



DIVERSITY OF YEASTS IN VETIVER PHYLLOPLANE IN THAILAND AND THEIR CAPABILITY TO PRODUCE INDOLE-3-ACETIC ACID, A PLANT GROWTH PROMOTER



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Phylloplane / Phyllosphere

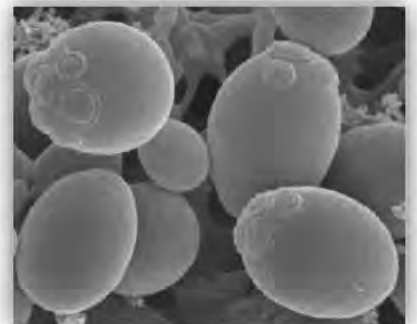
- External surface of plant leaf
- Important habitat for microorganisms
- Growth of microorganisms on phylloplane depend on nutrients derive
 - **endogenously from** plant exudates (provide organic substances e.g. simple sugars (glucose, fructose and sucrose), organic acids, amino acids and methanol
 - **exogenously** from compounds found in materials that drop on plant leaf, mostly are inorganic nutrients

(Tukey, 1971; Fonseca and Inacio 2006)



Yeasts

- **Unicellular fungi (eukaryotic microorganism)**
- **Industrial important microorganisms:**
 - **Alcoholic drinks, Bioethanol and Biodiesel (Fuel), Bakery Products, Probiotics and Prebiotics etc.**
- **Saprophytic yeasts can colonize phylloplane**
 - **act as natural barrier against plant pathogens**
 - **help to promote plant growth by producing plant growth promoter**





Yeasts (continued)

- **Phylloplane yeasts** are in both phyla *viz.* **Basidiomycota** and **Ascomycota**
 - **Basidiomycota:** *Bullera japonica*, *Cryptococcus laurentii*, *Cryptococcus fabianii*, *Pseudozyma antarctica*, *Rhodotorula mucilaginosa*, *R. taiwanensis* etc.
(Inacio *et al.* 2002; Glushakova *et al.* 2006; Limtong *et al.* 2014a, 2014b; Nasanit *et al.* 2015)
 - **Ascomycota:** *Candida tropicalis*, *Kodamaea ohmeri*, *Kluyveromyces thermotolerans*, *Meyerozyma caribbica*, *Metschnikowia koreensis*, *Torulaspora delbrueckii* etc.
(Spencer and Spencer, 1997; Peter *et al.*, 2007; Limtong and Koowadjanakul 2012; Limtong *et al.* 2014a, 2014b)



Indole-3-acetic acid (IAA)

- IAA is an auxin hormone that regulates various developmental and physiological processes in plants
- Plant growth can be stimulate by IAA produced by plant cells and by IAA secreted by plant-associated microorganisms
- Microorganisms produce plant growth promoters including bacteria, actinomycetes, fungi and yeasts have been isolated and identified to elucidate their role to plant growth



Objectives

- To study diversity of yeasts in phylloplane of vetiver grass in Thailand by culture-dependent method
- To investigate indole-3-acetic acid (IAA) producing capability of vetiver phylloplane yeasts



Research outline

1. Collection of vetiver grass leaf

2. Yeast isolation

3. Yeast identification

4. Determination of IAA production



Sample collection

- 34 samples of green and healthy leaf of vetiver grass were collected from 8 provinces, in Thailand

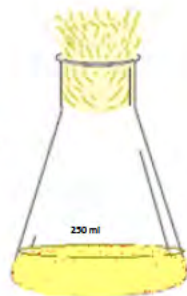




Yeasts Isolation: (Enrichment technique)



vetiver leaves (3 g)



Yeast extract malt extract (YM)
broth (50 ml/250):
250 mg/L sodium propionate
200 mg/L chloramphenicol

shake at 150 rpm, $30 \pm 2^\circ\text{C}$ for 2 days



Streak on **YM agar**

(+ sodium propionate and chloramphenicol)

