Diversity of Nitrogen-Fixing Bacteria Associated with Yam (*Dioscorea* spp.)

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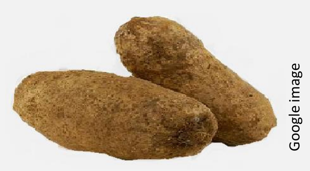

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STUDY BACKGROUND

Yam (*Dioscorea* spp.) is a tropical crop mainly produced for it tuber (underground organ), a good source of carbohydrates. More than 600 species have been identified within this genus, with *Dioscorea alata*, *D. rotundata*, *D. esculenta*, *D. bulbifera, D. trifida* and *D. dumetorum* being the most cultivated. 95% of World production is held in West Africa where at least 60 million people depend on it for their food (Asiedu and Sartie, 2010).

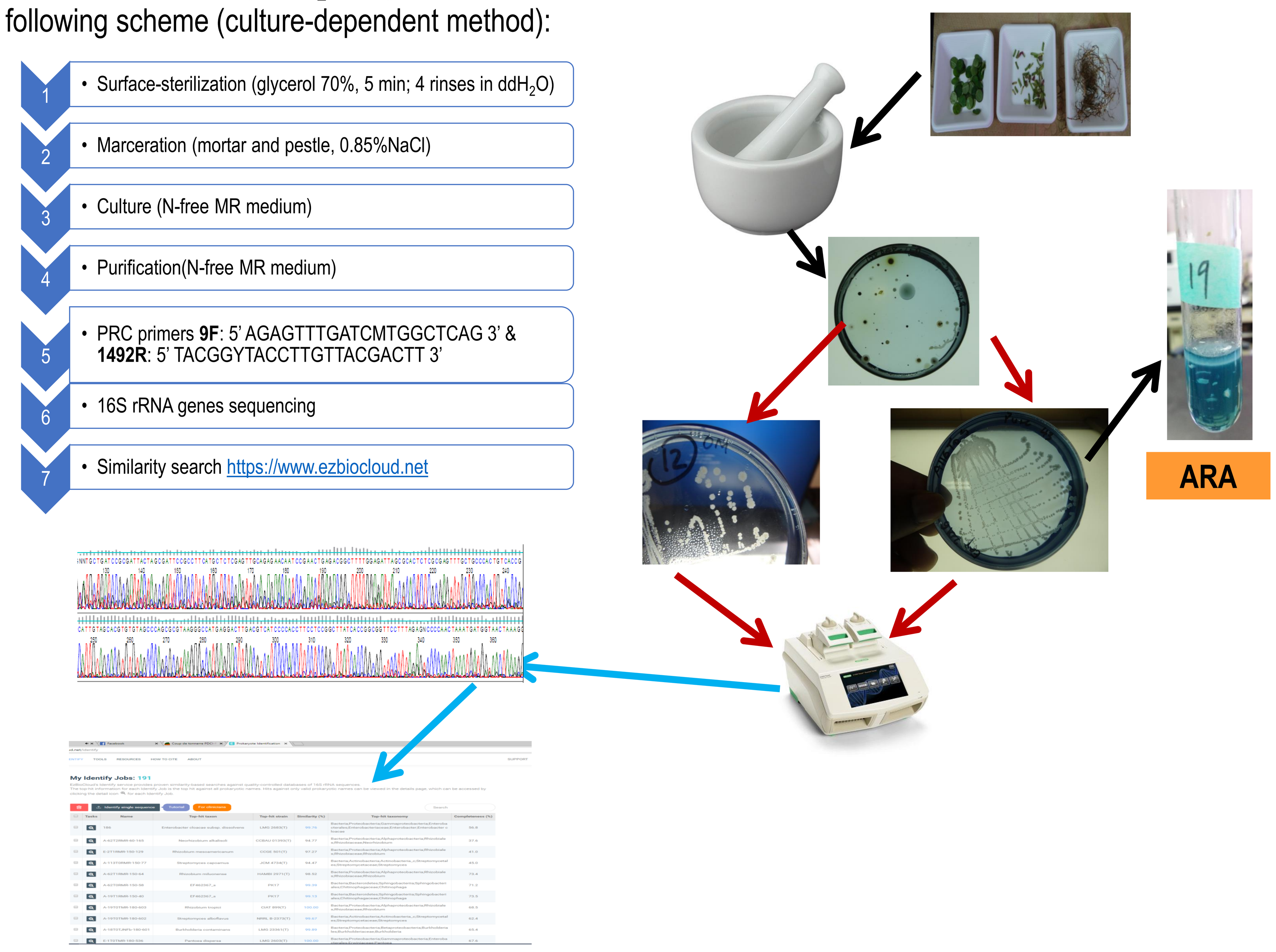
Yam cultivation needs nutrient-rich soils. Research outputs on mineral Nitrogen (N) applications showed inconsistent results. Shiwachi *et al.* (2015) reported strong effects and differences of genotypes on yam nutrition, and Takada *et al*. (2017) observed that accession A-19 in *D. alata* is able to grow in unfertile soil with the help of some N_2-fixing endophytic bacteria, as reported for other non-leguminous plants (rice, sugarcane, sweet potato). Later, symbiotic relationship between N_2-fixing endophytic bacteria and accession E-2 in *D. esculenta* was reported (Rezaei *et al*., 2017) .

