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Proteomics profile of *Hanseniaspora uvarum* enhanced with trehalose involved in the biocontrol efficacy of grape berry

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ABSTRACT

This present study tested the extent to which 2% w/v trehalose enhanced the proteins expression profile of *Hanseniaspora uvarum* Y3. Furthermore, it explored the relative gene expression of stilbene synthase (*StSy*), one of the vital defense-related genes found in the skin of grapes. The proteomics profile revealed that 29 proteins were differentially expressed out of which 26 were significantly up-regulated and 3 were download-regulated. The pathogenesis related (PR) and other protein spots were visible at 97.4 kDa and 14.4 kDa. Peroxiredoxin TSA1 and superoxide dismutase were the main proteins involved in defense response and both proteins were significantly up-regulated. The carbohydrate and energy metabolism proteins were also significantly up-regulated. The results revealed that the treatments were associated with substantial increase in peroxidase activity compared to the control. *StSy* relative gene expression level was observed to increase by 2.5-fold in grapes treated with the pre-enhanced *H. uvarum* compared to the control.

1. Introduction

Postharvest losses continue to deprive the world populace of adequate food security particularly with fruits and vegetables. Currently, postharvest losses of agricultural produce is between 29 and 38% for developed and higher for developing countries (Gustavsson, Cederberg, Sonesson, & Emanuelsson, 2013). In an attempt to curb the canker, several methods such as physical (Gündüz, Juneja, & Pazır (2015), chemical (Boukaew, Prasertsan, Troulet, & Bardin, 2017) and biological (Wicaksono, Jones, Casonato, Monk, & Ridgway, 2017) have been employed to control postharvest diseases of fruits and vegetables. Over the past few years, biological control has been touted as the best method for controlling postharvest diseases of fruits and vegetables (Schirra, D'Aquino, Cabras, & Angioni, 2011). As a result several biological control agents and bioactive compounds that play complementary roles in postharvest disease control have been studied (Zhang, Chen, & Wang, 2013). A better understanding of the mechanisms of postharvest biocontrol agents on fruit, is critical for the advancement of the development of successful biocontrol products.

Hanseniaspora uvarum exhibited industrially important antagonistic features against the growth of molds responsible for fruits decay (López, Mateo, & Maicas, 2015). Previous study indicated that *H. uvarum* Y3, a non *Saccharomyces cerevisiae* showed biocontrol efficacy against *Aspergillus tubingensis* (Apaliya et al., 2017). Similarly results showed that *H. uvarum* Y3 significantly inhibited grape rots caused by *A. tubingensis* with no negative effect observed on the quality parameters (unpublished data). However, the mechanism(s) behind the biocontrol was not investigated. In this wise, the proteome profile of the antagonist has been explored. Trehalose is a non-reducing sugar, which has successfully shown to act against oxidative stress, heat shock, dehydration, harmful chemicals and nutrient starvation (Argüelles, 2000). Trehalose can protect enzymes, liposomes, antibodies, and microorganisms during drying and or storage (Gancedo & Flores, 2004). Our previous results indicated that *H. uvarum* enhanced with 2% w/v trehalose demonstrated biocontrol efficacy against *Aspergillus tubingensis* in grapes (Apaliya et al., 2017).

Previously, phenylalanine ammonia lyase, CAT, chitinase, ascorbate peroxidase and polyphenoloxidase which are involved in defense-related and antioxidant activities were significantly regulated (Apaliya et al., 2017). Proteomics are among the most important tools employed to investigate the mechanism(s) involved in biocontrol. To this end, the aim of this study was to: (i) Investigate stilbene synthase genome, peroxidase, superoxide dismutase, hydrogen peroxide and malondialdehyde expression levels in table grapes treated with *H. uvarum* enhanced with trehalose in NYDB. (ii) Investigate the proteomics profiles of *H. uvarum* pretreated with/without 2% w/v trehalose.

