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ORIGINAL ARTICLE

Endophytic Yeasts Possibly Alleviate Heavy Metal Stress in their host *Phragmitesaustralis* Cav. (Trin.) ex Steud. through the Production of Plant growth Promoting Hormones

Roland M. Hipol*[1], Teresita U. Dalisay[2], Edna Y. Ardales[2], Maria Lourdes O. Cedo[3] and Virginia C. Cuevas[4]
Department of Biology, College of Science, University of the Philippines Baguio [1]
*Corresponding author: rmhipol@gmail.com
Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños[2]
Crop Physiology Division, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños[3]
Environmental Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños[4]

ABSTRACT
Three endophytic yeasts were isolated from the lower stem and roots of Phragmitesaustralis Cav. (Trin.) ex Steud. observed to be dominant along the banks of the tailings pond 5A of the Lepanto Consolidated Mining Co. (LCMC) in Mankayan, Benguet. These three yeast isolates were tested for their growth promoting properties in-vitro to establish the possible contribution of these endophytes to the survival of their hosts in this inhospitable environment. The growth promoting plant hormone auxin was measured for each isolate and the highest producer was Debaryomyceshansenii Yph4. All yeast isolates have the capacity to promote growth in paclobutrazol (GA synthesis inhibitor) treated PSB RC10 rice variety from the Philippine Rice Research Institute as measured through total plant length. The best yeast isolate to stimulate growth of the shoot was Candida parapsilosis Yph5.
Keywords: IAA, auxins, gibberellic acid, paclobutrazol, yeast endophytes, Phragmitesaustralis

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INTRODUCTION

The sparse literature on yeasts indicates that this group of fungi does contribute to the growth and development of the plants they are associated with. They have been found to produce auxins including indole-3-acetic acid and indole-3-pyruvic acid, gibberellins and polyamines; albeit induced in vitro. So-called plant growth promoting yeasts include *genera* such as *Candida, Cryptococcus, Rhodotorula, Sporobolomyces, Trichosporon, Williopsis* and Yarrowia [1].

In the study of Amprayn *et al*. [2], a yeast isolate identified as *Candida tropicalis* CtHY tested positive to the production of indole acetic acid(IAA), 1-aminocyclopropane-1-carboxylate (ACC)deaminase, phytase and polyamines, along with the ability tosolubilisetricalcium phosphate. It also promoted shoot and root growth in the tested riceseedlings.Yeast isolates from *Populustrichocarpa* have also been found to produce auxins[3]. The researchers also found that IAA production in their isolates exclusively followed thetryptophan dependent biosynthesis of IAA.

Gibberellic acid production by yeast isolates has also been suggested by El- Tarabily & Sivasithamparam[1]. They cited the capacity of *Candida valida*, *Rhodotorulaglutinis* and *Trichosporonasahii* from the rhizosphere to promote growth by producing IAA and GA_3. Thisis one publication available that provides evidence regarding the capacity of yeasts to augmentplant growth through the production of gibberellic acid.

Metallophyte growth promotion by symbiotic organisms greatly increases the adaptivecompetence of these plants to high heavy metal (HM) concentrations. By increasing the biomassof plants, a diluting effect in the plant tissues is observed. In this case the microorganismscontribute to the reduction of stress due to metal toxicity[4].

Three endophytic yeasts have been isolated from the roots and lower stems of *Phragmitesaustralis* Cav. (Trin.) ex Steud. Several *P. australis* stands were found thriving anddominating the vegetation in the tailings pond 5A of the Lepanto Consolidated MiningCompany. In view of the capacity of yeasts to promote growth, it is therefore interesting todetermine if these yeast isolates contribute to *P. australis* growth in this inhospitable habitat. Inthis research, this property of the isolated endophytic yeasts from *P. australis* was investigated in-vitro. Growth promoting properties of the isolates may help shed light on the reasons why *P.australis* survive and tolerate adverse conditions in its tailings pond habitat.

The objectives of this research include: (1) detection and measurement of IAA producedby the isolated yeasts; (2) determination of growth promotion of yeast isolates to paclobutrazol-treated (a GA inhibitor) rice seedlings [5].

MATERIALS AND METHODS

Collection, isolation and molecular identification of the endophytic yeasts are elaborated in Hipol [6]. The fermentation set-up is also detailed in this publication.

