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Exploration of indigenous rhizobacteria: in search for their potential as plant growth promoting bacteria at two potato producing areas in West Sumatra

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Abstract. An experiment aiming at exploring and identifying indigenous rhizobacteria from Nagari Alahan Panjang and Nagari Batagak, two potato growing field at the Province of West Sumatra, has been conducted from July to October 2018. Soil was collected from the potato growing areas at Municipality Solok and Municipality Agam. One hundred grams of soil was collected from 25 spots of land at each area. Ten gram of soil was added to 9 mL of sterile distilled water prior to thorough mix in a vortex. The suspension was then subject to series of dilution to get 10⁻⁵ or 10⁻⁶ solution. Then, 0.1 mL of the final solution was poured into a test tube containing liquid NA medium and mixed thoroughly. The mixture was incubated for 48 hours at ambient temperature. Bacterial colonies went through series of re-culture until pure isolate was obtained and were observed for their morphological and physiological characters. Based on hypersensitivity reaction on leaves of *Mirabilis jalapa*, seventeen isolates found at Municipality Solok and 49 isolates were identified from Municipality Agam. These isolates did not cause leaf-tissue damage of *Mirabilis jalapa*. Different types and characters of the rhizobacteria is a broad range of biodiversity which will be potential for further screening for bio assays against major weeds in potato cultivation and may be used to open the windows to the natural immunity and wellness through induced resistant to major weeds.

1. Introduction

A major challenge in agricultural production system is continuously providing food for an ever-growing world population. Increasing food production cannot merely rely on intensive agricultural practices on arable land. This arable land constantly decreases around the world due to demand for other purposes such as housing, industrial expansion, and estate plantation. For decades, farmers rely on synthetic fertiliser and pesticides as well irrigation to keep food production which has been affected by the environmental factors, biotic as well as abiotic. Therefore, [1] proposed that the future world food production will need next-generation crop production systems and at the same time reducing dependence on synthetic fertiliser and pesticides.

Competition in land use for housing and a constant loss of arable land due to salinity and post-mining abandoned land will continuously reduce productive land for agriculture [2]. Furthermore, land conversion from its natural ecosystem to agricultural field results in serious impact to the environment such as global carbon cycle, global hydrological cycle, disruption in bio-diversities, and soil health and property [2; 3; 4; 5]. However, efforts should be sought to optimise agricultural practices to meet world demand for food while improving ecological balances.



As for other crops, potato interacts with unwanted organisms that exist in its vicinity such as pest, diseases, and weeds. Potato yield loss for weed-crop interaction have been reported. Reference [6] reported that weed control at rapid vegetative growth stage of potato (3 weeks after emergence) increased yield around 30-50% compared to that of non-controlled weeds. Other reports stated that weed reduced potato yield up to 45-65%; although the reduction varied between varieties and places [7; 8]. The most effective and efficient mean of weed control in potato production system is through improvement of agricultural practices which may reduce the green colour on potato tubers due to exposure of sun light [6]. However, this practice may reduce yield due to loss of lateral roots and soil compaction. Therefore, many farmers rely on the application of herbicides to control weeds in potato. For instance, farmers applied herbicide linuron (up to 960 kg/ha active ingredient) that sometimes was not enough to control weeds. Farmers, sometimes, mixed herbicides such as linuron and metribuzin, pendimethalin and clomazone, metribuzin and flufenacet [9] to increase the efficacy. Intensive use of herbicide will result in environmental problems such as residue in soil and development of weed resistant to herbicide. Alternative means to herbicide application should be sought including beneficial rhizobacteria.