This opened new technology in yam cropping systems by introduction of use of beneficial bacteria for sustainable production. However, there is a lack of knowledge about the remaining several yam accessions and varieties.

The aim of this study is to investigate the diversity of N_2-fixing endophytic bacteria associated with yam.

METHODOLOGY

Nineteen (19) accessions from different origins were tested: *D. alata* (16) and *D. esculenta* (3) in pot experiment in Miyako island, Okinawa Prefecture (Japan). Plants were grown in nutrient-poor deep soil for 160 days after planting (DAP) and treated with urea or without urea (control). Nitrogen content of plant was determined. Endophytic N_2-fixing bacteria were isolated from roots, stems and leaves as described in the following scheme (culture-dependent method):



RESULTS

1. Nitrogen in yams *(Dioscorea* spp.)

Table1. Nitrogen content (%) and uptake (mg) in yam species at 160 DAP

Yam species	Treatment	N (%)		N uptake (mg)		
		above-ground	below-ground	above-ground	below-ground	Total N (mg/plant)
D. alata	control	1.7ᵃ	1.4ᵃ	80.2ᵃ	85.9ᵃ	166.1ᵃ
	urea	2.1ᵃ	1.8ᵃ	94.1ᵃ	119.4ᵃ	213.4ᵃ
D. esculenta	control	1.6ᵃ	1.4ᵃ	122.0ᵃ	140.8ᵃ	282.3ᵃ
	urea	2.1ᵃ	2.1ᵃ	136.0ᵃ	165.7ᵃ	301.8ᵃ

In column, means followed by same letter are not significantly different at p=5% (LDS) using Tukey test.

→ Urea application did not significantly increase plant N content and uptake as compared to non-treated plants in both yam species.

2. Endophytic N_2-fixing bacteria of yams *(Dioscorea* spp.)

Table2. Effect of accession origins on number of endopytic N_2-fixing bacteria isolated in *Dioscorea* spp.

Origin of accessions (Acc.)	Nb of Acc.	Nb of isolates	[%]	Isolate/Acc.
Indonesia	1	2	3.6	2.0
Japan	6	32	57.1	5.3
Miyanmar	3	11	19.6	3.6
PNG	1	5	8.9	5.0
Taiwan	2	4	7.1	2.0
Unknown (Kagoshima, JP)	1	2	3.6	2.0

- 17 isolates (control)
- 39 isolates (urea)

In total, 56 endophytes were found, belonging to 17 bacterial genera.