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2. Materials and methods

2.1. Source of fruit

Grape (*Vitis vinifera* cv. 'Hongti') berries were harvested from a project farm in Zhenjiang, Jiangsu Province, China. The grapes were harvested at commercial maturity in the vineyard and selected based on features such as absence of blemishes and uniformity of sizes. Subsequently, the grapes were sent to the laboratory and surface disinfected using 0.1% v/v sodium hypochlorite for 1 min. The grapes were then air dried at room temperature after rinsing twice with tap water, stored in an incubator at room temperature and 95% relative humidity.

2.2. Microbial antagonist

Hanseniaspora uvarum strain Y3 was isolated from the surface of grapes in a vineyard in Zhenjiang, Jiangsu Province, China by our research team. Standard methods based on cell morphology and colony formation were used for preliminary characterization (Kurtzman, Fell, & Boekhout, 2011). Afterward, sequence analysis of 5.8S internal transcribed spacer (ITS) ribosomal RNA (rRNA) region was used to identify the yeast (Apaliya et al., 2017).

2.3. Grapes treaments

Commercially matured 'Hongti' cv. (*Vitis vinefera*) were wounded (3 mm diameter × 3 mm deep) with sterile cork borer and the following treatments inoculated into the wounds: (1) 30 µl sterile distilled water (SDW) as control, (2) 30 µl of *H. uvarum* (1×10^8 CFU/ml), (3) 30 µl 2% w/v trehalose and (4) 30 µl *H. uvarum* T (1×10^8 CFU/ml) pretreated with 2% w/v trehalose in NYDB. The grapes were dried in plastic baskets, wrapped with plastic film and incubated at 20 °C at 95% relative humidity. The samples were taken and enzyme activity analyzed at 0, 24, 48 and 72 h.

2.4. Peroxidase activity

Peroxidase (POD) activity was determined using the method described by Lurie, Fallik, Handros, and Shapira (1997). The reaction mixture composed of 0.2 ml supernatant (crude enzyme extract) and guaiacol 2.2 ml of 0.3% (50 mM sodium phosphate buffer, pH 6.4) was incubate at 30 °C for 5 min. Then 0.6 ml of 0.3% H_2O_2 (50 mM sodium phosphate, pH 6.4) was added and incubated at 30 °C for 5 min. Subsequently, POD activity was measured at 470 nm every 30 s for 3 min. The blank was prepared by replacing the crude enzyme with 0.2 ml 50 mM sodium phosphate buffer (pH 6.4). The POD activity was expressed as unit milligram/g fresh weight (Unit mg/FW).

2.5. Superoxide dismutase (SOD)

SOD activity was assayed according to the method described by Wang, Tian, Xu, Qin, and Yao (2004). First, 3 ml of a reaction mixture composed of cold sodium phosphate buffer (50 mM, pH 7.8), 10 µl EDTA, 75 µM nitroblue tetrazolium (NBT), 13 mM methionine, 2 µM riboflavin and 0.1 ml crude enzyme extract were mixed. Then, a fluorescent lamp was illuminated through the sample for 10 min and absorbance determined at 560 nm. A unit of SOD was defined as the quantity of enzyme extract that caused a 50% decrease of SOD-nitroblue tetrazolium reduction. SOD activity was expressed as unit mg/FW.

2.6. Malondialdehyde (MDA)

To determine MDA activity, 2 ml of 0.5% thiobarbituric acid (TBA) in 15% trichloroacetic acid was added to 1 ml of the enzyme extract. The mixture was heated for 20 min at 95 °C, cooled in ice for 5 min then centrifuged at 12,000 rpm for 10 min. The absorbance was determined at 532 nm and subtracted from non-specific absorbance (600 nm). The extinction coefficient used for the MDA calculated was 155 mM cm/1. The protein content was determined using the method of Bradford (1976) with bovine serum albumin as the protein standard.

2.7. Hydrogen peroxide (H_2O_2)

The H_2O_2 content of the grapes was determined according to the method of Patterson, MacRae, and Ferguson (1984). Briefly, 3 g of the grape tissues was used to extract H_2O_2 and the sample homogenized with 5 ml of cold 100% acetone. The sample was then centrifuged at 12,000 rpm for 20 min at 4 °C. The absorbance of the sample was determined at 412 nm and the results expressed in µmol/g fresh weight (FW).