Assay for Auxin Production

One mL of culture filtrate (CF) from the centrifugation of the potato dextrose broth (PDB) was mixed with 2mL of Salkowski reagent (2 mL 0.5 M $FeCl_3$, 98 mL 35% $HClO_4$). The intensity of the pink color that developed in the mixture after 30 m was quantified using a UV-VIS Spectrophotometer (Shimadzu UV mini 1240 UV-VIS, Germany) at 530 nm wavelength. The negative control was Type 1 water.The weights of the previously dried cell pellets were used for normalizing indole produced per unit dry wt of the yeast isolates to quantify the auxin produced. Similarly, pink color was also developed for a series of IAA standard solutions (5ppm, 10ppm, 15ppm, 20ppm; $R^2 = 0.9915$) to establish a standard curve[3].

Rice Seedling Assay for Gibberellin Production

The method of Khan et al., [7] was used in this assay however modified to use the shortest rice variety from PhilRice (PSB RC10) instead of gibberellic acid (GA) mutant rice. Rice seeds are used as it can easily grow under controlled and sterilized conditions using autoclaved water-agar media. Previous reports have also cited that rice shoot growth stimulation or suppression can be attributed to the activity of plant growth promoting or inhibiting secondary metabolites present in the fungal culture filtrate [8].

Rice seeds of PSB RC10 variety from the Philippine Rice Research Institute were surface sterilized with 2.5% sodium hypochlorite for 1h, rinsed with Type 1 water and then incubated for 24 hr with 20-ppm paclobutrazol (an anti- GA compound) to obtain equally germinated seeds. These were then transferred to glass growth chambers with Hoagland's basal salt solution:agar medium (0.8% w/v) under aseptic conditions. Fifteen plants composed of 3 replicates of 5 plants each were prepared for each treatment. At the two-leafed stage, 50 µl of culture filtrate of each yeast isolate was applied at the apex of the rice seedlings. One week after treatment at ambient conditions under artificial light, the total plant length were measured. The total plant fresh weights were also recorded and compared with negative (water and broth) and positive controls (20 ppm IAA and 20ppm GA) [7]. All the treatments and controls were set up in triplicates.

Statistical Analyses

The values were subjected to Analysis of Variance to determine if there are significant differences between the measured plant lengths using SPSS® version 17.0. The Scheffe post-hoc test algorithm of the same software was used to show the different homogenous subsets thatare significantly different from each other.

RESULTS

Three culturable endophytic yeasts were successfully isolated. These were isolates Yph4 (unidentified), Yph5 identified as *Debaryomyceshansenii* and Candida parapsilosis. Yph3 wasunsuccessfully queried into the GenBank database due to the unsuccessful PCR protocol employed for this isolate.The sequences for *D. hansenii* Yph4and *C. parapsilosis* Yph5 were submitted to GenBank and were given the accession numbers KJ908845 and KJ908846 respectively.

Assay for Auxin Production

Colorimetric assay for indoles showed that all isolates were able to produce auxin asmeasured with a UV-VIS spectrophotometer at 530nm as compared to the control (Fig. 1). Theisolate with the highest indoles produced per ml of broth was *D. hansenii* Yph4 at 3x the controlat 29.8ppm. The other isolates Yph3 and *C. parapsilosis* Yph5 also produced auxins beingstatistically different from the control.

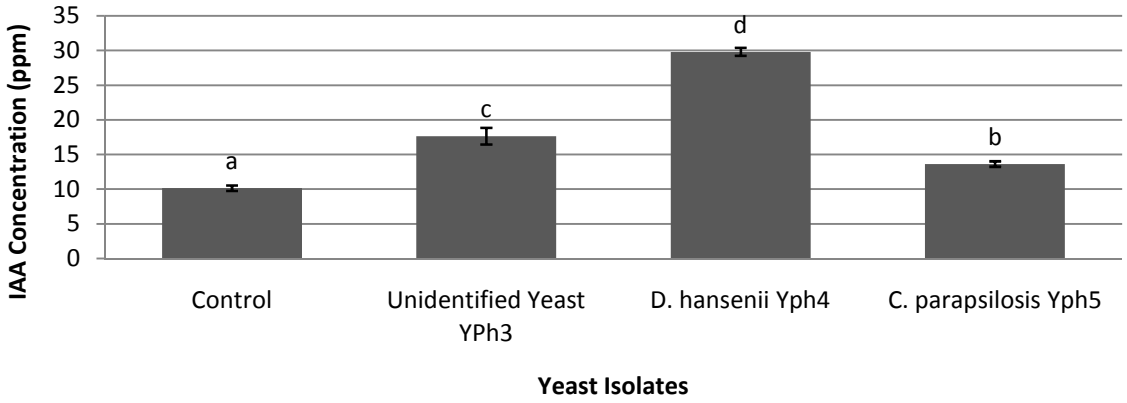

Figure 1: IAA production of the different yeast isolates. Error bars are standard deviations. Letters denote homogeneous groups at p=0.05.