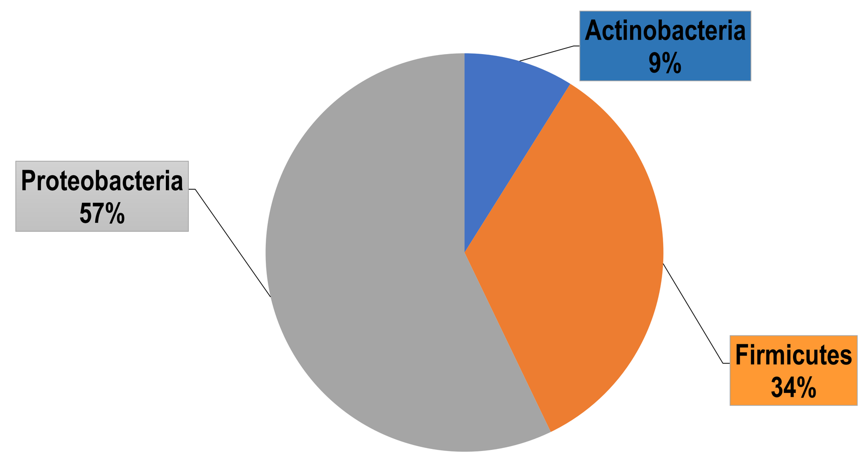


Fig1. Bacterial phyla found in N_2-fixing endophytic bacteria of yam (*Dioscorea* spp.)

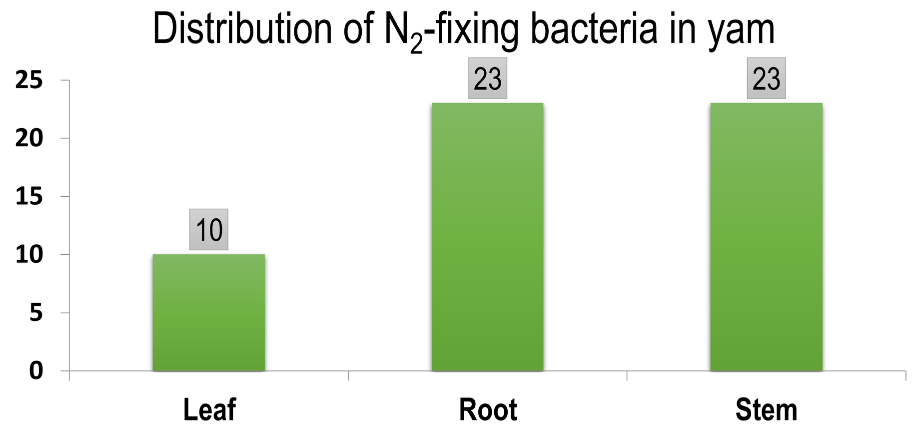


Fig2. Number of bacteria isolated in different organs of yam (*Dioscorea* spp.)

Urea application did not reduce number of endophytic bacteria in yam unlike reported in some studies (Wemheuer *et al*., 2017).

Strains of Proteobacteria accounted for 57.14 % of total isolates, followed by Firmicutes (33.92%) and Actinobacteria (8.92%). Most endophytes were isolated from stems (23) and roots (23), while only 10 were from the leaves.

Four genera (*Bacillus*, *Curtobacterium*, *Rhizobium* and *Sphingomonas*) belonged to both yam species (Fig3.)