Various microorganisms live surrounding roots of plants and many of them had been reported for their benefit to support plant growth as well as to improve plant adaptation to unpleasant environment such as low nutrient [10]. Plant roots produce exudates needed by the rhizobacteria as sources of nutrition [11]. This may play role as antagonistic agents and suppress soil born pathogen as well as enriching the composition and diversity of indigenous microorganisms [12; 13]. Rhizobacteria are often found at rhizosphere and mostly species- or location-specific. Some rhizobacteria are reported for their ability to increase plant growth and wellness through the absorption of Fe and Se and increased the chlorophyll content as much as 28% in *Arabidopsis thaliana* [14]; increase tolerance to drought in maize [15], induce tolerance to salinity stress [16], and induce plant immune system through interference in the function of auxin [17]. These will open the window for plant wellness improvement in attempts to increase plant competition to weeds. Research reported here is aimed at exploring and identifying indigenous rhizobacteria from two potato producing areas in the Province of Sumatera Barat and will be used for improvement of potato wellness against weed competition. Rhizobacteria that are associated with plant roots can be easily found in all agro-ecosystem [18].

2. Material and Method

Soil was collected from two potato producing areas in West Sumatra, namely Nagari Alahan Panjang at Municipality of Solok and Nagari Batagak at Municipality of Agam. The isolation and characterisation of rhizobacteria was conducted at the Laboratory of Microbiology of Faculty of Agriculture, Andalas University Padang from July to October 2018. About one hundred grams of soil was collected from each spot from 25 spots of the potato grown land at each municipality.

Debris such as roots and twigs were removed from the soil. The 10 g of soil was added to 10 mL of sterile distilled water prior to proper mixing for 3-5 minutes in a vortex. This suspension was then marked as 10-1 solution. One mL of the (10-1) soil suspension was transferred to test tube with 9 mL of sterile distilled water then being mixed at a vortex and was labeled as 10-2 solution. The dilution process was repeated until 10-6 solution has been obtained. Then, 0.1 mL (100 μ L) final soil suspension was poured into a test tube containing liquid NA medium prior to thoroughly mixed in a vortex. The mixture was then poured into a Petri dish, sealed, and incubated for 48 hours at ambient temperature.

Bacterial colony formed transparent zone of bacterial isolate. The isolate was then re-cultured onto fresh media using a loop ose. The re-culture was conducted several times until pure isolate was obtained. The pure isolates were then observed and identified for their morphological characters (color, shape, colony, colony surface, and shape of colony edge). The selected colony was then purified in streak plate using loop ose and was incubated for 48 hours at ambient temperature. A single colony of bacteria was then aseptically moved into a microtube containing 1 mL of sterile distilled water for preservation purposes. The tubes were kept in a refrigerator for later use.

Gram test for bacteria was conducted to separate the rhizobacteria either Gram-positive or Gram-negative. Another bacterial character tested was hiper-sensitive reaction (HR-pathogenesis) using

leaves of four o'clock flower (*Mirabilis jalapa*) following [19] with some modification. The isolate is pathogenic when causing necrotic on the leave of *Mirabilis jalapa* two weeks after exposure. Data were not statistical analysed as this research was more on exploration and observation of the morphological and physiological characters of the rhizobacteria.

3. Results and Discussion

There are 18 isolate of indigenous rhizobacteria found in the soil of potato field at NagariAlahanPangaj, Municipality of Solok. One isolate demonstrated to be toxic to the target plant (*Mirabilis jalapa*). However, there are more isolate of rhizobacteria found in the soil collected from NagariBatagak, Municipality of Agam. Fourty nine of 53 isolates are non-pathogenic to the target plants. Morphological characters of rhizobacteria are presented in Tables 1 and 2.

Table 1. Morphological and physiological characters of Rhizobacteria from Nagari Alahan Panjang, Municipality of Solok