2.8. Stilbene synthase RNA extraction and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Matured table grapes were prepared for the assay according to the method described by Apaliya et al. (2017). Total mRNA was extracted according to the manufacturer's instructions (Sigma-Aldrich, Shanghai, China). qRT-PCR was conducted with the Cdna (1000 ng of mRNA was used for cDNA synthesis) and the gene-specific primers *StSy* (F: 5′ CTCTTAGGTCTTGAAACATCGGT 3′; R: 5′AAGACCCATCACCAAA AGG 3′) and *actin* (F: 5′ TTCAATAAGGAGAAGATGGTG GA 3′; R: 5′ TTGGT GAGGTAGTCTGTGAGGTC 3′) were conducted with an Agilent 2100 Bioanalyzer and an ABI StepOnePlus Real-Time PCR System (Applied Biosystems, USA) (Zhang, Wang, Hu, & Liu, 2015).

The amplified products were examined after the PCR reaction through Melting curve analyses. The melting cycle condition was: 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. The ACTIN gene of the grapes was used as an internal control to normalize the expressed data (Liu & Ekramoddoullah, 2006). The $2^{-\Delta\Delta Ct}$ method was used to calculated relative expression level of the identified genes (Livak & Schmittgen, 2001). The RT-qPCR analysis was conducted twice and the standard deviation was determined among three biological replicates.

2.9. Protein assay

H. uvarum Y3 was dispensed into two separate 250 ml Erlenmeyer flasks containing NYDB with/without 2% w/v trehalose and cultured in a rotary incubator (180 × g) at 28 °C for 24 h. The cell suspensions of the yeasts were then harvested from both media (NYDB and NYDB containing 2% w/v trehalose) by centrifugation at 10,000 rpm for 10 min at 4 °C. Subsequently, the yeast cells were washed thrice using 4 °C SDW in order to get rid of all residual media. The protein samples were prepared for two-dimensional gel electrophoresis according to the method described by Zhao, Zhang, Lin, Zhang, and Ren (2013).

In brief, the samples were freeze dried with liquid nitrogen and ground to fine powder using a mortar and pestle. The powder was homogenized in cold 10 ml TE buffer (0.073 g EDTA, 1.518 g Tris-base adjusted to pH 8.0 with 1 M HCl). The buffer was dissolved using 250 ml MilliQ H_2O and kept at 4 °C. Then, 174 µl phenylmethane sulfonyl fluoride (PMSF) was added to the homogenate and stored for 30 min at 4 °C in order to make the proteins inactive. Thereafter, 174 µ1 of 1 mM/ml PMSF was again added to the homogenate and vortexed for 0.5 min, and placed in ice for a min. A volume of 50 µg/ml RNase A and 200 µg/ml DNase were used to hydrolyze the RNA and DNA in the homogenate for 30 min at 4 °C. After centrifugation for 30 min, the supernatant was transferred into a micro tube.

The homogenates were then stored overnight and 3 ml of acetone (20% trifluoroacetic acid which was pre-chilled −20 °C) added to precipitate the proteins. The mixture was centrifuged for 60 min and the precipitated proteins retained by discarding the supernatant. Subsequently, the proteins were washed twice using acetone for 30 min,

and centrifuged at 15,000 rpm for 30 min at °C. Afterward, the sedimented proteins were air-dried for 5 min, solubilized in 100 µl of lysis buffer (4% w/v CHAPS, 2 M thiourea, 65 mM DTT, 7 M urea, and 0.2% v/v) and stored at −80 °C until used. The protein concentration was determined using the method of Bradford (1976) with slight modifications using bovine serum albumin as a standard.

The 2-DE and Image Analysis were conducted to the method described by Wang et al. (2005). Briefly, isoelectric focusing (IEF) was performed using Immobiline™ and GE Ettan IPGphor 3 dry strip gels. A GE Healthcare with a nonlinear pH gradient ranging from 3 to 10 based on the manufacturer's manual was used. IPG gel strips of analytical grade were subsequently loaded with 1 mg of total protein that have been passively rehydrated with rehydration buffer (2 M thiourea, 7 M urea, 4% w/v CHAPS, 65 mM DTT, and 0.2% v/v of carrier ampholytes) overnight in a IPG-box (GE Healthcare). GE Ettan IPGphor 3 was used to focus the rehydrated strips in a manifold focusing tray at a current limit of 50 µA/strip.