$30 \pm 2^\circ\text{C}$ for 1-2 day



Pick and re-streak
on **YM agar**



Pure yeast
cultures

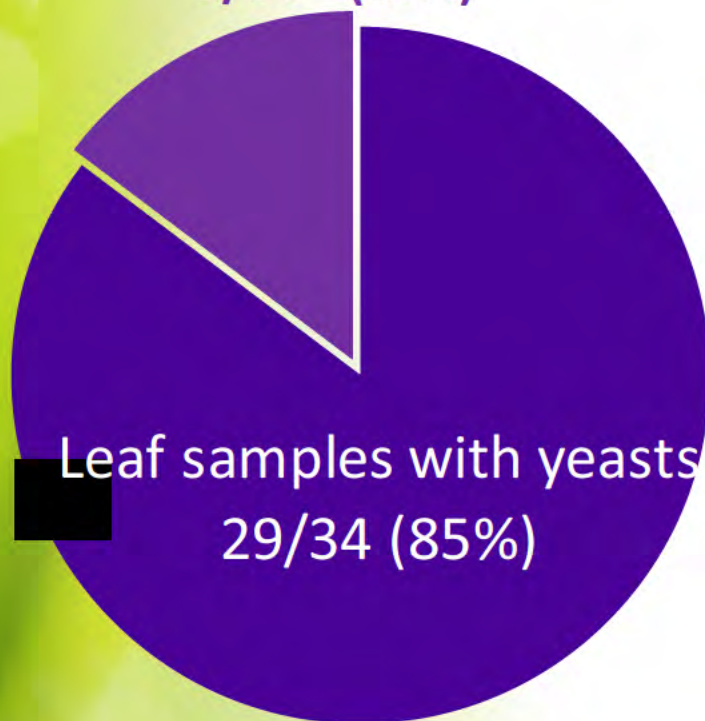




Result of yeast isolation

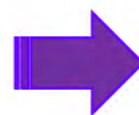
Leaf samples without yeasts

5/34 (15%)



Leaf samples with yeasts

29/34 (85%)



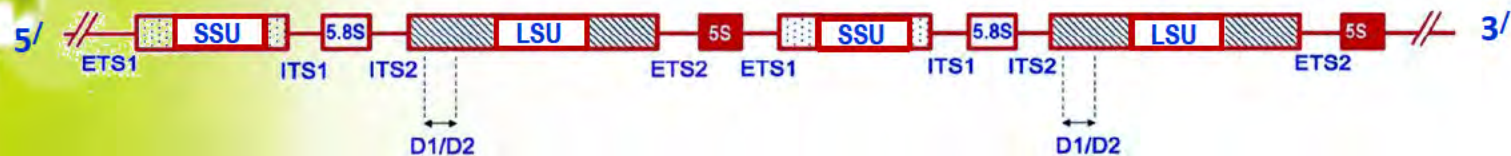
40 yeast strains





Yeast identification: Molecular taxonomy

Analysis of the D1/D2 region of the large subunit (LSU) rRNA gene sequences similarity



- ca. 600 nucleotides at the 5' end of the LSU rRNA gene
- Two rapidly evolution regions and account for most of the sequence divergence between LSU rRNA gene

