INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–1923/2019/22–1–167–177
DOI: 10.17957/IJAB/15.1047
http://www.fspublishers.org

Full Length Article

Phenotypic Plasticity in *Aureobasidium pullulans* Isolates

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Abstract

Phenotypic plasticity is the ability of organisms to change phenotypes in response to environmental selection pressures. With the characteristic of no mobility, the ability to respond to fluctuating environments plays a crucial role in the evolutionary success of microorganisms. *Aureobasidium pullulans* (de Bary) Arnaud is a yeast-like fungus found widespread and it is notable for its morphological variance. However, mechanisms through which *A. pullulans* adapts in different ecological niches remain unknown. The present study, phenotypic plasticity of *A. pullulans* was investigated. The traits of *A. pullulans* were examined under different environmental conditions. For cell colonization, indole-3-acetic acid (IAA) production is a strategy of interference competition. We observed that IAA production increased with the increasing concentration of the exogenous precursor tryptophan. Regarding nutrient acquisition, the production of siderophores, which scavenge ferrous ions, increased when the concentration of ferrous ions was low and vice versa. This suggests the role of siderophore-mediated antagonism of competitors (exploitative competition). Regarding the phosphate-solubilizing ability used for acquiring phosphorous, strains could more efficiently dissolve dicalcium phosphate and calcium triphosphate hydroxide compared with phosphorite. However, all *A. pullulans* strains used in this study could dissolve all the three types of phosphates. We also tested their abilities to protect themsleves against environmental pressures. The biofilm formation ability, which is useful in protection and fixation, was higher at 19°C and 28°C but lower at 37°C. The antioxidant capacitydecreased with increasing salinity. The results of this study demonstrated the phenotypic plasticity and genetic variation of *A. pullulans* and suggested that both play important roles in the adaptation of *A. pullulans* in fluctuating environments. © 2019 Friends Science Publishers

Keywords: Antioxidant capacity; Biofilm; Indole-3-acetic acid; Phosphate-solubilizing; Siderophore

Introduction

Organisms encounter stress from different sources while living in the wild environment (Thomas *et al*., 2002). The source of stress can be divided into two types: biotic factors such as competition, predation and parasitism (Worm and Chapman, 1998; Frost *et al*., 2016; Frainer *et al*., 2018) and abiotic factors such as temperature, moisture and ultraviolet light (Deryng *et al*., 2014; Pedersen *et al*., 2016). Organisms counteract and survive under these stress factors through two mechanisms. The first is genetic diversity (Bersabé *et al*., 2015; Ellegren and Galtier, 2016), which means that various types of genotypes exist that allow organisms with appropriate genotypes to survive. The second is phenotypic plasticity (Cortesi *et al*., 2015; Radice *et al*., 2018), which is the ability of an organism to change its phenotype in response to fluctuating environmental conditions. It is a crucial strategy for species to survive and adapt in the wild. For example, corkystem passion flower, *Passiflora suberosa* L., accumulates more anthocyanin when exposed to a high intensity of ultraviolet light than when under normal conditions (Barp *et al*., 2006). The accumulation of anthocyanin forms a protective layer, preventing photoinhibition in leaves. Another example is the changes occurring in the shape of the fish *Cyprinella venusta* in different habitats (Franssen *et al*., 2013). *C. venusta* is more streamlined in the stream than in reservoirs, which offers advantages in swimming or preying. This ability is particularly crucial for plants and microorganisms whose sessile lifestyle requires them to deal with ambient conditions (Searle *et al*., 2015).

Compared with animals and plants, microorganisms live in micro-niches that are considerably influenced by the fluctuating environment. Environmental changes and associated selective pressures may affect how microorganisms develop an appropriate phenotypic response and influence the genetic variation within the microbial population (Peltier *et al*., 2018). Evolutionary pressures from

To cite this paper: Xiao, H.S., R.Y. Chen, T.L. Cheng, W.T. Fang, C.H. Hsu and J.Y. Chou, 2019. Phenotypic plasticity in *Aureobasidium pullulans* isolates. *Intl. J. Agric. Biol*., 22: 167–177

fluctuating environments can result in the evolvement of phenotypic plasticity, in which individuals respond to environmental changes by modifying their expression of morphology, physiology, life-history and behavioral traits (Tadrowski *et al*., 2018). Phenotypic plasticity allows microorganisms to counteract diverse stressors in the environment. The dynamic process of fluctuating selection and genetic recombination allows genome diversity to be maintained in the microbial population and encountering diverse stressors in the environment between generations may act as a mechanism for maintaining phenotypic plasticity. Although many studies have investigated environmental or genetic variations in individual phenotypes or genes, the underlying meaning of traits and their correlations remains to be elucidated and discussed (Corwin *et al*., 2016).

Aureobasidium pullulans is also called "black yeast" because of its ability to produce melanin (Gadd, 1980). *A. pullulans* is named for its capability to produce the polysaccharide "pullulan." Pullulan, an extracellular product produced by *A. pullulans*, is widely used in food, biomedical, and pharmaceutical industries (Saber-Samandari and Gazi, 2015; Shah *et al*., 2016; Prasongsuk *et al*., 2018. Numerous studies have focused on the biotechnological application of *A. pullulans*; however, few have investigated its microbial ecology (Cooke, 1959; Pechak and Crang, 1977; Slepecky and Starmer, 2009). *A. pullulans* can be found in different habitats, such as on plants or insects, and even in extreme environments, such as hypersaline water, glaciers and rocks. The habitats of *A. pullulans* range from terrestrial to aquatic and from high-nutrition to barren and stressed niches (Urzi *et al*., 1999; Zalar *et al*., 2008; Gunde-Cimerman *et al*., 2009). The morphological and physiological properties of *A. pullulans* vary considerably among different strains. In addition, *A. pullulans* is notable for its phenotypic plasticity, and its colony morphology changes in response to environmental fluctuations. For instance, *A. pullulans* exhibits different levels of melanin accumulation and colonial appearance in divergent environments (Slepecky and Starmer, 2009). However, mechanisms through which *A. pullulans* grows throughout the world and adapts to different environments still requires further study. Thus, *A. pullulans* is a favorable candidate for studying phenotypic plasticity.

In this study, we quantified the expression of the following traits and determined their plasticity under different conditions: indole-3-acetic acid (IAA) production (morphological changes and interference competition), siderophore production (nutrient uptake and fungal inhibition), phosphate-solubilizing ability (nutrient acquisition), biofilm formation (cell fixation), and antioxidant capacity (cell damage prevention). In addition, we examined the performance of different strains in the same environment that may result from genetic variations. Briefly, this study investigated (1) the performance distribution of different strains in the same environment (genetic diversity) and (2) the variance of traits in response to environmental changes (phenotypic plasticity).

Materials and Methods

Yeast Isolation

Yeasts were collected from various sources such as insects, flowers and plants. Samples were collected using sterilized tweezers, stored in clean tubes, and maintained at 4°C. After mixing the samples with sterilized water, 100 μL of the mixture was plated on the selective medium YPD + LA agar (10 g of yeast extract, 20 g of peptone, 20 g of dextrose and 20 g of agar per liter, supplemented with 2–3 mL of lactic acid). Yeasts that exhibited different colonial appearances were extracted and then plated on YPD agar to isolate strains. Purified yeast strains were suspended in YPD medium supplemented with 15% v/v glycerol and maintained at 80°C. All samples were incubated overnight and cultures were refreshed in YPD medium for 3 h at 28°C to reach the exponential phase for use in further experiments.