Comparing the different isolates in terms of the amount of indoles produced per mg of dry wt, isolate Yph3 was the best isolate producing 0.89 µg IAA/mg (Fig. 2). This amount is almost twice the amount produced by isolate with the lowest yield which is *D. hansenii* Yph4 at 0.44ug/mg. *C. parapsilosis* Yph5 produced intermediate amounts at 0.65 0.89 µg IAA/mg of its DW.

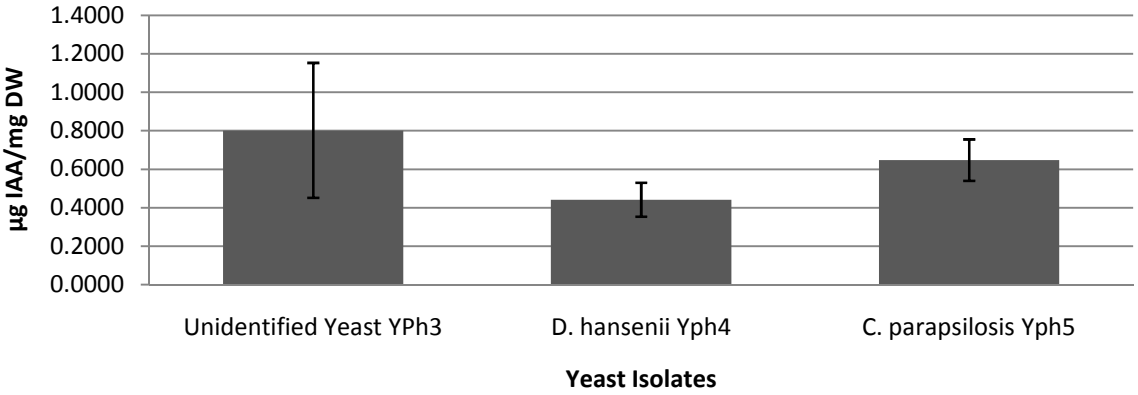

Figure 2: Auxin (IAA) production of the different yeast isolates per mg dry wt. Error bars are standard deviations. Letters denote homogeneous groups at p=0.05

Rice Seedling Assay for Growth Promotion

Effect on plant length.

In-vivo growth promotion of the yeast endophytes was tested on paclobutrazol (anti- GA synthesis) treated rice seedlings. Total plant length, as the sum of root and shoot length, was measured. Results show that isolate *C. parapsilosis* Yph5 treated seedlings were the longest at 27.2 cm; 23.6% longer than the negative control (water) (Fig. 3). Expectedly, the positive control treated with GA was the longest but was also characterized with the typical spindly growth of the stem as effected by increased GA. The effect of *C. parapsilosis* Yph5 was comparable to the growth promoting effect of one ofthe positive controls – 20 ppm IAA. All the other isolates still promoted growth; albeit low and not significantly different from the negative controls. This observed induction of growth by the yeast isolates are still notable in view of the seedlings being treated with paclobutrazol. The growth that was observed more than the negative controls is attributable to the yeast isolates. The increase in plant length for Yph3 was 18.1% while D. hansenii Yph4 was 25.5% of the average of the two controls.