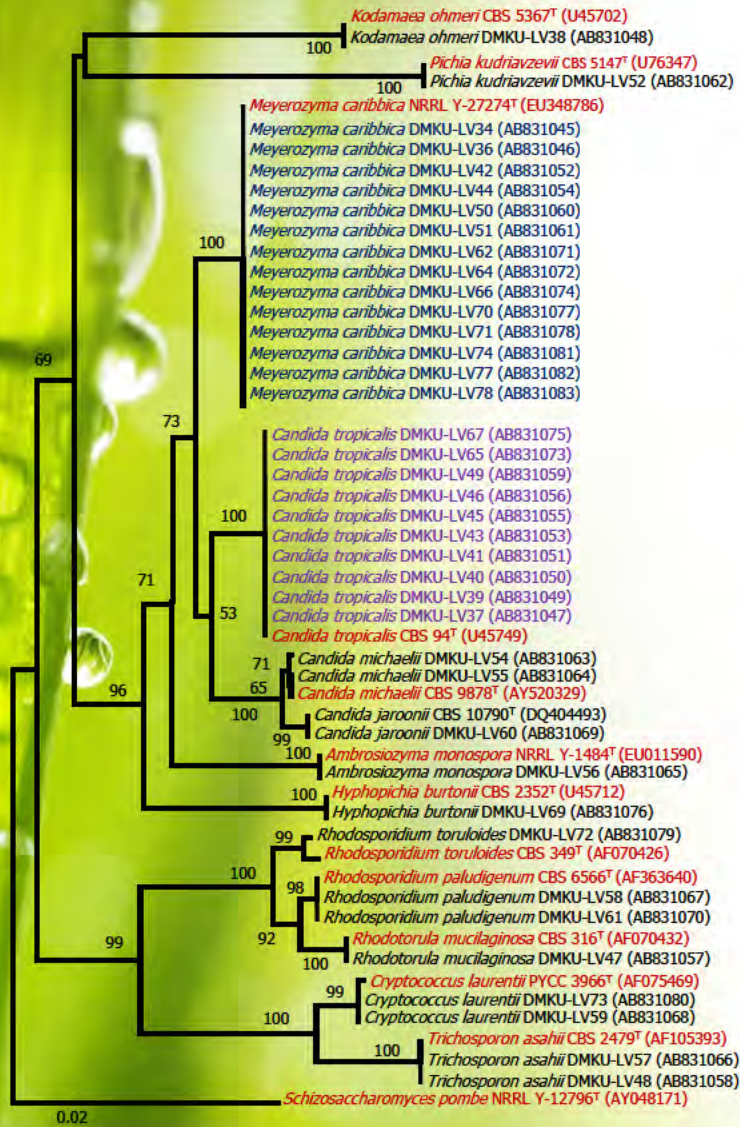
Guidelines:

- Kurtzman and Robnett (1998): ascomycetous yeast strains with 0–3 nucleotide differences are conspecific or sister species and strains showing > 6 nt substitutions are different species
- Fell et al. (2000): basidiomycetous yeast strains that differ by 2 or more nucleotide substitutions are different taxa



Yeast identification: Phylogenetic taxonomy

- Phylogenetic tree based on the sequences of the D1-D2 region was constructed by neighbor joining method (Saitou & Nei, 1987) with Kimura's two parameter correction (Kimura, 1980. Confidence levels of the clades were estimated from bootstrap analysis (1000 replicates) (Felsenstein, 1985).





Result of yeast identification

- 39 strains were identified to be 13 known yeasts species in both phyla
 - Phylum Ascomycota
 - Phylum Basidiomycota
- 1 strain represented a new yeast species closest to *Rhodosporidium toruloides* (Basidiomycota)