Yeast Identification

The genomic DNA of all the collected yeasts was extracted and subsequently amplified using a polymerase chain reaction as described in our previous study (Sun *et al*., 2014). A pair of universal primers, ITS-1 (5′-TCCGTAGGTGAACCTGCG-3′) and NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′), were used to amplify the large subunit, ITS regions and small-subunit ribosomal DNA (White *et al*., 1990; Kurtzman and Robnett, 1997). The DNA sequencing of these samples was performed at Tri-I Biotech, Inc. Sequences were compared using the nucleotide–nucleotide BLAST (blastn) search with the default setting except that sequences were not filtered for low complexity. Yeast identification was based on differences in sequences, and yeast strains with ≤1% differences in sequences were considered to be conspecific species. The voucher specimens of fungi used in this study were deposited in the Bioresource Collection and Research Center, Hsinchu City, Taiwan (http://www.bcrc.firdi.org.tw/).

Quantification of IAA Production

To examine the phenotypic plasticity of IAA production, the precursor of IAA, tryptophan (Trp), was chosen as an influencing factor. In this experiment, three different incubation environments were designed here: YPD medium only, YPD medium with 0.1% Trp medium and YPD medium with 1% Trp medium. The samples were cultured in YPD medium at 28°C overnight and then refreshed in the same medium for 3 h. The number of cells was adjusted to 1.85×10^7/mL by evaluating their absorbance at OD_{660}. Subsequently, the samples were transferred into the aforementioned three incubation environments and cultivated at 28°C for 3 days. The

cultures were centrifuged at 15000 g for 1 min and the supernatant was transferred into a new tube and mixed with an equal amount of the Salkowski reagent (2% of 0.5 *M* iron (III) chloride and 98% of 35% perchloric acid) (Gordon and Weber, 1951). After incubation for 30 min in a dark environment, the color (red) of the sample was quantified using a Multiskan GO microplate spectrophotometer (Thermo Scientific) by determining the absorbance at 530 nm. The concentration of IAA was determined using an IAA standard curve ($y = 0.0014x + 0.0419$, $R^2 = 0.994$) by using commercial IAA (Sigma-Aldrich, Co.).

Quantification of Siderophore Production

Siderophore is a type of compound that is secreted to scavenge ferrous ions in the environment. In this experiment, the concentration of exogenous ferrous ions was considered as the influencing factor and different strains were cultivated in YPD medium only, YPD medium with 100 μM ferrous ions and YPD medium with 900 μM ferrous ions, respectively. The samples were cultured at 28°C for 3 days and then centrifuged at 15 000 g for 1 min. The supernatant was transferred into new tubes and mixed with an equal amount of the CAS blue dye at room temperature for 40 min following the protocol used by Louden *et al*. (2011) The absorbance was determined at 630 nm by using a Multiskan GO microplate spectrophotometer (Thermo Scientific) and the absorbance value represented the relative abundance of each strain.

Quantification of the Phosphate-solubilizing Ability

Phosphorous exists in the wild environment as different complexes and most of these can not be directly used by organisms. In this experiment, three types of phosphate were used to examine if the yeast strains could dissolve the complex. After refreshing the strains, they were inoculated in a medium modified from Pikovskaya's agar (Sundara and Sinha, 1963), which contained dicalcium phosphate (DCP), calcium phosphate tribasic (CPT), or phosphorite as the substrate. The media without yeast inoculation incubated under the same conditions served as the blank. After incubation at 28°C for 3 days, free phosphate ions formed by dissolving of the complex were measured using the ascorbate method (Ames, 1966) and a Multiskan GO microplate spectrophotometer (Thermo Scientific). The standard curve was prepared using K_2HPO_4 solution ($y = 15.104x - 0.0113$, $R^2 = 0.99973$).

Quantification of Biofilm Formation

A biofilm is a barrier that protects microorganisms from external threats in various approaches and help cells to adhere to surfaces (Fux *et al*., 2005). To measure the biofilm formation ability of strains, 200 μL of the refreshed culture was added into a 96-well plate and cultivated at different temperatures (19°C, 28°C and 37°C) for 2 days. The plate was washed with sterile water three times to remove nonattached cells and 200 μL of 0.1% crystal violet was then added for 30 min to stain the biofilm. After the excess dye was washed away with sterile water three times, 200 μL of 10% sodium dodecyl sulfate was added for 30 min to dissolve cells. Subsequently, the absorbance of the solution was measured at 570 nm using the Multiskan GO microplate spectrophotometer (Chen *et al*., 2018). When the absorbance value was over 1, the sample was considered to be diluted. The differences in the absorbance values between the sample and blank (empty wells treated using the same procedure) was regarded as being representative of the biofilm formation ability of each strain.

Quantification of Antioxidant Capacity

The production of reactive oxygen species (ROS) is related to environmental stress and causes an increase in oxidative pressure, resulting in cell damage. To estimate the antioxidant capacity of strains, the concentration of sodium chloride (NaCl) was adjusted to affect osmotic pressure. After the strains were refreshed, they were inoculated in YPD medium only, YPD medium with 1% NaCl, or YPD medium with 3% NaCl at 28°C for 24 h. The cell density of the culture was adjusted to approximately 1 at OD_{660} nm. Then, 1 mL of the culture was centrifuged at 15000 g for 1 min and washed twice with sterile water. The pellet was mixed with 400 μL of phosphate-buffered saline (PBS) and 500 μL of a reagent (0.4 M DPPH in anhydrous methanol) (Gil-Rodríguez *et al*., 2015). After 30 min of reaction in the dark, the mixture was centrifuged at 15000 g for 1 min. The absorbance of the supernatant was measured at 517 nm using the Multiskan GO microplate spectrophotometer, and the differences in the absorbance values between the sample and blank (400 μL of PBS with 500 μL of the reagent) was regarded as denoting the relative antioxidant capacity of each strain.

Statistical Analysis

The values in each experiment were expressed as the mean of six independent repeats, and the significance among different groups was determined using a one-way analysis of variance (ANOVA) with Tukey's (variance is homogeneous) or the Games–Howell (variance is heterogeneous) post hoc test. Significance was confirmed when the P value was below 0.05. To quantify the plasticity of each phenotype, the shift in the percentage of yeasts between treatments was calculated using the following equation:

Shift in the percentage range of yeast from condition A to condition B = [the production range between the first quartile (Q1) and the third quartile (Q3) in condition A/the production range between Q1 and Q3 in condition B] × 100.

Results

Yeast Identification

The partial sequence of internal transcribed spacer 1, complete sequence of 5.8S ribosomal RNA gene and internal transcribed spacer 2 and partial sequence of large subunit ribosomal RNA gene confirmed that we have isolated 25 strains of *A. pullulans* from different niches. Information on relevant sequence databases can be found in the GenBank sequence database (GenBank accession numbers MK156684 ~ MK156692, MK334632 ~ MK334647). The voucher specimens of *A. pullulans* isolates used in this study were deposited in the Bioresource Collection and Research Center, Hsinchu City, Taiwan (http://www.bcrc.firdi.org.tw/). Any requests should be addressed to the corresponding author.