No	Isolate Codes	Dilution	Form	Margin	Elevation	Size (cm)	Number of colony	Color	Gram Test	HR Test
1	L1 S1.1	10 ⁻⁵	Circular	Entire	Flat	0.1	1	White	(+)	(+)
2	L1 S2.2	10 ⁻⁵	Irregular	Undulate	Flat	1.5	3	Putih	(+)	(-)
3	L1 S3.1	10 ⁻⁵	Filamentous	Filiform	Flat	2.6	1	Putih	(+)	(-)
4	L1 S3.2	10 ⁻⁵	Irregular	Undulate	Flat	5.2	1	White	(+)	(-)
5	L1 S3.3	10 ⁻⁵	Circular	Entire	Convex	0.1	4	Yellow	(-)	(-)
6	L1 S3.4	10 ⁻⁵	Circular	Entire	Flat	1	1	White	(+)	(-)
7	L1 S4.2	10 ⁻⁵	Circular	Entire	Flat	1	1	White	(-)	(-)
8	L1 S4.3	10 ⁻⁵	Filamentous	Filiform	Flat	2	1	White	(+)	(-)
9	L1 S4.4	10 ⁻⁵	Circular	Entire	Flat	0.1	1	Yellow	(-)	(-)
10	L1 S4.5	10 ⁻⁶	Circular	Entire	Flat	0.3	5	Yellow	(+)	(-)
11	L2 S1.1	10 ⁻⁵	Filamentous	Filiform	Flat	3.5	1	White	(+)	(-)
12	L2 S1.2	10 ⁻⁵	Circular	Entire	Convex	0.2	9	White	(+)	(-)
13	L2 S2.3	10 ⁻⁵	Irregular	Undulate	Flat	0.1	1	Yellow	(+)	(-)
14	L2 S2.4	10 ⁻⁶	Irregular	Lobate	Flat	1	1	White	(+)	(-)
15	L2 S3.1	10 ⁻⁶	Filamentous	Filiform	Flat	2	3	White	(+)	(-)
16	L2 S3.4	10 ⁻⁵	Irregular	Undulate	Flat	1	1	White	(+)	(-)
17	L2 S3.5	10 ⁻⁵	Circular	Entire	Flat	0.2	1	Transpa- rent	(+)	(-)
18	L2 S3.6	10 ⁻⁵	Circular	Entire	Flat	0.1	2	Yellow	(+)	(-)

Remarks: HR test, (-) non pathogenic; (+) pathogenic

Table 2. Morphological and physiological characters of Rhizobacteria from Nagari Batagak, Municipality of Agam