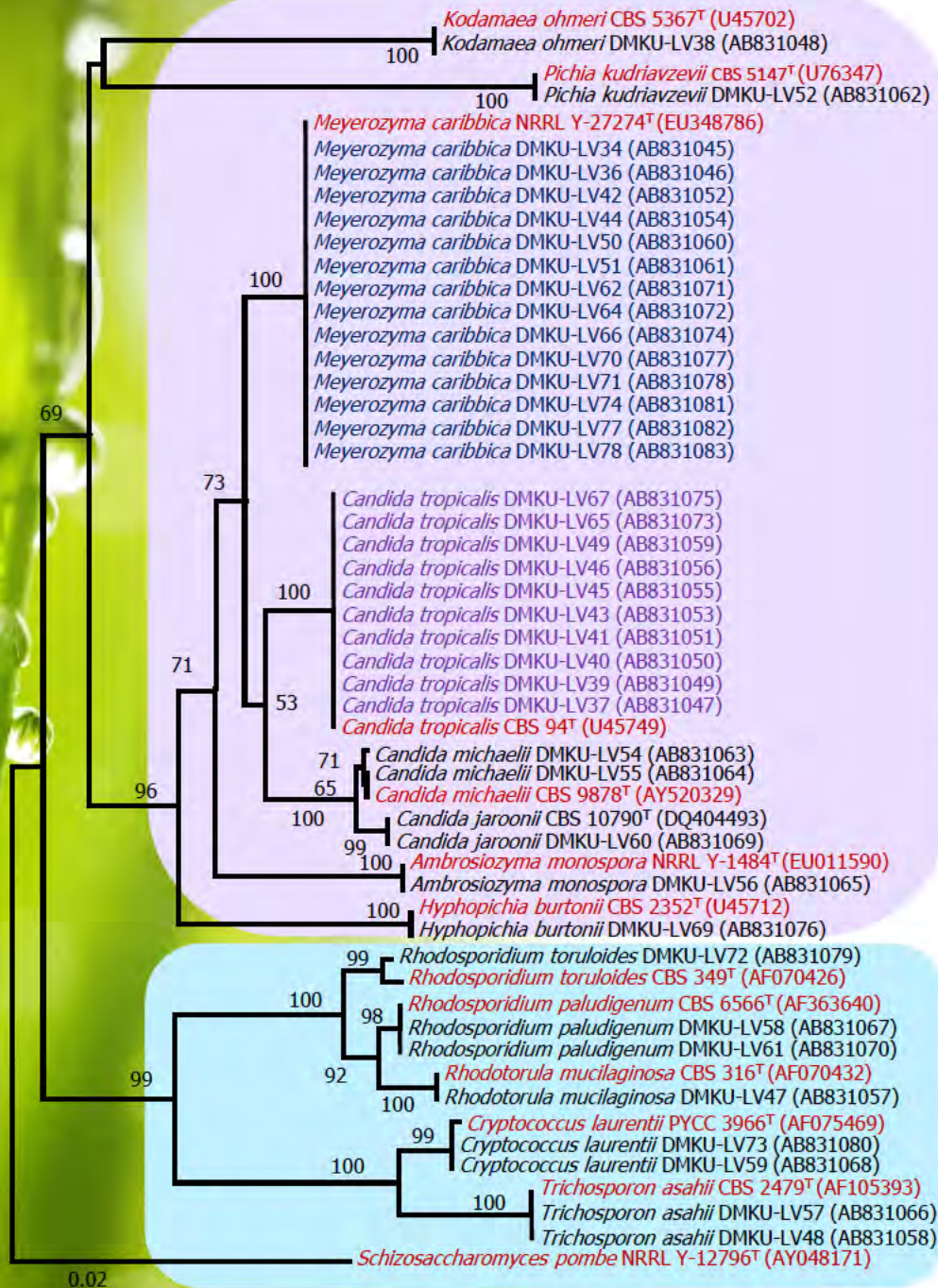
Result of yeast identification: Known yeasts species (13 species)

Ascomycota (9 species)

- *Ambrosiozyma monospora*
- *Candida fukuyamaensis*
- *C. jaroonii*
- *C. michaelii*
- *C. tropicalis*
- *Hyphopichia burtonii*
- *Kodamaea ohmeri*
- *Meyerozyma caribbica*
- *Pichia kudriavzevii*

Basidiomycota (4 species)

- *Cryptococcus laurentii*
- *Rhodospiridium paludigenum*
- *Rhodotorula mucilaginosa*
- *Trichosporon asahii*