Then, the strips were covered with 7 ml mineral oil and IEF filters placed at the terminals. For the second dimension separation, strips were equilibrated by shaking them continuously in the IPG gels equilibration buffer (6 M urea, 2% SDS, 32% w/v glycerol, 0.375 M Tris–HCl and pH 8.8). The IPG strips were incubated for 15 min in 130 mM DTT buffer and then alkylated for 20 min in 135 mM iodoacetamide buffer in order to reduce the proteins. Thereafter, the strips were washed using electrophoresis buffer and transferred into a 12.5% acrylamide SDS-PAGE gels (24 × 18 × 1 cm).

The gels were covered with agarose solution (0.5% w/v agarose, 192 mM glycine, 0.1% SDS, 0.001% bromophenol blue (BPB), 25 mM Tris–base and pH 8.8) and (GE Healthcare), Ettan DALT system was used to run the polyacrylamide gels in order to separated proteins. The gels obtained after the electrophoresis were stained with coomassie blue for 2 h and then washed (45% of methanol, 45% of MilliQ water, 10% of glacial acetic acid and 2.5 g of R250) continuously until the protein spots became clear. Imagery assay was conducted with modifications (Zhang et al., 2016). The washed gels were scanned using Ettan DALT System (GE Healthcare, USA) with a resolution of 500 dpi. The analysis of the images was carried out with Image Master 7.0 analysis software (GE Healthcare, Piscataway, NJ, USA) for the detection and matching of the protein spots. Quantitative comparative analysis between the two samples (treatment and the control) were performed taking into consideration the statistically significant protein spots. Protein spots that had an average fold change > 1.5 ($p < 0.05$) were regarded as differentially expressed proteins. The experiment consisted of three biological replicates and each was repeated twice.

The protein In-Gel digestion was carried out according to Zhang et al. (2011). Briefly, differentially expressed protein spots of the gels were excised manually and put in a tube. Afterwards, each protein spot was washed twice using 350 µl deionized H_2O for 10 min. This step was repeated till all the blue stains were washed off. Subsequently, the gel was dehydrated for 10 min with 50 µl acetonitrile and 10 mM $(NH_4)_2CO_3$ (1:1 v/v). The gel pieces were dehydrated once again with 50% acetonitrile and with 25 mM ammonium carbonate. Three (3) µl containing 10 ng/µl of trypsin solution in 25 mM ammonium carbonate was added to the samples and stored for 30 min at 4 °C until the gel pieces absorbed all trypsin solution.

Then, 15 µl 25 mM $(NH_4)_2CO_3$ was added to the gel pieces and stored at 37 °C overnight for digestion and proteolysis. Afterwards, 2% TFA was added to the samples and centrifuged instantly at 4 °C for 10 min at 10,000 rpm. Then, 1 µl peptide was marked in all the wells in perforated plates and vaporized at room temperature. This phase was repeated. Mass calibrations were performed using a standard peptide mixture. Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Germany) was used to ran the mass spectra and database queries used to identify all proteins (Wang et al., 2005). MASCOT search link (http://www.matrixscience.com) with NCBInr and Swissport databases were used to identify the proteins. Allowed modifications, carbamidomethyl of cysteine (fixed), oxidation of methionine (variable), all series, taxonomy, and peptide tolerance, ± 0.3 Da. were the MS parameters used. Only proteins with the highest Mowse score were considered for identification, and was significant ($p < 0.05$) when protein scores were N88 (NCBInr) or 70 (Swissport).

