the maximum ranging from 300 to 562.50 % of solubilization efficiency percentage (SE). Also, data revealed that the isolates RN58, HHN109, HHN105, RRN155, TN12, HN33 and HHN127 has a high efficiency to dissolve phosphate; this result may be due to their ability to exert organic acids. Similar data were observed by[29,15]. As well as, results emphasized that the highly phosphate solubilization efficiency isolates were the highly producer of dissolved phosphate amount. Total of 18 isolates were showed negative results. These type of endophytic isolates which has the ability to dissolve phosphate are particularly of great interest

Table 5. Phosphate solubilization by the selected endophytic bacterial isolates

Isolate no.	Solubilization efficiency of phosphate %	Amounts of dissolved P ppm	Isolate no.	Solubilization efficiency of phosphate %	Amounts of dissolved P ppm
TN10	400.00	114.54	HHN98	350.00	86.66
TN11	-	ND	HHN99	172.73	ND
TN12	414.28	121.44	HHN102	227.27	88.32
TN15	-	ND	HHN103	208.33	28.29
HN23	312.50	77.28	HHN105	475.00	117.3
HN28	240.00	82.44	HHN109	537.50	122.78
HN32	146.67	ND	HHN114	181.82	ND
HN33	422.22	112.24	HHN117	300.00	88.78
HN35	250.00	18.63	HHN127	400.00	100.01
HN37	200.00	113.16	RRN128	166.67	ND
HN38	190.90	83.49	RRN130	181.82	ND
HN40	236.36	27.6	RRN138	220.00	44.12
HN43	280.00	ND	RRN140	153.85	ND
HN44	300.00	86.25	RRN141	300.00	111.32
HN45	200.00	26.61	RRN142	190.00	ND
HN48	133.33	ND	RRN143	171.43	ND
RN55	191.67	40.71	RRN148	312.50	78.39
RN56	277.78	71.76	RRN149	385.71	105.09
RN58	562.50	241.5	RRN150	233.33	ND
RN62	227.27	42.10	RRN153	388.88	121.90
RN64	388.89	75.67	RRN154	422.22	124.66
RN67	333.33	99.31	RRN155	428.57	128.34
RN68	140.00	ND	RRN160	227.27	27.85
TTN72	125.00	ND	RRN164	227.27	22.69
TTN77	240.00	21.85			
TTN78	-	ND			
TTN79	344.44	62.33			
TTN85	222.22	55.92			
TTN89	180.00	ND			
TTN90	257.14	89.73			
HHN97	220.00	40.99			

ND. Not detected

to agriculture land as it can improve the availability of phosphorus for plant growth[18]

Determination of siderophores production

Data in Table 6 showed that the selected 55 endophytic isolates were experimented to grow on tryptic soya agar medium. Results recorded that about 32 isolates were able to produce siderophores and gave positive results of growth also, 41 isolates were observed colorimetrically with yellow color as an indication of catecholate-tybe of siderophares positive result, whereas 14 isolates not able to produce siderophores. Both of TN12, HN32 and RN62 isolates were have the highest values of siderophores (catecholate-types). Results reveal that the RN62 isolate has a high possibility of root colonization, IAA and siderophors production[10] reported among all PGP traits of the bacteria, the frequency of IAA-producers was found much higher than other PGP traits.

Indol acetic acid (IAA) production

The results in Table 7 show that out of 55 endophytic selected isolates only 12 produced IAA

Table 6. Determination of siderophores production by the selected endophytic bacterial isolates

Isolate no.	Siderophores		Isolate . no	Siderophores	
	Qualitative detection	Catecholate-type detection		Qualitative detection	Catecholate-type detection
TN10	++	+	HHN97	++	+
TN11	+++	++	HHN98	+++	+
TN12	+++	+++	HHN99	-	+
TN15	+	++	HHN102	-	-
HN23	-	-	HHN103	-	-
HN28	+++	++	HHN105	++	++
HN32	+++	+++	HHN109	-	-
HN33	-	++	HHN114	-	-
HN35	-	-	HHN117	-	+
HN37	+	-	HHN127	-	-
HN38	-	+	RRN128	+++	+
HN40	+++	+	RRN130	+	+
HN43	-	-	RRN138	+	++
HN44	-	-	RRN140	-	+
HN45	+++	++	RRN141	-	+
HN48	+	-	RRN142	+++	+
RN55	-	+	RRN143	+	-
RN56	-	+	RRN148	+++	+
RN58	-	+	RRN149	+++	+
RN62	+++	+++	RRN150	+++	++
RN64	+++	++	RRN153	++	+
RN67	+	+	RRN154	-	+
RN68	+	+	RRN155	-	+
TTN72	+++	+	RRN160	-	-
TTN77	+++	+	RRN164	+++	+
TTN78	-	-			
TTN79	-	+			
TTN85	++	+			
TTN89	+++	+			
TTN90	**+++**	**+**			