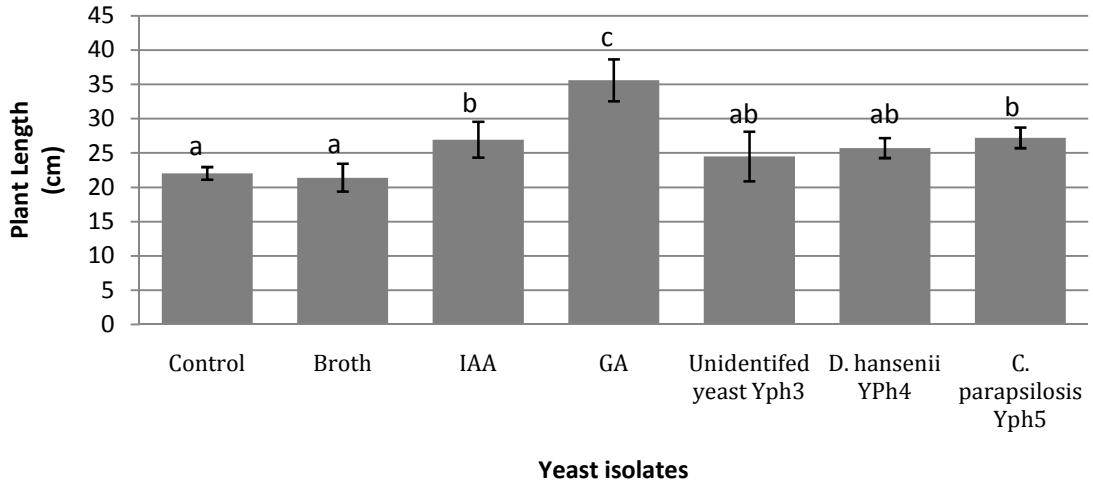

Figure 3: Growth promotion measured as total plant length of paclobutrazol–treated rice seedlings amended with the different yeast isolates. Letters denote significantly different values at p=0.05.

Effect on plant biomass

Figure 4 present the effect the different isolates on the biomass of the seedlings. All values for the total plant fresh weight were not significantly different from the positive and negative controls despite the differences seen in the measured plant lengths. However, it may be good to mention that Yph3 and *D. hansenii* Yph4 caused an increase in biomass. Isolate Yph3 and *D. hansenii* Yph4 increased weight by 9.4% and 10.5% respectively.

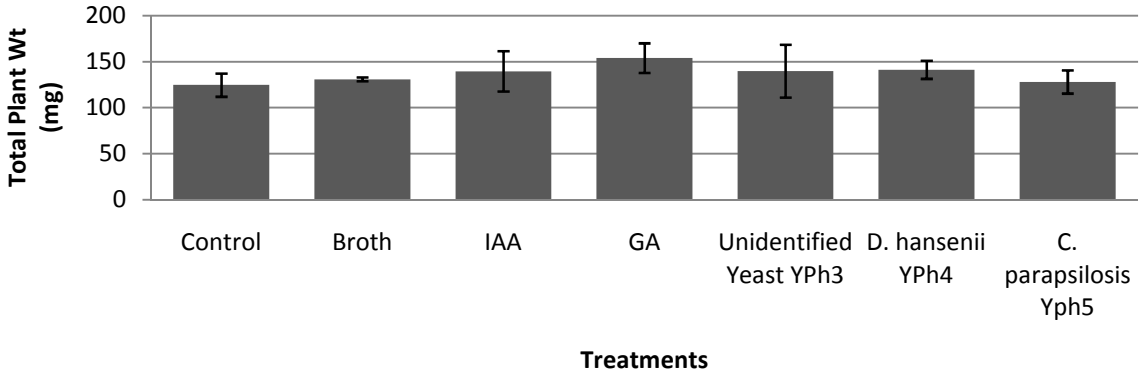

Figure 4: Growth promotion measured as fresh wt of paclobutrazol–treated rice seedlings amended with the different yeast isolates.

DISCUSSION

It has been established that fungal endophytes, including yeasts contribute to the growth and development of their hosts. Various modes of promoting growth include the production of growth promoting hormones (IAA and GA) [1]. In this study, these hormones were investigated in three isolated endophytic yeasts from apparently healthy populations of *P. australis* observed to be occurring in the tailings pond 5A of the Lepanto Consolidated Mining Co. (LCMC) in Mankayan, Benguet, Philippines.

The highest producer of indoles was isolate *D. hansenii* Yph4 at 29.8 µg/ml. However, this value is only around a third of that produced by the plant associated Fusarium sp. studied by Tsavkelova et al. [8]. In the assays, L-tryptophan was added to the culture broth. Xin et al. (2009) discovered that yeasts, together with bacteria, synthesize IAA through the tryptophan dependent pathway. They add that with tryptophan available in the host, the endophytic yeasts do not have to spend high energy for the synthesis of this amino acid themselves. At the same time, the ability to convert tryptophan to IAA by the endophytes would, in return, benefit the tryptophan provider, which may be seen as a mutually advantageous plant–microbe example.