Quantification of IAA Production

All the strains were inoculated in three types of medium containing different concentrations of the precursor Trp: YPD medium only, YPD medium with 0.1% Trp, or YPD medium with 1% Trp. The production of IAA ranged from 0 to 114.1 ± 31.0 μg/mL in the YPD medium only, from 13.4 ± 8.1 to 150.3 ± 11.5 μg/mL in the YPD medium with 0.1% Trp and from 47.4 ± 15.9 to 1035.6 ± 276.7 μg/mL in the YPD medium with 1% Trp (Fig. 1A). Furthermore, some strains could secrete IAA without Trp. The mean values of IAA production were 32.6 ± 39.2, 53.2 ± 32.8 and 374.3 ± 246.9 μg/mL in YPD medium only, YPD medium with 0.1% Trp and YPD medium with 1% Trp, respectively. The production range between Q1 and Q3 was 45.7 μg/mL in the YPD medium only, 33.8 μg/mL in the YPD medium with 0.1% Trp and 238.6 μg/mL in the YPD medium with 1% Trp. The shift in the percentage was approximately 74% between the YPD medium only and YPD medium with 0.1% Trp, approximately 522% between the YPD medium only and YPD medium with 1% Trp, and approximately 705% between the YPD medium with 0.1% Trp and YPD medium with 1% Trp. The difference in the amount of IAA produced among the different groups was significant ($P < 0.05$) and IAA production increased with an increasing concentration of exogenous Trp (Fig. 1B). As presented in Fig. 1C, the frequency distribution of IAA production in the YPD medium only indicated that the majority (~32%) of strains could not produce IAA in the absence of Trp and approximately 20% of strains produced approximately 50 μg/mL of IAA. The frequency distribution of IAA production in the YPD medium with 0.1% Trp indicated that most (~36%) strains and approximately 32% of strains produced approximately 70 μg/mL and 30 μg/mL of IAA, respectively. The frequency distribution of IAA production in the YPD medium with 1% Trp indicated that most (~24%) strains produced approximately 400 μg/mL of IAA.

Quantification of Siderophore Production

All the strains were inoculated in a medium containing different concentrations of exogenous ferrous ions: YPD medium only, YPD medium with 100 μM Fe^{2+}, or YPD medium with 900 μM Fe^{2+}. The siderophore production ability was expressed as the absorbance value and ranged from 0.051 ± 0.024 to 0.35 ± 0.005 in the YPD medium only, from 0.078 ± 0.105 to 0.68 ± 0.012 in the YPD medium with 100 μM Fe^{2+} and from 0 to 0.141 ± 0.023 in the YPD medium with 900 μM Fe^{2+} (Fig. 2A). The mean values of siderophore production were 0.21 ± 0.011, 0.333 ± 0.024 and 0.053 ± 0.02 in the YPD medium only, YPD with 100 μM Fe^{2+} and YPD medium with 900 μM Fe^{2+}, respectively. The production range between Q1 and Q3 was 0.063 in the YPD medium only, 0.06 in the YPD medium with 100 μM Fe^{2+} and 0.044 in the YPD medium with 900 μM Fe^{2+}. The percentage shift was approximately 95% between the YPD medium only and the YPD medium with 100 μM Fe^{2+}, approximately 71% between the YPD medium only and that with 900 μM Fe^{2+}, and approximately 74% between the YPD medium with 100 μM Fe^{2+} and that with 900 μM Fe^{2+}. The difference in the amount of siderophores produced among different groups was significant ($P < 0.05$). Siderophore production increased when the concentration of ferrous ions was low and decreased when the concentration of exogenous ferrous ions was considerably high (Fig. 2B). As shown in Fig. 2C, the frequency distribution of siderophore production in the YPD medium only indicated that most (~32%) strains and approximately 24% of strains had absorbance values of approximately 0.25 and 0.16, respectively. The frequency distribution of siderophore production in the YPD medium with 100 μM Fe^{2+} indicated that most (~28%) strains and approximately 24% of strains had absorbance values of approximately 0.35 and 0.3, respectively. The frequency distribution of siderophore production in the YPD medium with 900 μM Fe^{2+} indicated that most (~20%) strains and approximately 16% of strains had absorbance values of approximately 0.06 and 0.01, respectively.

Quantification of Phosphate-solubilizing Ability

Different types of phosphate were used in this experiment to determine the ability of *A. pullulans* to dissolve phosphates. The phosphate-solubilizing ability was determined as the concentration of inorganic phosphorous dissolved by *A. pullulans*. The concentration of inorganic phosphorous ranged from 0.48 ± 0.14 to 1.12 ± 0.38 μM in DCP, from 0.50 ± 0.20 to 0.82 ± 0.06 μM in CPT and from 0.15 ± 0.02 to 0.38 ± 0.05 μM in phosphorite (Fig. 3A and B). The mean values in terms of phosphate-solubilizing ability were 0.81 ± 0.14, 0.67 ± 0.09 and 0.27 ± 0.06 μM in DCP, CPT and phosphorite, respectively. The range of the phosphate-solubilizing ability between Q1 and Q3 was 0.11 in DCP, 0.10 in CPT and 0.06 in phosphorite. Most of the strains had

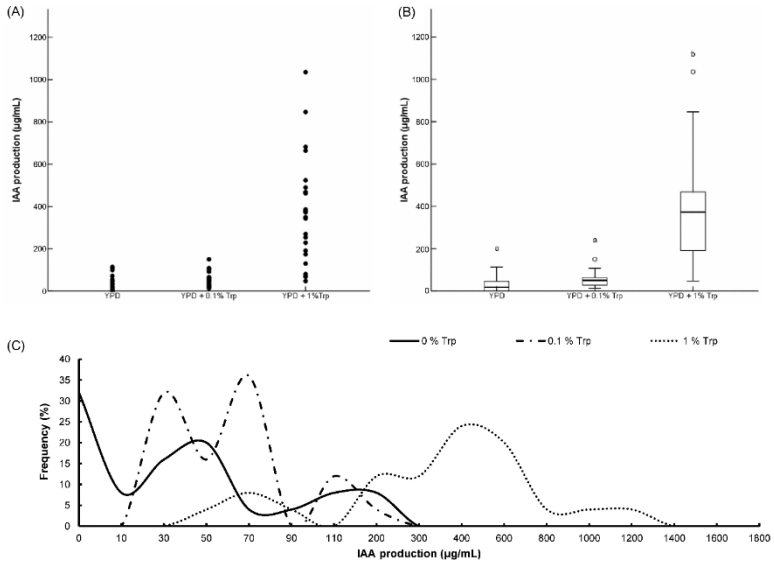

Fig. 1: IAA production of *A. pullulans* in the YPD medium only, YPD medium with 0.1% Tro or YPD medium with 1% Trp. (**A**) Scatter plot, (**B**) bar chart of IAA production (μg/mL) of *A. pullulans* and (**C**) Frequency distribution of IAA production (μg/mL) of *A. pullulans* in three experimental media (YPD medium only, YPD medium with 0.1% Trp, or YPD medium with 1% Trp). Data are the mean of six independent experiments. Means with the same letter are not significantly different from each other according to a one-way ANOVA with the Games–Howell post hoc test (N = 25, df = 2, F = 43.337, $P < 0.05$)

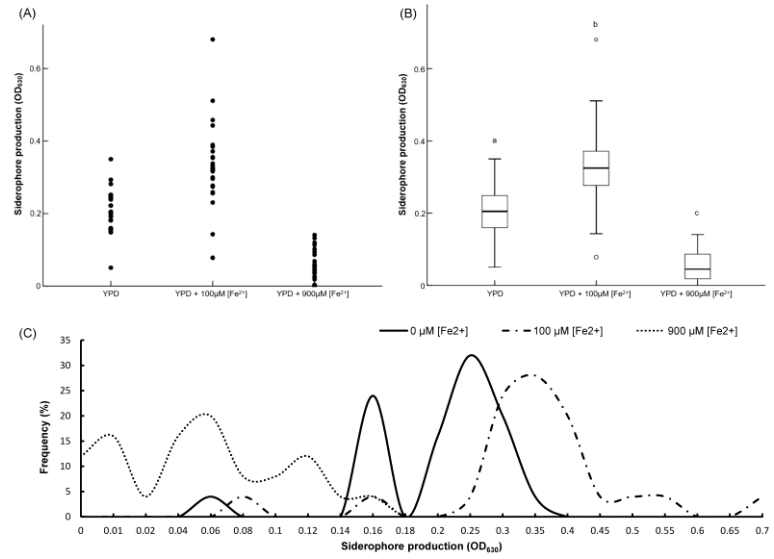

Fig. 2: Siderophore production of *A. pullulans* in YPD medium only, YPD medium with 100 μM Fe^{2+}, or YPD medium with 900 μM Fe^{2+}. (**A**) Scatter plot, (**B**) bar chart of siderophore production of *A. pullulans* and (**C**) Frequency distribution of siderophore production of *A. pullulans* in three experimental media (YPD medium only, YPD medium with 100 μM Fe^{2+}, or YPD medium with 900 μM Fe^{2+}). Data are expressed as absorbance values and are the mean of six independent experiments. Means with the same letter are not significantly different from each other according to a one-way ANOVA with Games–Howell post hoc test (N = 25, df = 2, F = 78.228, $P < 0.05$)