2.10. Statistical analysis

To test the significance of the effect of all treatments, one-way analyses of variance (ANOVA) using OriginPro version 2016 (Original, Northampton, USA) was performed. A two-way ANOVA was also used to determine the effect of treatment and time on gene expression level. Mean separations were performed by Tukey's test for comparison among treatments at regular time intervals. Differences at $p < 0.05$ were considered significant. All experiments were performed in triplicates and each experiment repeated. Bars represent the mean expressed from three independent experiments (± standard deviation).

3. Results

3.1. Effect of H. uvarum and H. uvarum T on POD, SOD, MDA and H_2O_2 in grapes

In Fig. 1A, the results indicated that POD activity significantly ($p < 0.05$) increased in all the treated grapes compared to the control after inoculation and storage at 20 °C. All the treatments increased exponential from 0 to 48 h of storage and then declined at 72 h. At 48 h, the activity of POD in the grapes that were treated with *H. uvarum* T reached 20 mg per fresh weight (mg/FW).

Besides the *H. uvarum* T significantly ($p < 0.05$) impacted on SOD activity from 0 to 48 h (Fig. 1B). At 48 h after inoculation, SOD activity reached a peak of 21.7 mg/FW. *H. uvarum* treated grapes was also significantly different ($p < 0.05$) from the control. It was observed that the control recorded the least SOD activity throughout the experiment. There was no significant difference ($p < 0.05$) between 2% w/v trehalose and the control treated grapes from 0 to 48 h after inoculation. MDA activity in all the grapes treated with the control, *H. uvarum* and the *H. uvarum* T increased gradually with increasing time of incubation till 48 h, after which there were steady declines (Fig. 1C). It was observed that there were no significant differences ($p < 0.05$) among all the treatments from 0 to 24 h. However, at 48–72 h all the treatments were significantly different in comparison with the control. MDA level in grapes that were inoculated with *H. uvarum* T remained the lowest throughout the period of the experiment. As shown in Fig. 1D, H_2O_2 content in the grapes treated with *H. uvarum* and the *H. uvarum* T were found to be generally low with maximum values below 1.0 µmol/FW. However, H_2O_2 activity in both the control and 2% w/v trehalose treated grapes increased sharply from 0 to 24 h and then declined. There were no significant differences ($p < 0.05$) in H_2O_2 content observed between the grapes treated with the control and 2% w/v trehalose. Contrarily, there were significant differences among grapes treated with *H. uvarum* and the *H. uvarum* T compared to the control and 2 w/v trehalose.

3.2. Effects of H. uvarum and H. uvarum T on stilbene synthase expression in grapes

The qRT-PCR assay on *StSy* relative gene expression levels in the grapes showed that *H. uvarum*, 2% w/v of trehalose, and *H. uvarum* T significantly ($p < 0.05$) enhanced the expression levels of *StSy* in the grapes compared to the control (Fig. 2). Indeed the two-way ANOVA revealed that time ($p < 0.05$) and treatment ($p < 0.05$) and their interaction significantly influenced the gene expression level of *StSy*. The results also indicated that *H. uvarum* T grapes was significantly higher ($p < 0.05$) compared to the 2% w/v trehalose and *H. uvarum*. It was