The effect of the growth promoting metabolites of the different endophytic yeast isolates were investigated on paclobutrazol treated PSB RC10 variety from the Philippine Rice Research Institute. Use of

rice plants for growth promotion screening is suggested by Hamayun et al. [8] (2009), as they easily grow under controlled and sterilized conditions, using hoagland's basal salt solution-agar media. Treatment of its seeds with paclobutrazol, a GA biosynthesis retardant [9], suppresses GA production. Shoot elongation of these seedlings can thus be related to the activity of plant growth-promoting secondary metabolites from the broth cultures applied. Among the different isolates, *Candida parapsilosis* Yph5 had the caused the highest increase in plant length. This isolate may have produced the most GA among the isolates resulting to longer plants. The research by Azcón et al. [10] had also discovered the growth promoting property of *C. parapsilosis* isolated from the soil in consortium with Bacillus cereus and an arbuscular mycorrhizal fungus (AMF). The remaining isolates may have produced GA, albeit the effected growth may not have been great enough to be statistically different from the control.

CONCLUSION

The three endophytic yeasts of the metallophyte *P.australis* found dominating the banks of the copper rich active tailings pond of Lepanto Consolidated Mining Company in Mankayan, Benguet, Philippines possess the capacity to produce plant growth promoting hormones indole acetic acid and gibberellic acid. Under the stressful conditions of the tailings pond, these endophytes may have helped their host plants adapt to these conditions resulting to their hosts' luxuriant growth. By possibly contributing to the increased the biomass of *P. australis*, a diluting effect of the heavy metals in the plant tissues is possibly experienced by the hosts. In this case, the microorganisms contribute to the reduction of stress due to metal toxicity. This may partly explain the verdant growth of these plants under inhospitable conditions of their habitat.

In view of the dearth of information regarding the contribution of yeasts to plant growth [1], this study provides valuable contribution addressing this gap. To generate more useful information about these organisms, it is suggested that further research be done; e.g. characterization of associated genes and optimization of conditions to elicit the maximal growth promoting potentials of these isolates. These proposed researches may lead toward the development of possible agricultural or horticultural applications for these culturable yeast endophytes

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REFERENCES

1. El-Tarabily, KA & Sivasithamparam K. (2006). Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Mycoscience 47(1):25–35.
2. AmpraynK, RoseMT,KecskésM, PeregL, NguyenHT.& KennedyIR. (2012). Plant growth promoting characteristics of soil yeast (*Candida tropicalis* HY) and its effectiveness for promoting rice growth. Appl Soil Ecol, vol. 61, pp. 295–299.
3. XinG,GlaweD& DotySL. (2009). Characterization of three endophytic, indole-3-acetic acid-producing yeasts occurring in *Populu s*trees.Mycol Res 113:973–980.
4. LebeauT, Braud A & JézéquelK. (2008). Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review."Env Poll153:497–522.
5. LeeEH, ByunJA & WildingJ. (1985). A new gibberellin biosynthesis inhibitor, paclobutrazol (PPa33), confers increased so/ tolerance on snap bean plants. Environmental and ExpBiol 25(3):265–275.
6. HipolRM. (2014).Antioxidant potentials of culturable endophytic yeasts from *Phragmitesaustralis* Cav. (Trin) ex Steud.from copper-contaminated mining site in Mankayan, Benguet.PhilippSci Lett7(2):337-346.
7. KhanAL, Hamayun M, Kang SM, Kim YH, JungHY, Lee JH & LeeIJ. (2012). Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomycesformosus* LHL10. BMC Micro12(3): doi:10.1186/1471-2180-12-3,.
8. HamayunM, KhanSA, AhmadN, Tang DS, KangSM, NaCI & LeeIJ.(2009). "*Cladosporiumsphaerospermum* as a new plant growth-promoting endophyte from the roots of *Glycine max*(L.)Merr.World J MicrobioBiotech25(4):627–632.
9. TsavkelovaE, OeserB, Oren-YoungL, IsraeliM, SassonY, Tudzynski B & Sharon A. (2012). Identification and functional characterization of indole-3-acetamide-mediated IAA biosynthesis in plant-associated *Fusarium*species.Fungal Genet Biol49:48–57.
10. AzcónR, PerálvarezMD, RoldánA and BareaJM. (2010). Arbuscular mycorrhizal fungi, *Bacillus cereus*, and *Candida parapsilosis* from a multi contaminated soil alleviate metal toxicity in plants. Microbial Ecol59(4):668–77.

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