a greater ability to dissolve DCP and CPT and a lesser ability to dissolve phosphorite ($P < 0.05$). As shown in Fig. 3C, the frequency distribution of the phosphate-solubilizing ability in a medium containing DCP as the substrate indicated that most (~36%) strains dissolved approximately 0.75 μM of inorganic phosphorous. The frequency distribution of the phosphate-solubilizing ability in a medium containing CPT as the substrate indicated that most (~40%) strains dissolved approximately 0.65 μM of inorganic phosphorous. The frequency distribution of the phosphate-solubilizing ability in a medium containing phosphorite as the substrate indicated that most (~52%)

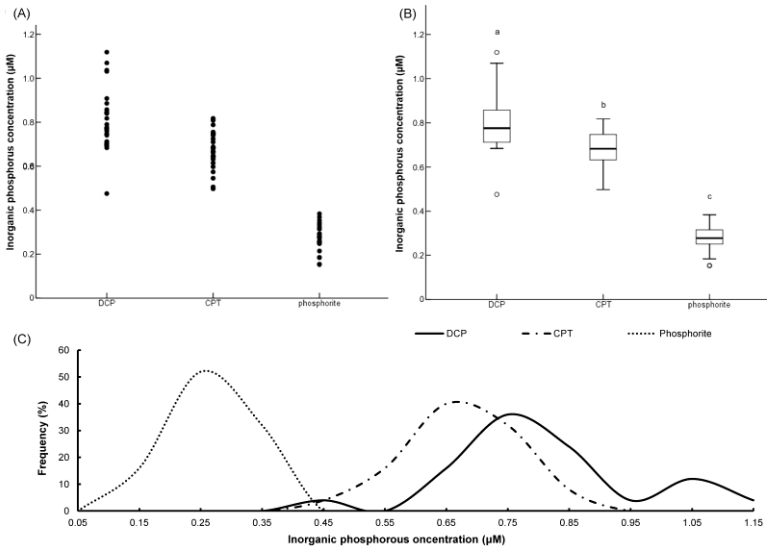

Fig. 3: Inorganic phosphorous concentration of *A. pullulans* in a medium containing DCP, CPT, or phosphorite as the substrate. (**A**) Scatter plot, (**B**) bar chart of the concentration (μM) of inorganic phosphorous dissolved by *A. pullulans* and (**C**) Frequency distribution of the concentration of inorganic phosphorous (μM) dissolved by *A. pullulans* in a medium containing DCP, CPT, or phosphorite as the substrate. Data are the mean of six independent experiments. Means with the same letter are not significantly different from each other according to a one-way ANOVA with Tukey's post hoc test (N = 25, df = 2, F = 184.189, $P < 0.05$)

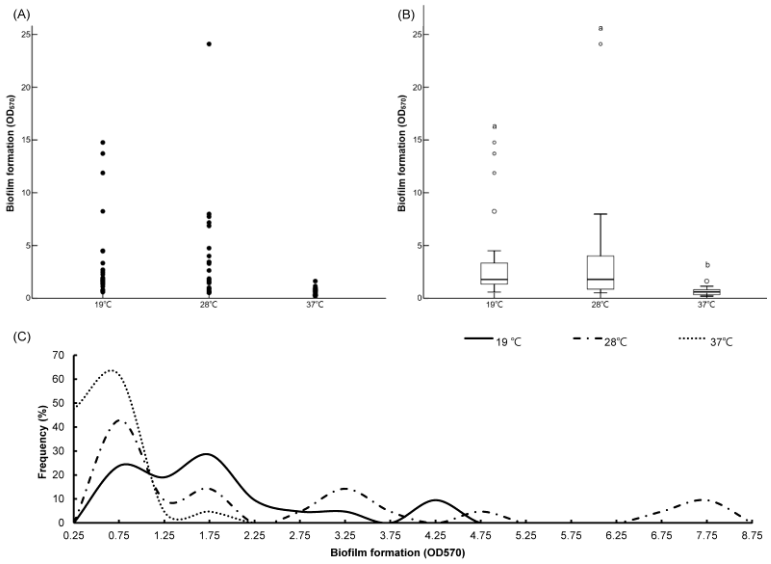

Fig. 4: Biofilm formation ability of *A. pullulans* at 19°C, 28°C, or 37°C. (**A**) Scatter plot, (**B**) bar chart of the biofilm formation ability of *A. pullulans* and (**C**) Frequency distribution of the biofilm formation ability of *A. pullulans* at three different temperatures (19°C, 28°C, or 37°C). Biofilm formation ability is expressed as an absorbance value. Data are the mean of six independent experiments. Means with the same letter are not significantly different from each other according to a one-way ANOVA with the Games–Howell post hoc test (N = 25, df = 2, F = 5.209, $P < 0.05$)

strains dissolved approximately 0.25 μM of inorganic phosphorous.

Quantification of Biofilm Formation

In this experiment, strains were inoculated in YPD medium at different temperatures (19°C, 28°C and 37°C). Biofilm formation ability is expressed as an absorbance value and the optical density (OD) ranged from 0.59 ± 0.25 to 14.77 ± 4.64 at 19°C, from 0.52 ± 0.18 to 24.09 ± 16.14 at 28°C and from 0.20 ± 0.19 to 1.63 ± 0.55 at 37°C. The mean OD values were 3.50 ± 4.11, 3.60 ± 4.9 and 0.63 ± 0.34 at 19°C, 28°C and 37°C, respectively (Fig. 4A and B). The OD range between Q1 and Q3 was 1.30 at 19°C, 2.53

at 28°C and 0.46 at 37°C. The percentage shift was approximately 194% between 19°C and 28°C, approximately 35% between 19°C and 37°C and approximately 18% between 28°C and 37°C. As shown in Fig. 4C, the frequency distribution of the biofilm formation ability at 19°C indicated that most (~29%) strains had an absorbance value of approximately 1.75. The frequency distribution of biofilm formation ability at 28°C indicated that the majority (~43%) of strains had an absorbance value of approximately 0.75. The frequency distribution of biofilm formation ability at 37°C indicated that the majority (~48%) of strains had an absorbance value of approximately 0.25. Most strains had a lower biofilm formation ability at 37°C.