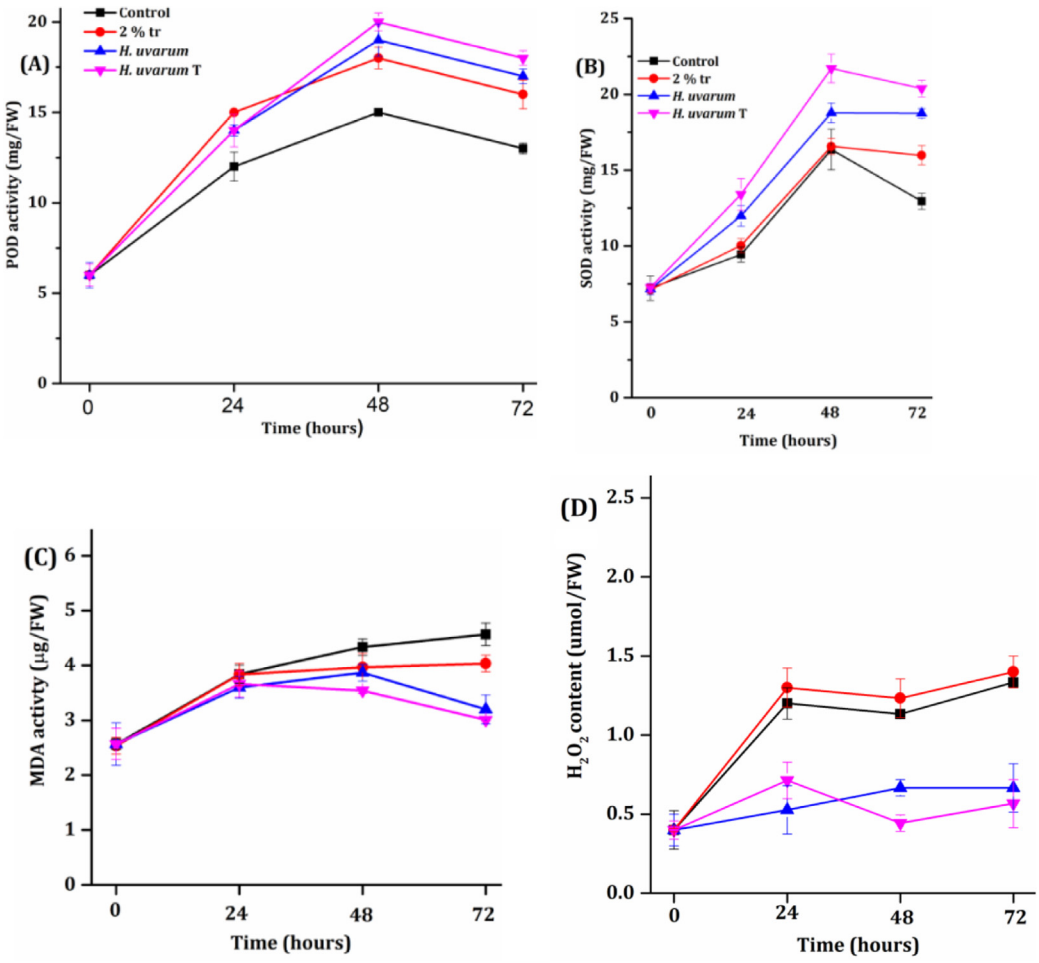

Fig. 1. Time course change of (a) peroxidase (POD), (b) superoxide dismutase (SOD), (c) malondialdehyde (MDA), and Hydrogen peroxide (H_2O_2) in table grapes incubated at 20 °C following treatment with (a) control (sterile distilled water), (b) 2% w/v tr (2% w/v of trehalose), (c) *H. uvarum* and *H. uvarum* T (*H. uvarum* pretreated with 2% w/v trehalose NYDB). The findings show the mean of the three (3) independent replicates. Error bars indicate the SD (n = 3).

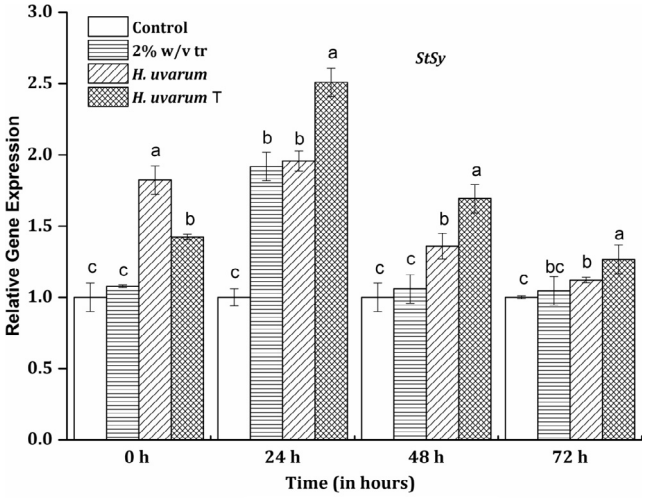

Fig. 2. Time course change of stilbene synthase (*StSy*). qRT-PCR was used to determine relative gene expression levels of table grapes inoculated and stored at 20 °C from 0 to 72 h. The treatments used were: control (sterile distilled water), 2% w/v tr (2% w/v of trehalose), *H. uvarum* and *H. uvarum* T (*H. uvarum* pretreated with 2% w/v trehalose in NYDB). 0 h (1 h after the treatments were performed). The results are the means of three independent replicates. Different letters on each bar indicate statistical significance at $p < 0.05$ among treatments. Bars represent the mean expressed from three independent experiments (± standard deviation).