No	Isolate Codes	Dilution	Form	Margin	Elevation	Size (cm)	Number of colony	Color	Gram Test	HR Test
1	L1.S1.1	10 ⁻⁵	Irregular Filamentous	Flat	Undulate	1.8	11	White	(-) (+)	(-)
2	L1.S1.2	10 ⁻⁵		Flat	Filiform	6.3	1	White	(+)	(+)
3	L1.S1.3	10 ⁻⁵	Circular	Raised	Entire	0.5	2	White	(+)	(-)
4	L1.S1.4	10 ⁻⁶	Circular	Flat	Entire	0.2	672	White	(+)	(-)
5	L1.S2.1	10 ⁻⁵	Irregular	Flat	Undulate	0.8	25	White	(+)	(-)
6	L1.S2.2	10 ⁻⁶	Circular	Flat	Entire	0.1	1540	White	(+)	(-)
7	L1.S3.1	10 ⁻⁶	Irregular	Flat	Lobate	0.6	50	White	(+)	(-)
8	L1.S4.1	10 ⁻⁵	Circular	Flat	Entire	0.4	48	White	(+)	(-)
9	L1.S4.2	10 ⁻⁵	Irregular	Raised	Filiform	2.5	31	White	(+)	(-)
10	L1.S4.3	10 ⁻⁶	Irregular	Flat	Undulate	2	25	White	(+)	(-)
11	L1.S5.1	10 ⁻⁵	Circular	Flat	Entire	0.2	1456	White	(-)	(-)
12	L1.S5.2	10 ⁻⁶	Irregular	Raised	Undulate	1.5	29	White	(+)	(-)
13	L2.S1.1	10 ⁻⁵	Irregular Filamentous	Flat	Undulate	3	28	White	(-)	(-)
14	L2.S1.2	10 ⁻⁵		Flat	Filiform	3	2	White Yellow	(+)	(-)
15	L2.S1.3	10 ⁻⁵	Circular	Raised	Entire	0.11	3	w	(-)	(-)
16	L2.S1.4	10 ⁻⁵	Circular	Flat	Entire	0.2	57	White	(+)	(-)
17	L2.S2.1	10 ⁻⁵	Irregular	Flat	Undulate	1.5	1	White Yellow	(+)	(-)
18	L2.S2.2	10 ⁻⁵	Circular	Conve	Entire	0.1	9	w	(+)	(-)
19	L2.S2.3	10 ⁻⁶	Circular Filamentous	Raised	Undulate	0.5	2	White	(+)	(-)
20	L2.S3.1	10 ⁻⁵		Flat	Filiform	1	3	White	(-)	(-)
21	L2.S3.2	10 ⁻⁵	Irregular	Raised	Undulate	0.8	49	White	(+)	(+)
22	L2.S3.3	10 ⁻⁵	Circular	Conve	Entire	0.1	1000	White	(-)	(-)
23	L2.S4.1	10 ⁻⁵	Circular	x	Entire	0.3	116	White	(-)	(-)
24	L2.S4.2	10 ⁻⁵	Irregular	Flat	Undulate	2	33	White	(+)	(-)
25	L2.S5.1	10 ⁻⁵	Circular Filamentous	Raised	Entire	0.3	323	White	(+)	(-)
26	L2.S5.2	10 ⁻⁵		Flat	Filiform	1.5	1	White	(+)	(-)
27	L2.S5.3	10 ⁻⁵	Irregular	Flat	Undulate	4	5	White	(+)	(-)
28	L3.S1.1	10 ⁻⁵	Circular	Flat	Entire	0.3	52	White	(-)	(+)
29	L3.S1.2	10 ⁻⁵	Irregular Filamentous	Flat	Undulate	1	30	White	(-)	(-)
30	L3.S1.3	10 ⁻⁶		Flat	Filiform	2.5	1	White Yellow	(-)	(-)
31	L3.S2.1	10 ⁻⁵	Circular	Flat	Entire	0.1	1124	w	(-)	(-)
32	L3.S2.2	10 ⁻⁵	Irregular	Flat	Lobate	1	15	Putih	(-)	(-)
33	L3.S3.1	10 ⁻⁵	Irregular	Flat	Entire	0.2	2440	Yellow	(-)	(-)

No	Isolate Codes	Dilution	Form	Margin	Elevation	Size (cm)	Number of colony	Color	Gram Test	HR Test
34	L3.S3.2	10 ⁻⁶	Irregular	Flat	Undulate	0.6	39	White	(-)	(-)
35	L3.S4.1	10 ⁻⁵	Irregular	Flat	Undulate	0.7	168	Yellow	(-)	(-)
36	L3.S4.2	10 ⁻⁶	Circular	Raised	Entire	0.1	384	Red	(-)	(-)
37	L3.S5.1	10 ⁻⁵	Circular	Raised	Entire	0.1	88	White	(+)	(-)
38	L3.S5.2	10 ⁻⁵	Irregular	Raised	Undulate	1	11	White	(-)	(-)
39	Ag4.S1.1	10 ⁻⁵	Circular	Flat	Entire	0.2	2443	White	(-)	(+)
40	Ag4.S1.2	10 ⁻⁶	Irregular	Flat	Undulate	0.3	12	White	(-)	(-)
41	Ag4.S2.1	10 ⁻⁵	Irregular	Flat	Undulate	2	18	White	(-)	(-)
42	Ag4.S2.2	10 ⁻⁶	Circular	Convex	Entire	0.2	42	Yellow	(-)	(-)
43	Ag4.S2.3	10 ⁻⁶	Irregular	Convex	Undulate	0.2	2	Red	(-)	(-)
44	Ag4.S3.1	10 ⁻⁵	Irregular	Flat	Undulate	0.4	20	White	(+)	(-)
45	Ag4.S3.2	10 ⁻⁶	Circular	Raised	Entire	0.1	4	White	(-)	(-)
46	Ag4.S4.1	10 ⁻⁵	Circular	Raised	Entire	0.1	4	Yellow	(-)	(-)
47	Ag4.S4.2	10 ⁻⁵	Circular	Flat	Entire	0.2	36	Yellow	(-)	(-)
48	Ag4.S4.3	10 ⁻⁵	Irregular	Flat	Undulate	0.6	8	White	(+)	(-)
49	Ag4.S4.4	10 ⁻⁶	Filamentous	Flat	Filiform	3	1	White	(+)	(-)
50	Ag4.S5.1	10 ⁻⁵	Irregular	Flat	Undulate	4	14	White	(+)	(-)
51	Ag4.S5.2	10 ⁻⁵	Filamentous	Flat	Filiform	3	2	White	(+)	(-)
52	Ag4.S5.3	10 ⁻⁶	Filamentous	Raised	Filiform	0.5	3	Broke	(+)	(-)
53	Ag4.S5.4	10 ⁻⁶	Circular	Convex	Entire	0.3	40	White	(-)	(-)