Quantification of Antioxidant Capacity

In this experiment, strains were inoculated in YPD medium containing different concentrations of NaCl (0%, 1%, or 3%). Some of the strains were inhibited by osmotic pressure and their cell density was too low for inclusion in further experiments. Thus, those strains were not used in this experiment, and the number of strains used in the YPD medium with 0%, 1% and 3% salinity was 25, 25 and 24, respectively. Antioxidant capacity is expressed as the absorbance value and ranged from 0.342 ± 0.024 to 0.415 ± 0.01 in the YPD medium only, from 0.184 ± 0.025 to 0.338 ± 0.013 in the YPD medium with 1% NaCl and 0.161 ± 0.044 to 0.331 ± 0.004 in the YPD medium with 3% NaCl. The range of antioxidant capacity between Q1 and Q3 was 0.014 in the YPD medium only, 0.043 in the YPD medium with 1% NaCl and 0.041 in the YPD medium with 3% NaCl. The percentage shift was approximately 308% between the YPD medium only and that with 1% NaCl, approximately 307% between YPD medium only and that with 3% NaCl and approximately 100% between the YPD medium with 1% NaCl and that with 3% NaCl. The mean values of antioxidant capacity were 0.378 ± 0.019, 0.26 ± 0.045 and 0.26 ± 0.041 in the YPD medium only, YPD medium with 1% NaCl and YPD medium with 3% NaCl, respectively (Fig. 5A and B). As shown in Fig. 5C, the frequency distribution of antioxidant capacity in the YPD medium only indicated that most (~80%) strains had an absorbance value of approximately 0.375. The frequency distribution of antioxidant capacity in the YPD medium with 1% NaCl indicated that most (~36%) strains had an absorbance value of approximately 0.225. The frequency distribution of antioxidant capacity in the YPD medium with 3% NaCl indicated that most (~46%) strains had an absorbance value of approximately 0.275. The antioxidant capacity in the YPD medium only was significantly higher than that in the YPD media with 1% and 3% NaCl ($P < 0.05$; Fig. 5C).

Discussion

A. pullulans widely grows in different microhabitats worldwide. Similar to *A. pullulans*, a plant pathogen, *Botrytis cinerea*, is found in different habitats around the world. The high genetic variation and notable phenotypic plasticity of *B. cinerea* have been reported in numerous studies (Vlugt-Bergmans *et al*., 1993; Kerssies *et al*., 1997;

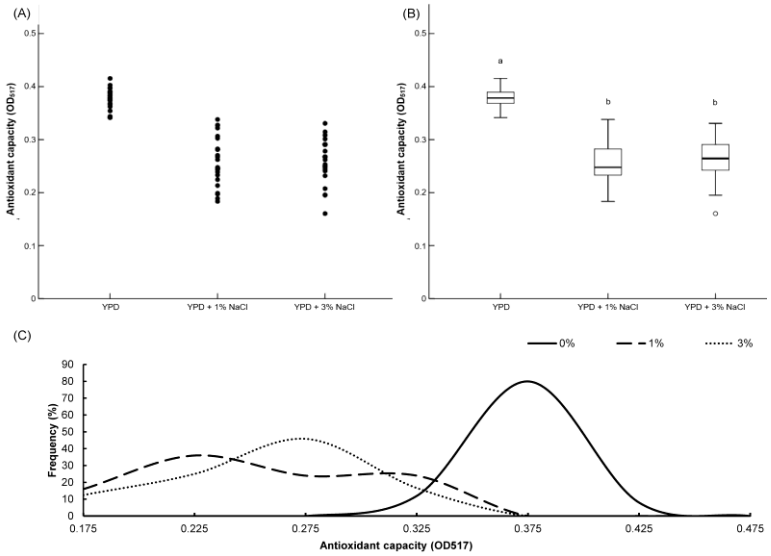

Fig. 5: Antioxidant capacity of *A. pullulans* in YPD medium only, YPD medium with 1% NaCl, and YPD medium with 3% NaCl. (**A**) Scatter plot, (**B**) bar chart of the antioxidant capacity of *A. pullulans* and (**C**) Frequency distribution of the antioxidant capacity of *A. pullulans* in the YPD medium only, YPD medium with 1% NaCl, or YPD medium with 3% NaCl. The antioxidant capacity is expressed as its absorbance value. Data are the mean of six independent experiments. Means with the same letter are not significantly different from each other according to a one-way ANOVA with the Games–Howell post hoc test (N = 25, df = 2, F = 85.381, $P < 0.05$)

Ma and Michailides, 2005; Blanco-Ulate *et al*., 2015). A study (Corwin *et al*., 2016) reported that the pathogenic ability (lesion formation on plants) and survivability (sclerotia formation and sporulation) of *B. cinerea* are controlled by its phenotypic plasticity and genetic variation of its traits. In the following paragraphs, the roles of phenotypic plasticity of *A. pullulans* playing in coping with environmental challenges will be discussed.

In this study, the fungal strains were inoculated in three concentrations of the IAA precursor Trp. Trp is considered a precursor for IAA biosynthesis, and its addition in a culture medium enhances IAA production both in bacteria and fungi (Hoffman *et al*., 2013; Sun *et al*., 2014). Our results indicated that an increase in the Trp concentration from 0 to 1% significantly increased the amount of IAA produced. Our results are in agreement with those reported by Khalid *et al*. (2004); who added Trp to a culture medium and further stimulated the auxin biosynthesis of rhizobacteria. In our study, some strains could still produce IAA in the absence of Trp, indicating that those *A. pullulans* strains used a Trp-independent pathway for synthesizing IAA (Normanly *et al*., 1993). The Trp-independent pathway for IAA synthesis has been proposed to be present in several bacterial and yeast species (Spaepen *et al*., 2007; Rao *et al*., 2010). Thus, these species can still synthesize IAA even in the absence of Trp. The results of the present study supported the hypothesis of a previous study that the inhibition of the growth of competitors by secreted IAA is an example of interference competition among yeast species (Liu *et al*., 2016). In each yeast species, an optimal concentration of IAA promotes its growth, and such effects are dependent on the strain. Thus, IAA production may be a crucial strategy for wild fungi to increase their competitiveness. In this study, we found high plasticity of IAA production in *A. pullulans*; the production of IAA was approximately 7–8 fold between environments with and without Trp. In addition, we noted a considerable degree of diversity in IAA production among strains even under the same condition. These findings provide insights into how *A. pullulans* remains one of the dominant members in the epiphytic microbial community and grows and colonizes on leaves (Abdelfattah *et al*., 2015).

Bacteria and fungi synthesize IAA as part of a system to communicate with their host plant, and many of them use IAA in pathogenic interactions such as in tumors and hairy roots (Spaepen *et al*., 2007). Gea *et al*. (1994) suggested that IAA facilitates the loosening of plant cell walls, allowing the fungus to enter the host's roots and manipulate the host's physiology. Mehmood *et al*. (2018) reported that the ability of the fungus *Fusarium oxysporum* to produce IAA is responsible for the colonization of the roots of corn. They inhibited IAA production by using the IAA biosynthesis inhibitor yucasin, which efficiently reduced the colonization of *F. oxysporum* in maize roots. Their findings suggest an IAA crosstalk between the two partners, and the inhibition of IAA limits their ability to interact. Thus, IAA plays crucial roles in fungal infection in plants. This characteristic may explain why *A. pullulans* is frequently found in association with diverse plants, such as in the phyllosphere, as an epiphyte or endophyte (Schena *et al*., 2003; Dimakopoulou *et al*., 2008; Martini *et al*., 2009; Sun *et al*., 2014). *A. pullulans* is recognized as an endophyte of considerable importance for plant hosts and its role in protecting plants against insects, nematodes and pathogenic microorganisms has also been well demonstrated (Saikkonen *et al*., 1998).