noticed that at 0 h after incubation *H. uvarum* T increased by 1.80-fold compared to the control. At 24 h, the relative gene expressions of *H. uvarum* T attained a peak of 2.5-fold increase compared to the control.

3.3. Differentially expressed protein profile

To study the possible mechanisms of action involved in the different activities showed by *H. uvarum* and *H. uvarum* T on grapes, the proteomic profile of both microorganisms was performed. The 2-DE analysis indicated that 93 protein spots were visualized after the stain buffer was washed off. Unclear protein spots and those with undefined shapes were not considered. Quantitative analysis showed that a total of 40 protein spots from the *H. uvarum* T were compared to spots from the control. The best resolved protein spots in the gel were distributed between 14.4 and 97.4 kDa with a pH of 7.0 (Fig. 3).

Among the 40 protein spots, 29 protein spots (72.5%) were clearly identified through MALDI-TOF/MS and subsequently searched in NCBInr database. The findings showed that out of the 29 differentially expressed proteins, 26 were significantly up-regulated while 3 were down-regulated compared to the control. Hence, Fig. 3 shows two-dimensional pattern of intracellular proteins of *H. uvarum* and *H. uvarum* T respectively. These protein spots (Fig. 3) correspond to those stated in the first column of Table 1. The proteins identified were classified based on their functions such as cell structure and signal transduction, carbohydrate and energy metabolism, defense response, protein biosynthesis and degradation, amino acid and fatty acid metabolism and unclassified proteins. Majority of the differentially expressed proteins

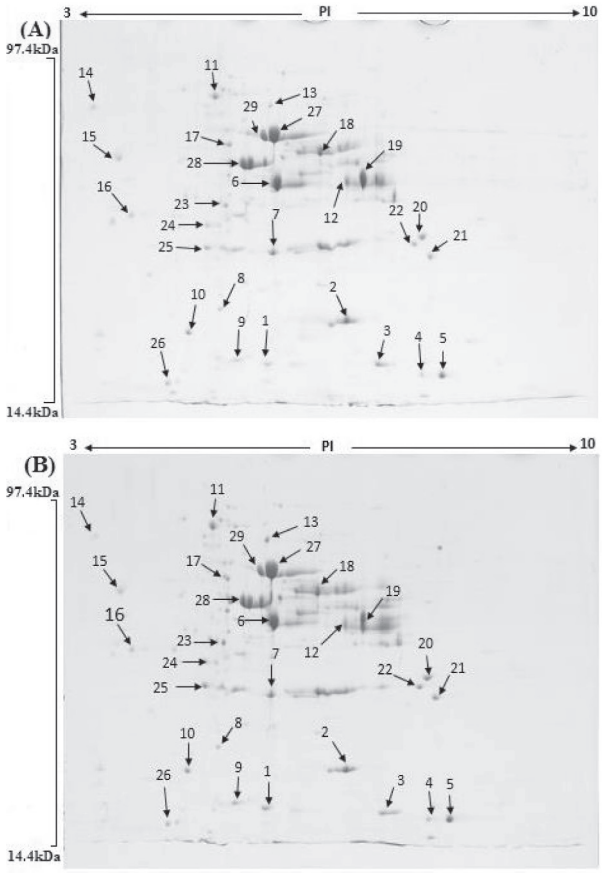

Fig. 3. Two-dimensional gels of intracellular proteins expressed in *H. uvarum* Y3 after culturing in NYDB for 24 h (a) *H. uvarum* (b). *H. uvarum* T (*H. uvarum* pretreated with 2% w/v trehalose in NYDB). Protein spots were excised on 24 cm strips.

were involved in carbohydrate and energy metabolism constituted 28.0% of the total proteins identified. Additionally, 20.0% of the differentially expressed proteins were classified under cell structure and signal transduction proteins. Eleven proteins could not be identified following the mass spectrometry analysis that was performed and this made it difficult to characterize their functions. However, these proteins considered as either unknown or hypothetical may play various vital functions directly or indirectly in response to the application of the trehalose.