Good +++ Moderate ++ Weak + Negative -

more than 25 µ/ml in the presence of the precursor tryptophan. The maximum amount of IAA was recorded by the isolate RN62, the isolate produced value of 92.52 µ/ml of IAA compared with other isolates. On the other hand, the lowest amount of IAA was produced by RRN164.The isolate RN62 not only produce IAA as a growth promoting, but also increased the rate of colonization of lupine root plant when used for colonization assay the results agree with[10, 31]

HCN and ammonia production

The results of HCN production showed that 45 endophytic bacteria out of 55 isolates were capable of producing HCN (81.8%), while, 42 isolates of endophytic isolates were produced ammonia (Table 8). Data detected that 12 isolates were gave high level (good) of positive HCN more than other isolates. Forty two isolates were able to produce ammonia while 13 isolate were unable to produce. Ammonia can be produced by several processes such as, nitrite ammonification[32], degradation of various amino acids and decarboxylation of amino acids to produce biogenic amines as well as ammonia [26].The production of ammonia is another characteristic of PGPR that indirectly influence development of plant[23].

Root colonization in legumes and non-legumes

Data in Table 9 showed that the selected endophytic isolates were detected to the plant root colonization of legumes and non-legumes plants such as faba been, peas, lupine, common beans, fenugreek and rice respectively, on the MS medium. The plant parameters root length, shoot length and root fresh weight were observed, as well as log CFU of endophytic bacteria inside the roots compared to control treatment. Recorded results indicated that the inoculated plants were enhanced in the root length, shoot length and root fresh weight. Similar results were observed

Table 7. IAA production (µg /ml) by the selected endophytic isolates from legumes and non-legumes plants

Isolate no.	values	Isolate no.	values	Isolate no.	values
		TTN77	1.12	RRN150	2.72
TN10	25.28	TTN78	ND	RRN153	47.60
TN11	11.64	TTN79	48.96	RRN154	10.16
TN12	23.2	TTN85	7.36	RRN155	41.00
TN15	0.45	TTN89	11.60	RRN160	1.4
HN23	45.6	TTN90	28.16	RRN164	0.40
HN28	45.00	HHN97	14.52		
HN32	1.04	HHN98	20.32		
HN33	20.64	HHN99	ND		
HN35	7.00	HHN102	4.60		
HN37	8.60	HHN103	1.90		
HN38	8.96	HHN105	11.96		
HN40	40.00	HHN109	21.9		
HN43	1.88	HHN114	11.80		
HN44	7.08	HHN117	7.64		
HN45	25.92	HHN127	24.16		
HN48	0.51	RRN128	6.84		
RN55	1.52	RRN130	1.24		
RN56	23.28	RRN138	8.04		
RN58	41.4	RRN140	6.64		
RN62	92.52	RRN141	9.12		
RN64	8.56	RRN142	2.52		
RN67	10.24	RRN143	1.20		
RN68	1.48	RRN148	27.12		
TTN72	1.52	RRN149	9.20		

ND: not detected

by[40] who reported that the bacteria to have direct influence on root length, root volume, number of secondary roots, increase the elongation zone, and dry weight due to the production of IAA. The highest values of log CFU recorded was $6.4x10^5$ of isolate TN10 inside the roots of faba bean plant, then the log CFU $83.6x10^4$ of isolates RN56 and RN58 respectively, inside lupine plant roots, while the isolate RN62 significantly increased the rate of root length, shoot length, root fresh weight and log CFU more than other selected endophytic isolates. The lowest number obtained was $25x10^2$ CFU plant root^{-1} of isolate RRN160. The main reason that attracts high concentration of bacteria around plant root is the presence of root exudates which contain free amino acids, proteins, carbohydrates, vitamins, and hormones[36, 17]. The increasing in root or shoot biomass was not correlated with the existence of a strain as endophytic but this was dependent on the ability of strain for growth promotion[13].