Remarks: HR test, (-) non pathogenic; (+) pathogenic

The number of colony of rhizobacteria from Municipality of Agam was nearly triple than that of Municipality Solok. This may resulted from intensive application of pesticide for horticultural crops in Solok (personal communication with some farmers and observation, 2017 and 2018) which would in turn reduce beneficial microorganisms in the soil.

Farmers rotate potato cropping system with shallot, tomato, and cabbage with various pesticides applied to the crops and land. This was intended to cut the pest and disease life cycle in the field. However, rotating crops from similar family group will enhance the life and environment suitable for the pest and diseases. Careful practices should then be taken into account when rotating plants.

Higher number of isolates and colonies of rhizobacteria from Nagari Batagak at Municipality Agam may, to some extent, may be resulted from the increased awareness of farmers to reduce pesticides. Some farmers and farmers' group tried to apply bio-pesticides to protect their crops. They are in the process of shifting from conventional to organic farming in producing potatoes. Better environment for soil microorganisms including rhizobacteria would be achieved by less residue of pesticides and high organic matter [20] that provide organic compounds to support the diversity and activity of soil microbia [21]. This is in accordance with the finding of [22] who demonstrated that organic matter increased soil microbial biomass, activity, and diversity. The increase was observed within a 20-day period afterwards declined over a longer time.

Soil quality determines its capacity to function in an ecosystem to support biological productivity, to maintain the quality of environment, and to facilitate the health of animals and plants in that ecosystem [23], and is important for the survival of microorganisms [24]. Intensive uses of pesticide may result in imbalance of soil biology. It is common that the number and type of rhizobacteria will vary according to the distance from roots due to root exudates released and utilised as nutrient source for the rhizobacteria [11; 25]. Moreover, certain rhizobacteria will associate with certain crop depends on the compatibility of the two species. This will open the windows to the natural plant immunity and wellness through induced resistance [26].

The complex mechanism of plant and soil bacteria may lead to the production of plant growth regulators. The chemical substances range from secondary metabolites to enzymes [27]. They signal molecules to act as chemical communication leading to supporting the growth and development of the host plants [28]. Ref. [29] reported that rhizobacteria isolated from the roots of sweet potato produced IAA and solubilised phosphate. The solubilisation of inorganic phosphate was detected as an increase of phosphate content in the potato shoot systems. They recommend the utilisation of the isolates as potential bioinoculants to reduce dependency on chemical fertiliser. This practices support environmentally-friendly agricultural system.