Competition is categorized into two modes: exploitative and interference. Exploitative competition is passive in the sense that one organism depletes its surroundings of nutrients, thereby preventing competitors from gaining access to those resources. Herein, we suggest that the production of siderophores by *A. pullulans* is one of their exploitative competition strategies to increase their fitness. Siderophores are expected to be secreted by microorganisms in a ferrous-limiting environment. To save energy, siderophores should not be secreted when ferrous ions are adequate in the environment. Our study revealed that siderophore production decreased with an increase in the concentration of ferrous ions (0 to 900 μM). However, we observed that a maximization of siderophore production does not occur in the absence of ferrous ions but does in the presence of a low concentration of ferrous ions. To explain this phenomenon, we offer two suggestions. The first is regarding the cost of siderophore production. When the concentration of ferrous ions is limited in the environment, a small amount of siderophores is adequate to scavenge ferrous ions. By contrast, microorganisms can stimulate their growth and inhibit competitors by gathering ferrous ions (Calvente *et al*., 1999). For example, the bacterium *Vibrio anguillarum* can produce siderophores to scavenge ferrous ions and inhibit the growth of *V. ordalii*. *V. anguillarum* collects nearby ferrous ions and creates an iron-limiting environment, which is toxic for iron-sensitive *V. ordalii* (Pybus *et al*., 1994). The results of the aforementioned studies indicate that siderophores are helpful in nutrient acquisition and competition for shared resources. Thus, *A. pullulans* alternates its siderophore production in response to the changing concentration of ferrous ions in the environment, and this may be its strategy of exploitative competition.

Phosphorous is an essential element for the growth of organisms that mostly exists as different insoluble phosphate complexes. In this study, three commonly found complexes (DCP, CPT and phosphorite) were used to examine their phosphorous-solubilizing ability. The results showed that the solubilizing ability was higher for DCP and CPT than for phosphorite; this result is consistent with those reported in relevant studies (Gulati *et al*., 2008; Xiao *et al*., 2009). One of the mechanisms through which microonganisms dissolve phosphate is secrete the organic acid to decrease the pH value and increase the solubility to acid. Thus, we suggest that a lower ability in dissolving

phosphorite results from its poor solubility in acid; phosphorite is considerably difficult to dissolve in acid compared with other complexes. In related studies, microorganisms with a phosphorous-solubilizing ability were obtained from different environments and screened. Of the total organisms, approximately 1.5% (1% of bacteria and 0.5% fungus) obtained from the soil of southern Alberta and approximately 9% (fungus) obtained from the soil of Ethiopia could solubilize phosphorous (Kucey, 1983; Gizaw *et al*., 2017). However, in our study, all *A. pullulans* strains could dissolve all the three types of phosphate, indicating that *A. pullulans* is more likely to survive in a phosphorus-limited environment.

Biofilm, which is composed of polysaccharides, is a functional product used for cell localization and protection from antibiotic or other environmental pressures (Flemming, 1993; Davies, 2003). Temperature has been considered an important factor affecting biofilm formation. In this study, we chose three temperatures (19°C, 28°C and 37°C) to examine biofilm formation ability. The biofilm formation ability at 37°C was lower than that at other temperatures; this finding may be attributable to two factors. First, temperature affects the growth of microorganisms (Casman and Rettger, 1933). A high temperature inhibits the growth of microorganisms, which is related to polysaccharide production (Bueno and Garcia-Cruz, 2006). Second, the biofilm formed at 37°C group could easily fall off from the surface, possibly due to decreases in elasticity and viscosity at high temperatures (Rühs *et al*., 2013). In addition to the aforementioned functions, biofilm is helpful in several aspects, such as nutrient acquisition and energy sinking (Decho, 1990; Ortega-Morales *et al*., 2007). For nutrient acquisition, the highly adsorptive nature of biofilm helps microorganisms easily obtain nutrients adsorbed on the biofilm. In terms of energy sinking, biofilm is composed of exopolysaccharides that can serve as a storage substance for excess carbon. Thus, all strains in our experiment could form biofilm under three different conditions, indicating that *A. pullulans* can form biofilm at different temperatures and the biofilm formation can help *A. pullulans* in adapting to the environment.

Drought induces changes in salinity. This influences osmotic pressure, which in turn affects microbial growth, metabolism and ROS production (Fisher and Newell, 1986; Brito *et al*., 2000). In this study, we used different salinities to examine antioxidant capacity against ROS. The antioxidant capacity is expected to increase with a higher oxidative pressure caused by salinity. However, the antioxidant capacity of strains grown in YPD medium was only significantly higher than that of strains grown in YPD medium with 1% and 3% NaCl. A related study (Sharma and Sharma, 2017) demonstrated that salt stress increased the antioxidant capacity but inhibited growth at the same time. Our results indicate that growth inhibition may be more substantial than the improvement in antioxidant capacity (Queiroga *et al*., 2016). Although the antioxidant capacity of each cell increased, the total cell number decreased rapidly. By contrast, the decreasing antioxidant capacity was relative to ROS accumulation, which is crucial in yeasts for filament transition, cleistothecium (ascocarp) formation, and pathogenicity (Georgiou *et al*., 2000; LaraOrtíz *et al*., 2003; Leuthner *et al*., 2005; Brown *et al*., 2014).

Conclusion

The present study concludes that the performance of traits is affected by phenotypic plasticity and genetic variation. The findings of the studies on *A. pullulans* and *B. cinerea* suggest that phenotypic plasticity and genetic variation both play crucial roles in the adaptation of microorganisms to fluctuating environmental conditions.

Acknowledgements

We thank the members of the Chou Lab for their helpful discussion and comments on the manuscript. This work was supported by grants from the Ministry of Science and Technology (MOST 105-2311-B-018-001-MY3 to Chou JY; MOST 106-2119-M-003-003- to Fang WT). This manuscript was edited by Wallace Academic Editing.

References

Abdelfattah, A., M.G.L.D. Nicosia, S.O. Cacciola, S. Droby and L. Schena, 2015. Metabarcoding analysis of fungal diversity in the phyllosphere and carposphere of olive (*Olea europaea*). *PLoS One*, 10: e0131069

Ames, B.N., 1966. Assay of inorganic phosphate, total phosphate and phosphatases. *In: Methods in Enzymology*, pp: 115–118. Elizabeth, V.G. and F. Neufeld (Eds). Academic Press, Amsterdam, The Netherlands

Barp, E.A., G.L.G. Soares, G. Gosmann, A.M. Machado, C. Vecchi and G.R.P. Moreira, 2006. Phenotypic plasticity in *Passiflora suberosa* L. (Passifloraceae): induction and reversion of two morphs by variation in light intensity. *Braz. J. Biol*., 66: 853–862

Bersabé, D., A. Caballero, A. Pérez-figueroa and A. García-Dorado, 2015. On the consequences of purging and linkage on fitness and genetic diversity. *G3: Genes Genom. Genet*., 6: 171–181

Blanco-Ulate, B., K.C. Amrine, T.S. Collins, R.M. Rivero, A.R. Vicente, A. Morales-Cruz, C.L. Doyle, Z. Ye, G. Allen, H. Heymann, S.E. Ebeler and D. Cantu, 2015. Developmental and metabolic plasticity of white-skinned grape berries in response to *Botrytis cinerea* during noble rot. *Plant Physiol*., 169: 2422–2443

Brito, R., M.E. Chimal and C. Rosas, 2000. Effect of salinity in survival, growth and osmotic capacity of early juveniles of *Farfantepenaeus brasiliensis* (Decapoda: Penaeidae). *J. Exp. Mar. Biol. Ecol*., 244: 253–263

Brown, A.J., S. Budge, D. Kaloriti, A. Tillmann, M.D. Jacobsen, Z. Yin, I.V. Ene, I. Bohovych, D. Sandai, S. Kastora, J. Potrykus, E.R. Ballou, D.S. Childers, S. Shahana and M.D. Leach, 2014. Stress adaptation in a pathogenic fungus. *J. Exp. Biol.*, 217: 144–155

Bueno, S.M. and C.H. Garcia-Cruz, 2006. Optimization of polysaccharides production by bacteria isolated from soil. *Braz. J. Microbiol.*, 37: 296–301