CONCLUSIONS

This study focused on a suitable screening for selection the best endophytic isolates which capable to produce high values of cellulase, pectinase, solubilizing phosphate, sidrophore, ammonia, HCN, high colonization, and indol acetic

Table 8. HCN and ammonia detection for the selected bacterial endophytic isolates

Isolate no.	HCN Detection	NH_3 Production	Isolate no.	HCN Detection	NH_3 Production
TN10	++	+	HHN98	+++	-
TN11	++	+	HHN99	+++	+
TN12	-	+	HHN102	+++	+
TN15	-	+	HHN103	-	+
HN23	-	-	HHN105	+	+
HN28	++	+	HHN109	++	+
HN32	-	-	HHN114	++	+
HN33	++	+	HHN117	+	+
HN35	+	-	HHN127	+	-
HN37	+	+	RRN128	++	-
HN38	+	-	RRN130	-	+
HN40	+++	+	RRN138	+	+
HN43	+	-	RRN140	+	+
HN44	++	-	RRN141	+	+
HN45	+++	+	RRN142	+++	+
HN48	++	-	RRN143	+	+
RN55	++	-	RRN148	++	+
RN56	+++	+	RRN149	-	+
RN58	+	+	RRN150	++	+
RN62	+++	+	RRN153	++	+
RN64	+	+	RRN154	-	+
RN67	+	-	RRN155	++	+
RN68	-	+	RRN160	+	+
TTN72	+	+	RRN164	+++	+
TTN77	+	+			
TTN78	+++	+			
TTN79	++	+			
TTN85	+	+			
TTN89	-	+			
HHN90	+++	+			
HHN97	+++	-			

Good +++ Moderate ++ Weak + Negative -

Table 9. Root colonization in legumes and non-legumes plants inoculated with the selected bacterial isolates

Plants	Isolate no.	Root length (cm)	Shoot length (cm)	Root fresh weight (g/tube)	log CFU
Faba bean	Control	2.00	2.50	0.10	-
	TN10	4	2	0.14	6.4×10^{5}
	TN11	3	6.5	0.15	64.4×10^{4}
	TN12	3	3	0.15	60×10^{4}
	TN15	4	4	0.18	4×10^{3}
Pea	Control	2.50	4.50	0.10	-
	HN23	5	5.5	0.16	80×10^{4}
	HN28	4	8	0.20	45.6×10^{4}
	HN32	3	4.5	0.12	60×10^{3}
	HN33	3	10	0.18	42×10^{4}
	HN35	3.5	7	0.15	50×10^{3}
	HN37	3	10	0.12	50×10^{4}
	HN38	3	8.5	0.20	80×10^{3}
	HN43	3	8	0.10	2×10^{3}
	HN44	2	9	0.11	60×10^{4}
	HN45	3.5	8	0.16	79.6×10^{3}
	HN48	4	9	0.15	78.8×10^{3}
Lupine	Control	3.00	7.00	0.15	
	RN55	8	9	0.29	62×10^{3}
	RN56	4	8	0.37	83.6×10^{4}
	RN58	4	8	0.17	82.8×10^{4}
	RN62	4	15	0.35	68×10^{4}
	RN64	4.5	9	0.22	56×10^{3}
	RN67	4.5	8	0.17	39.2×10^{3}
	RN68	3	7	0.15	31.6×10^{3}
fenugreek	Control	2.50	6.50	0.02	-
	TTN72	3.5	7	0.04	75×10^{2}
	TTN77	4.5	6.5	0.03	56×10^{3}
	TTN78	4	7	0.05	37.2×10^{3}
	TTN79	4	6.5	0.03	35.2×10^{4}
	TTN85	3.5	7	0.03	42.4×10^{3}
	TTN89	3	6	0.04	82.4×10^{3}
	TTN90	4.5	8	0.05	76.4×10^{3}
Beans	Control	2.50	12.5	0.04	-
	HHN97	10	18	0.28	79.6×103
	HHN98	10	17	0.25	42×103
	HHN99	6	13	0.24	46×103
	HHN102	7	12	0.24	52×103
	HHN103	5	17	0.24	40×103
	HHN105	3	14	0.20	38×103
	HHN109	4	15	0.31	37.2×103
	HHN114	5	13	0.13	38×103
	HHN117	2	10	0.09	42×103
	HHN127	4	10	0.11	64×103
Rice	Control	1.29	8.00	0.01	-
	RRN128	1.5	8	0.01	58×102
	RRN130	2	10	0.08	50.4×103
	RRN138	1.5	9	0.07	51.6×103
	RRN140	1.3	10	0.07	55.6×103
	RRN141	1.3	9	0.06	48.4×103
	RRN142	2	10	0.01	46×104
	RRN143	2.5	9	0.01	80.8×103
	RRN148	3	10	0.01	80×103
	RRN149	3.5	9	0.05	43.2×103
	RRN150	3	10	0.01	70×102
	RRN153	2.5	8	0.06	39.6×103
	RRN154	3	10	0.07	42×103
	RRN155	1	8.5	0.007	59.6×103
	RRN160	2	8	0.008	25×102
	RRN164	2.5	13	0.07	84×103

**Initial inoculum of approx. 3.2×107log CFU mL-1 of each bacterial isolates were added

acid. Finding good candidates isolates can save time and to be applied as a biofertilizer for future agriculture, providing higher production yields, and reduced input costs due to the use of agro-chemical

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