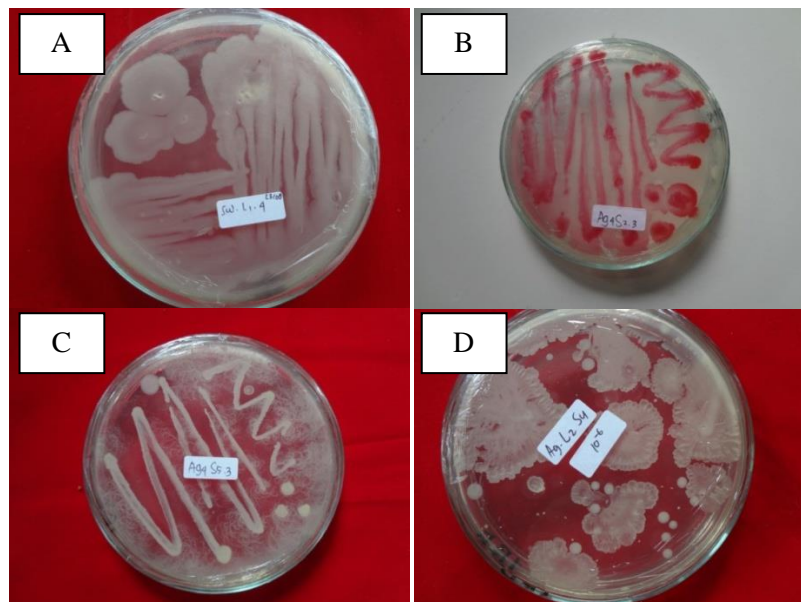


Figure 1. Isolate of rhizobacteria isolated from potato-growing areas at Municipality Agam. (A: Circular, flat, white, from Agam. B: Irregular, convex, red, from Agam. C: Filamentous, raised, broken white, from Agam. D: Circular, convex, white, from Agam)

The research reported here found isolates of rhizobacterial colony in various sizes ranging from 0.1 to 6.3 cm. Shape, color, margin, and elevation divers within the isolated bacteria. Most of the isolate are white but some are filamentous. Some isolates with different morphological characters are presented in Figure 1 and 2.

Various form and color of rhizobacteria was found from two potato producing areas. Some photos are presented in Figure 1 and 2.

Sixty six isolates of the total 71 isolates from both potato growing areas in West Sumatra did not cause any tissue damage on the leaves of four o'clock flower (*Mirabilis jalapa*). This is a good sign of bacteria and plants compatibility in nature. The rhizobacteria in association with potato plant roots, to some extent, would increase plant growth through various mechanisms including nutrient uptake and promoting the production of growth hormones. Reference [30] stated that hypersensitive is a defence mechanism develop in plant in response to microorganism infection. Infected cells often be characteristically observed through an increase in membrane permeability which lead to the death of host cells nearby the infected cells. Root exudation attracts soil microorganisms in such a complex mechanisms that might be interaction-specific and recognition processes depending upon the plant cultivars, environmental stress, and plant growth stage [31]. Therefore, different colonisation might be influenced by different root exudates and some sites are better colonised than others and resulted in spatial differences in colonisation [32]. This will support the exploration of indigenous rhizobacteria to be utilised within the specific areas.

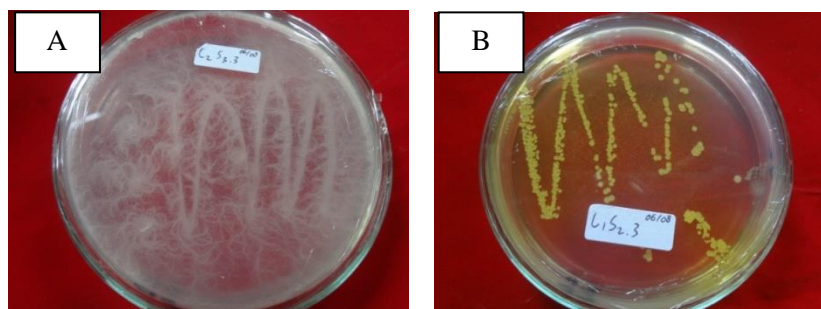


Figure 2. Isolate of rhizobacteria isolated from potato-growing areas at Municipality Solok. (A: Filamentous, filiform, white, from Solok. B: Circular, flat, yellow, from Solok)

4. Conclusion

Various isolates of rhizobacteria was found at two potato producing areas in West Sumatra. Seventeen isolates were from Municipality Solok and 49 isolates were identified from Municipality Agam. Different types and characters of the rhizobacteria is a broad range of biodiversity which will be potential for further screening for bio assays againts major weeds in potato cultivation.

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