Calvente, V., D. Benuzzi and M.S.D. Tosetti, 1999. Antagonistic action of siderophores from Rhodotorula glutinis upon the postharvest pathogen *Penicillium expansum*. *Intl. Biodeterior. Biodegrad.*, 43: 167–172

Casman, E.P. and L.F. Rettger, 1933. Limitation of bacterial growth at higher temperatures. *J. Bacteriol*., 26: 77–123

Chen, P.H., R.Y. Chen and J.Y. Chou, 2018. Screening and Evaluation of Yeast Antagonists for Biological Control of *Botrytis cinerea* on Strawberry Fruits. *Mycobiology*, 46: 33–46

Cooke, W.B., 1959. An ecological life history of *Aureobasidium pullulans* (de Bary) Arnaud. *Mycopathol. Mycol. Appl.*, 12: 1–45

Cortesi, F., W.E. Feeney, M.C. Ferrari, P.A. Waldie, G.A. Phillips, E.C. McClure, H.N. Sköld, W. Salzburger, N.J. Marshall and K.L. Cheney, 2015. Phenotypic plasticity confers multiple fitness benefits to a mimic. *Curr. Biol.*, 25: 949–954

Corwin, J.A., A. Subedy, R. Eshbaugh and D.J. Kliebenstein, 2016. Expansive phenotypic landscape of Botrytis cinerea shows differential contribution of genetic diversity and plasticity. *Mol. Plant-Microb. Interact.*, 29: 287–298

Davies, D., 2003. Understanding biofilm resistance to antibacterial agents. *Nat. Rev. Drug Discov.*, 2: 114–122

Decho, A.W., 1990. Microbial exopolymer secretions in ocean environments: Their role(s) in food webs and marine processes. *Oceanogr. Mar. Biol. Annu. Rev.*, 28: 9–16

Deryng, D., D. Conway, N. Ramankutty, J. Price and R. Warren, 2014. Global crop yield response to extreme heat stress under multiple climate change futures. *Environ. Res. Lett.*, 9: 1–14

Dimakopoulou, M., S.E. Tjamos, P.P. Antoniou, A. Pietri, P. Battilani, N. Avramidis, E.A. Markakis and E.C. Tjamos, 2008. Phyllosphere grapevine yeast *Aureobasidium pullulans* reduces *Aspergillus carbonarius* (sour rot) incidence in wine-producing vineyards in Greece. *Biol. Contr.*, 46: 158–165

Ellegren, H. and N. Galtier, 2016. Determinants of genetic diversity. *Nat. Rev. Genet.*, 17: 422–433

Fisher, W.S. and R.I. Newell, 1986. Salinity effects on the activity of granular hemocytes of American oysters, *Crassostrea virginica*. *Biol. Bull.*, 170: 122–134

Flemming, H.C., 1993. Biofilms and environmental protection. *Wat. Sci. Technol.*, 27: 1–10

Frainer, A., B.G. Mckie, P.A. Amundsen, R. Knudsen and K.D. Lafferty, 2018. Parasitism and the Biodiversity-Functioning Relationship. *Trends Ecol. Evol.*, 33: 260–268

Franssen, N.R., L.K. Stewart and J.F. Schaefer, 2013. Morphological divergence and flow-induced phenotypic plasticity in a native fish from anthropogenically altered stream habitats. *Ecol. Evol.*, 3: 4648–4657

Frost, C.M., G. Peralta, T.A. Rand, R.K. Didham, A. Varsani and J.M. Tylianakis, 2016. Apparent competition drives community-wide parasitism rates and changes in host abundance across ecosystem boundaries. *Nat. Commun.*, 7: 1–12

Fux, C., J.W. Costerton, P.S. Stewart and P. Stoodley, 2005. Survival strategies of infectious biofilms. *Trends Microbiol.*, 13: 34–40

Gadd, G., 1980. Melanin production and differentiation in batch cultures of the polymorphic fungus *Aureobasidium pullulans*. *FEMS Microbiol. Lett.*, 9: 237–240

Gea, L., L. Normand, B. Vian and G. Gay, 1994. Structural aspects of ectomycorrhiza of *Pinus pinaster* (Ait.) Sol. formed by an IAA-overproducer mutant of *Hebeloma cylindrosporum* Romagnési. *New Phytol.*, 128: 659–670

Georgiou, C.D., N. Tairis and A. Sotiropoulou, 2000. Hydroxyl radical scavengers inhibit sclerotial differentiation and growth in *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. *Mycol. Res.*, 104: 1191–1196

Gil-Rodríguez, A.M., A.V. Carrascosa and T. Requena, 2015. Yeasts in foods and beverages: *In vitro* characterisation of probiotic traits. *LWT-Food Sci. Technol.*, 64: 1156–1162

Gizaw, B., Z. Tsegay, G. Tefera and E. Aynalem, 2017. Phosphate solubilizing yeast isolated and characterized from Teff rhizosphere soil collected from Gojam; Ethiopia. *J. Bacteriol. Mycol.*, 5: 218–223

Gordon, S.A. and R.P. Weber, 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiol.*, 26: 192–195

Gulati, A., P. Rahi and P. Vyas, 2008. Characterization of phosphate-solubilizing fluorescent pseudomonads from the rhizosphere of seabuckthorn growing in the cold deserts of Himalayas. *Curr. Microbiol.*, 56: 73–79

Gunde-Cimerman, N., J. Ramos and A. Plemenitaš, 2009. Halotolerant and halophilic fungi. *Mycol. Res.*, 113: 1231–1241

Hoffman, M.T., M.K. Gunatilaka, K. Wijeratne, L. Gunatilaka and A.E. Arnold, 2013. Endohyphal bacterium enhances production of indole-3-acetic acid by a foliar fungal endophyte. *PLoS One*, 8: e73132

Kerssies, A., A.I. Bosker-Van Zessen, C.A.W. Wagemakers and J.A.L.V. Kan, 1997. Variation in pathogenicity and DNA polymorphism among *Botrytis cinerea* isolates sampled inside and outside a glasshouse. *Plant Dis.*, 81: 781–786

Khalid, A., M. Arshad and Z. Zahir, 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol.*, 96: 473–480

Kucey, R., 1983. Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. *Can. J. Soil Sci.*, 63: 671–678

Kurtzman, C. and C. Robnett, 1997. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5'end of the large-subunit (26S) ribosomal DNA gene. *J. Clin. Microbiol.*, 35: 1216–1223

LaraOrtíz, T., H. RiverosRosas and J. Aguirre, 2003. Reactive oxygen species generated by microbial NADPH oxidase NoxA regulate sexual development in *Aspergillus nidulans*. *Mol. Microbiol.*, 50: 1241–1255

Leuthner, B., C. Aichinger, E. Oehmen, E. Koopmann, O. Muller, P. Müller, R. Kahmann, M. Bölker and P.H. Schreier, 2005. A H_2O_2-producing glyoxal oxidase is required for filamentous growth and pathogenicity in *Ustilago maydis*. *Mol. Genet. Genom.*, 272: 639–650

Liu, Y.Y., H.W. Chen and J.Y. Chou, 2016. Variation in indole-3-acetic acid production by wild *Saccharomyces cerevisiae* and *S. paradoxus* strains from diverse ecological sources and its effect on growth. *PLoS One*, 11: e0160524

Louden, B.C., D. Haarmann and A.M. Lynne, 2011. Use of Blue Agar CAS Assay for Siderophore Detection. *J. Microbiol. Biol. Edu.*, 12: 51–53

Ma, Z. and T.J. Michailides, 2005. Genetic structure of *Botrytis cinerea* populations from different host plants in California. *Plant Dis.*, 89: 1083–1089