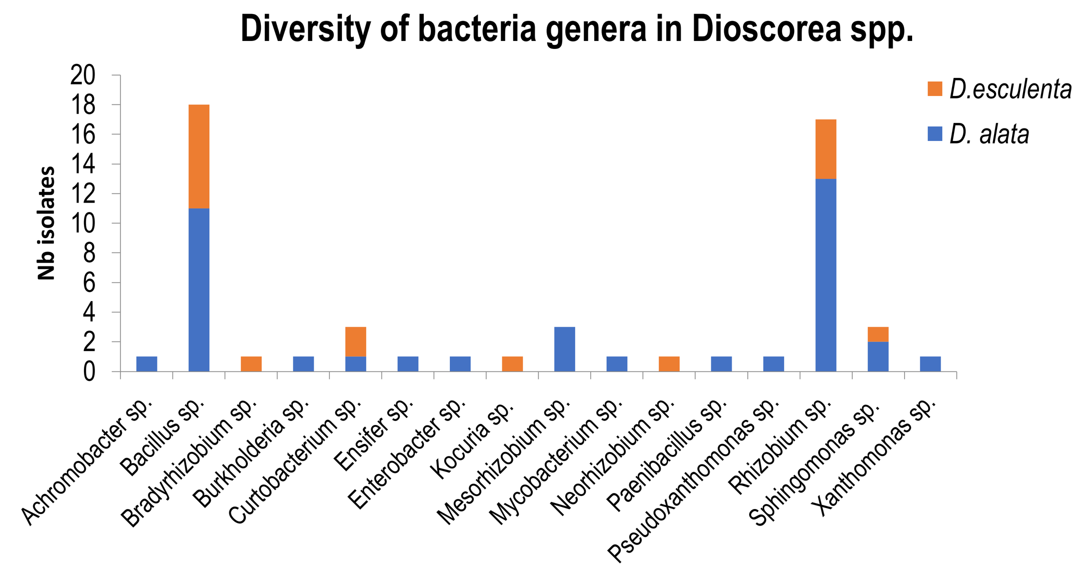
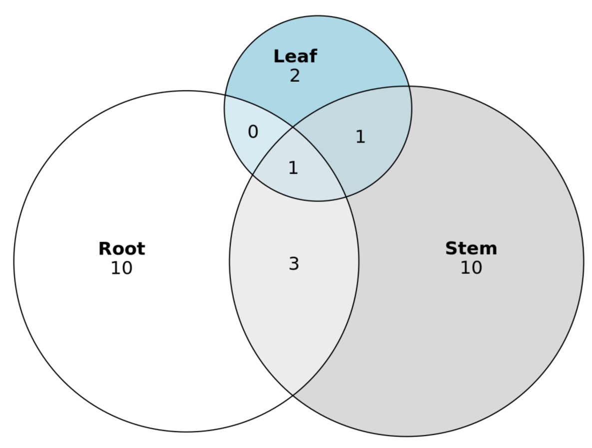


Fig3. Distribution of bacteria genera in *Dioscorea alata* and *Dioscorea esculenta*

Bradyrhizobium, *Kocuria* and *Neorhiobium* were found only in accessions of *D. esculenta,* and the remaining 8 genera belonged to accessions of *D. alata* Species richness was higher in *D. alata* (3.15) than in *D. esculenta* (2.30). However, diversity of endophytic diazotrophic bacteria was similar (0.14).

Important differences were observed between yam species in relation to the distribution of bacterial isolates in plant organs. In *D. alata*, 51.28% of isolates were located in the roots followed by the stem (30.77%). On the opposite, most colonized organ was the stem (64.71% of the 17 isolates), and roots and leaves harbored same number of isolates (3).

Our results indicate possible significant differences in physiology and nutrient transportation between these two yam species that might affect bacteria movement between roots and aboveground organs.



Overall, only one bacterial genus was found in all plant organs, 3 were found in roots and stems and 1 genus was in leaves and stems. No isolates were common to leaves and roots. Number of genera was similar in root and stem samples, the leaves harboring the lowest number of generas as reported in previous studies.

Fig 4. Venn Diagram showing similarities of bacterial genera in organs of yam (*Dioscorea* spp.)

CONCLUSION

This study aimed at revealing types of N_2-fixing endophytic bacteria and their diversity in two yam species. The results show that several types of N_2-fixing endophytic bacteria can colonize tissues (roots, stems and leaves) of the crop. Several isolates were closely related to already identified diazotrophs of non-legumes. However, strains of the genera *Curtobacterium, Ensifer, Kocuria, as well as Mesorhizobium camelthorni, Mycobacterium cosmeticum, Pseudoxanthomonas indica, Paenibacillus panacisoli* and *Neorhizobium huautlense* were reported for the first time as potential nitrogen-fixing bacteria of crops.

Because bacteria species diversity was similar and species richness was different between yam species, more studies are needed to confirm our findings. The effective plant growth promoting activities of the isolated bacteria strains is needed in order to evaluate their true beneficial properties in this symbiotic relationship.

Our study opens way for introducing new technology in sustainable yam production systems that needs to be more investigated for a full understanding of all factors involved in this symbiosis.

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