Ascomycota

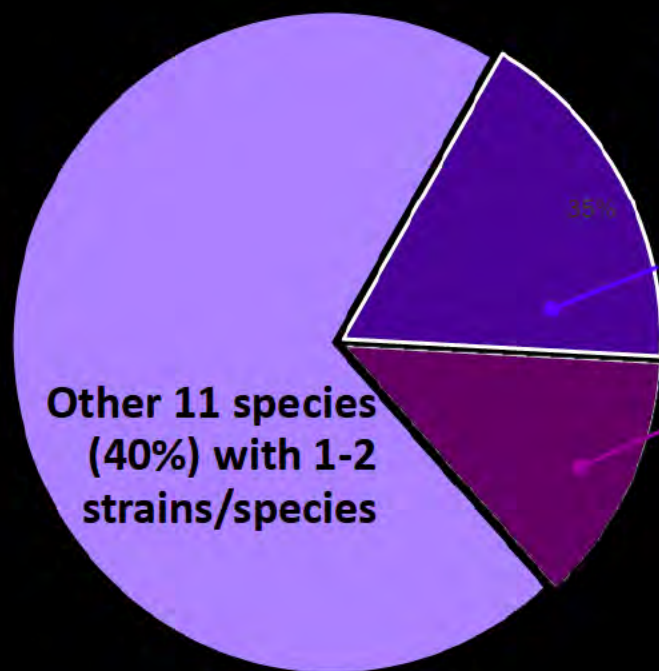
Fig. Phylogenetic tree based on the sequences of the D1-D2 region of the known yeast species

Basidiomycota



occurrence (OF)

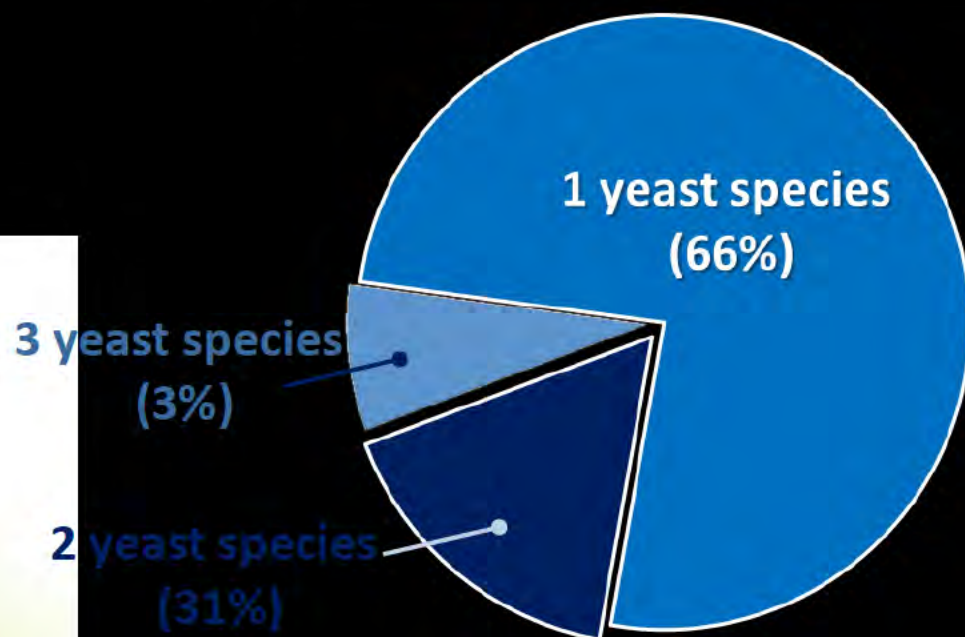
FO = no of leaf sample from which that species was isolated/Number of plant leaf sample examined X 100