Martini, M., R. Musetti, S. Grisan, R. Polizzotto, S. Borselli, F. Pavan and R. Osler, 2009. DNA-dependent detection of the grapevine fungal endophytes *Aureobasidium pullulans* and *Epicoccum nigrum*. *Plant Dis.*, 93: 993–998

Mehmood, A., M. Irshad, N. Khan, M. Hamayun, Ismail, Husna, A. Javed and A. Hussain, 2018. IAA Producing Endopytic Fungus *Fusariun oxysporum* wlw Colonize Maize Roots and Promoted Maize Growth Under Hydroponic Condition. *Eur. J. Exp. Biol.*, 8: 1–7

Normanly, J., J.D. Cohen and G.R. Fink, 1993. *Arabidopsis thaliana* auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. *Proc. Natl. Acad. Sci. USA*, 90: 10355–10359

Ortega-Morales, B.O., J.L. Santiago-García, M.J. Chan-Bacab, X. Moppert, E. Miranda-Tello, M.L. Fardeau, J.C. Carrero, P. Bartolo-Pérez, A. Valadéz-González and J. Guezennec, 2007. Characterization of extracellular polymers synthesized by tropical intertidal biofilm bacteria. *J. Appl. Microbiol.*, 102: 254–264

Pechak, D.G. and R.E. Crang, 1977. An analysis of *Aureobasidium pullulans* developmental stages by means of scanning electron microscopy. *Mycologia*, 69: 783–792

Pedersen, O., T.D. Colmer, J. Borum, A. ZavalaPerez and G.A. Kendrick, 2016. Heat stress of two tropical seagrass species during low tides–impact on underwater net photosynthesis, dark respiration and diel *in situ* internal aeration. *New Phytol.*, 210: 1207–1218

Peltier, E., V. Sharma, M.M. Raga, M. Roncoroni, M. Bernard, V. Jiranek, Y. Gibon and P. Marullo, 2018. Dissection of the molecular bases of genotype x environment interactions: a study of phenotypic plasticity of *Saccharomyces cerevisiae* in grape juices. *BMC Genomics*, 19: 1–20

Prasongsuk, S., P. Lotrakul, I. Ali, W. Bankeeree and H. Punnapayak, 2018. The current status of *Aureobasidium pullulans* in biotechnology. *Fol. Microbiol.*, 63: 129–140

Pybus, V., M. Loutit, I. Lamont and J. Tagg, 1994. Growth inhibition of the salmon pathogen *Vibrio ordalii* by a siderophore produced by *Vibrio anguillarum* strain VL4355. *J. Fish Dis.*, 17: 311–324

Queiroga, F.R., L.F. Marques-Santos, I.A.D. Medeiros and P.M.D. Silva, 2016. Effects of salinity and temperature on *in vitro* cell cycle and proliferation of *Perkinsus marinus* from Brazil. *Parasitology*, 143: 475–487

Rühs, P.A., L. Böni, G.G. Fuller, R.F. Inglis and P. Fischer, 2013. *In-situ* quantification of the interfacial rheological response of bacterial biofilms to environmental stimuli. *PLoS One*, 8: e78524

Radice, S., M. Alonso and M.E. Arena, 2018. *Berberis microphylla*: A species with phenotypic plasticity in different climatic conditions. *Intl. J. Agric. Biol*., 20: 2221–2229

Rao, R.P., A. Hunter, O. Kashpur and J. Normanly, 2010. Aberrant synthesis of indole-3-acetic acid in *Saccharomyces cerevisiae* triggers morphogenic transition, a virulence trait of pathogenic fungi. *Genetics*, 185: 211–220

Saber-Samandari, S. and M. Gazi, 2015. Pullulan based porous semi-IPN hydrogel: Synthesis, characterization and its application in the removal of mercury from aqueous solution. *J. Taiwan Instrum. Chem. Eng.*, 51: 143–151

Saikkonen, K., S.H. Faeth, M. Helander and T. Sullivan, 1998. Fungal endophytes: a continuum of interactions with host plants. *Annu. Rev. Ecol. Evol. Syst.*, 29: 319–343

Schena, L., F. Nigro, I. Pentimone, A. Ligorio and A. Ippolito, 2003. Control of postharvest rots of sweet cherries and table grapes with endophytic isolates of *Aureobasidium pullulans*. *Postharv. Biol. Technol.*, 30: 209–220

Searle, C., J. Ochs, C. Cáceres, S. Chiang, N. Gerard, S.R. Hall and M.A. Duffy, 2015. Plasticity, not genetic variation, drives infection success of a fungal parasite. *Parasitology*, 142: 839–848

Shah, N.N., C. Vishwasrao, R.S. Singhal and L. Ananthanarayan, 2016. n-Octenyl succinylation of pullulan: Effect on its physico-mechanical and thermal properties and application as an edible coating on fruits. *Food Hydrocoll.*, 55: 179–188

Sharma, A. and S.C. Sharma, 2017. Physiological basis for the tolerance of yeast *Zygosaccharomyces bisporus* to salt stress. *HAYATI J. Biosci.*, 24: 176–181

Slepecky, R.A. and W.T. Starmer, 2009. Phenotypic plasticity in fungi: A review with observations on *Aureobasidium pullulans*. *Mycologia*, 101: 823–832

Spaepen, S., J. Vanderleyden and R. Remans, 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol. Rev.*, 31: 425–448

Sun, P.F., W.T. Fang, L.Y. Shin, J.Y. Wei, S.F. Fu and J.Y. Chou, 2014. Indole-3-acetic acid-producing yeasts in the phyllosphere of the carnivorous plant *Drosera indica* L. *PLoS One*, 9: e114196

Sundara, W.V.B. and M.K. Sinha, 1963. Phosphate dissolving microorganisms in the rhizosphere and soil. *Indian J. Agric. Sci.*, 33: 272–278

Tadrowski, A.C., M.R. Evans and B. Waclaw, 2018. Phenotypic Switching Can Speed up Microbial Evolution. *Sci. Rep.*, 8: 1–10

Thomas, F.M., R. Blank and G. Hartmann, 2002. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. *For. Pathol.*, 32: 277–307

Urzi, C., F.D. Leo, C.L. Passo and G. Criseo, 1999. Intra-specific diversity of *Aureobasidium pullulans* strains isolated from rocks and other habitats assessed by physiological methods and by random amplified polymorphic DNA (RAPD). *J. Microbiol. Meth.*, 36: 95–105

Vlugt-Bergmans, C.J.B.V.D., B.F. Brandwagt, J.W.V. Klooster, C.A.M. Wagemakers and J.A.L.V. Kan, 1993. Genetic variation and segregation of DNA polymorphisms in *Botrytis cinerea*. *Mycol. Res.*, 97: 1193–1200

White, T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In: PCR Protocols: A Guide to Methods and Applications*, pp: 315–322. Innis, M.A., D.H. Gelfand, J.J. Sninsky and T.J. White (Eds.). Academic Press Inc., New York, USA

Worm, B. and A.R. Chapman, 1998. Relative effects of elevated grazing pressure and competition from a red algal turf on two post-settlement stages of *Fucus evanescens* C. Ag. *J. Exp. Mar. Biol. Ecol.*, 220: 247–268

Xiao, C., R. Chi, H. He, G. Qiu, D. Wang and W. Zhang, 2009. Isolation of phosphate-solubilizing fungi from phosphate mines and their effect on wheat seedling growth. *Appl. Biochem. Biotechnol.*, 159: 330–342

Zalar, P., C. Gostinčar, G.D. Hoog, V. Uršič, M. Sudhadham and N. Gunde-Cimerman, 2008. Redefinition of *Aureobasidium pullulans* and its varieties. *Stud. Mycol.*, 61: 21–38

[Received 28 Dec 2018; Accepted 21 Feb 2019; Published (online) 26 Apr 2019]