Meyerozyma caribbica: 35%

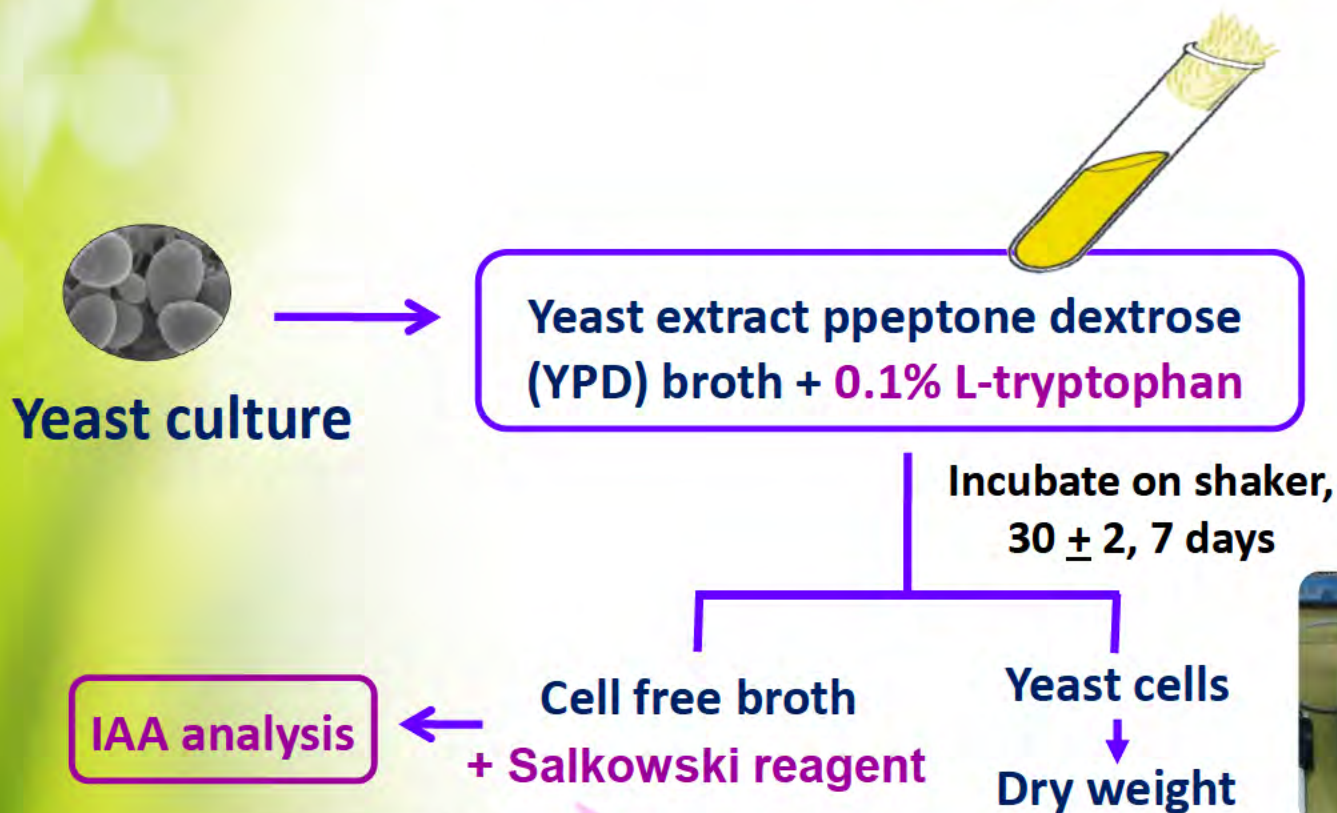
Candida tropicalis: 25%

Number of yeast species per one leaf sample





IAA production by vetiver phylloplane yeasts

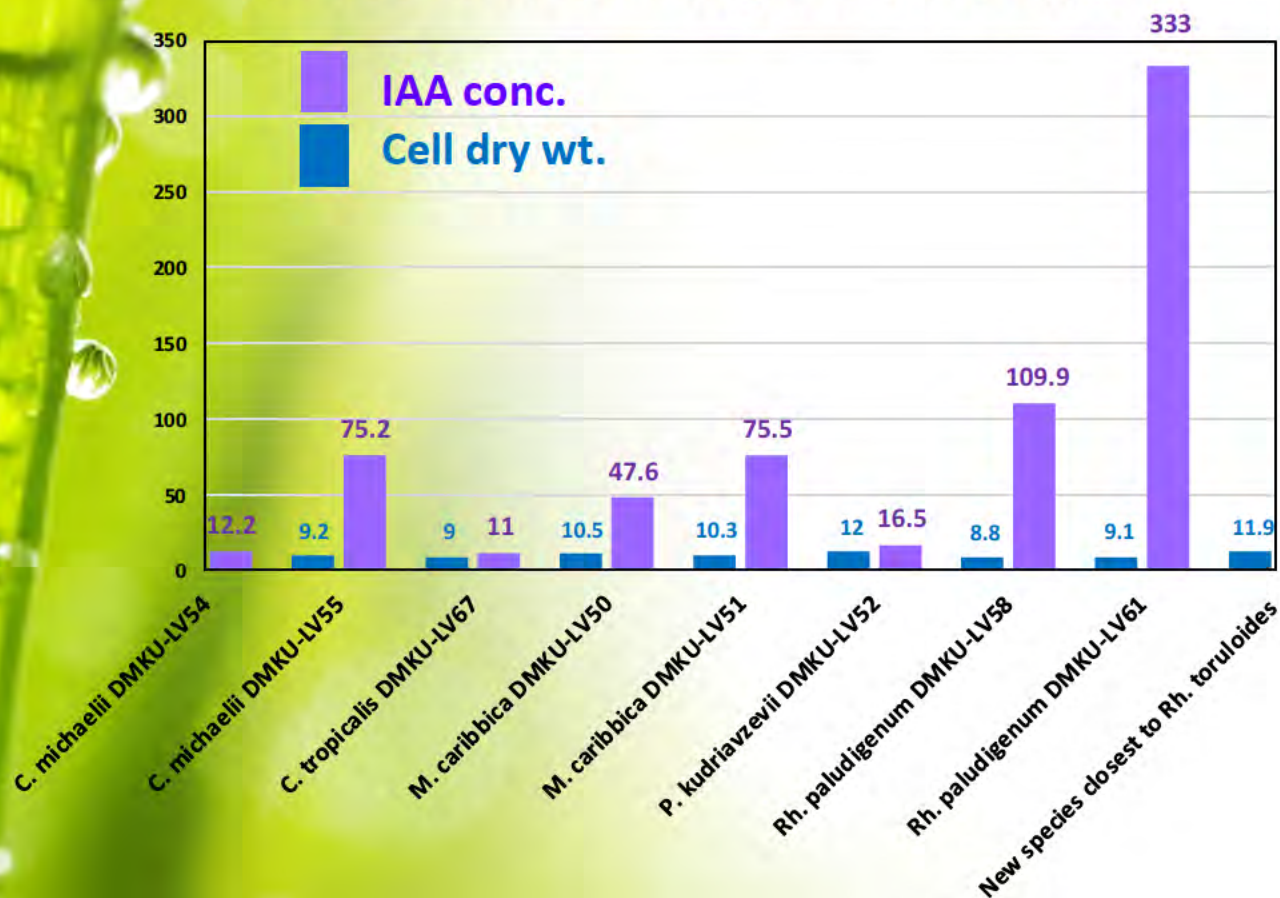


FeCl_3 12 g/l and H_2SO_4 7.9 M
(Glickmann and Dessaux, 1994)



Result of IAA production by vetiver phylloplane yeasts

- 9 strains (from 40 strains) could produce IAA and they produced in the range of 11-333 mg/L



Rhodosporidium paludigenum DMKU-LV61 produced the highest IAA of 333 mg/L



Conclusions

- The result of this study revealed that yeast species in the vetiver grass phylloplane were similar to that present in the phylloplane of the other plants species.
- *Meyerozyma caribbica* and *Candida tropicalis*, which frequently found in the sugarcane and rice phylloplane, were also presented in the phylloplane of vetiver grass at high frequency of occurrence.
- Some yeast strains could produce IAA which may possibly contribute to promote growth of vetiver grass in the field.



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