All such proteins were termed as unclassified proteins (Table 1). Glyceraldehyde-3-phosphate dehydrogenase (spot 1 and 2), pyruvate decarboxylase 1 (spot 8), saccharopine dehydrogenase (spot 9), phosphomannomutase, (spot 14), enolase 1 (spot 15), fructose-bisphosphate aldolase (spot 17) and GDP-mannose pyrophosphorylase spot 23 were all involved in carbohydrate and energy metabolism. It was also observed that peroxiredoxin TSA1 (spot 13) and SOD (spot 20) were involved in defense response. Based on the gene ontology (GO) analysis the differentially expressed proteins were categorized into biological process, molecular function and cellular component.

Under the biological process, 11 of the proteins were found to be involved in glycolytic process, oxidation-reduction process, glucose metabolic process, cellular oxidant detoxification, superoxide metabolic process, and metabolic process among others. Similarly, the GO query also revealed that most of the proteins were found to be involved in molecular functions such as NADP binding, metal ion binding, ATP binding, ATPase activity, SOD activity and antioxidant activity (Fig. 4). Regarding the cellular component, it was realized that majority of the differentially expressed proteins were located in the cytoplasm, nucleus, cytosol, mitochondrion and membrane.

4. Discussion

Genomics and proteomics are some of the useful 'omic' technologies that can be employed to characterize genes and proteins expression profiles of biocontrol agents and hosts in response to various treatments and other environmental conditions. POD is noted to control the balance of H_2O_2 in the cell wall of fruits, which is essential for the cross-linking of phenolic compounds in response to several external stress such as injuries, pathogens attack, and environmental stress (Passardi, Penel, & Dunand, 2004). In this study, the antioxidant enzyme POD was significantly increased in the table grapes, suggesting that the integrity of the cell wall firmness was enhanced, hence decreased the susceptibility of the grapes to fungal infection. Thus, the increase of POD in our findings is an indication of induced resistance in the grapes following the application of the *H. uvarum* T. Previous study indicated that CAT, ascorbate peroxidase, chitinase activities among others were noted to increase significantly in grapes compared to the untreated control (Apaliya et al., 2017). Trehalose as a sugar has shown to offer protection to yeasts in both dry and liquid formulations (Sui & Liu, 2014).

In the nutshell, SOD activity was found to increase following the treatment of the grapes with *H. uvarum* and the *H. uvarum* T. These results may suggest that both treatments have positive impacts in the inducement of SOD activity in the grapes (Qin et al., 2015). Typically, the metabolism of H_2O_2 is controlled by SOD together with other enzymes such as catalase and ascorbate peroxidase. Henceforth, a balance among these three enzymes is important in maintaining a steady level of H_2O_2 and $O_2^{\cdot -}$ (Chan & Tian, 2006).

MDA is a product formed as a result of the decomposition of polyunsaturated fatty acid hydroperoxide and mostly considered as a standard for determining lipid peroxidation caused by oxidative damage (Zhang et al., 2015). In this finding, grapes treated with *H. uvarum* and the *H. uvarum* T were very low and did not differ significantly in MDA content demonstrating that *H. uvarum* has no effect on MDA activity in the grapes (Zhang et al., 2015). Similarly, chitosan was observed to significantly reduce MDA level in strawberry (Zhang, Ge, Chen, Zhao, & Zhang, 2014). These results may be due to the sharp increase in POD and SOD activities which has been reported to suppress ROS (Fu, Zeng, Zheng, & Yu, 2015; Sharma, Jha, Dubey, & Pessarakli, 2012). The relatively low level of H_2O_2 in the treated grapes could be as a result of the upsurge of SOD and POD (Apaliya et al., 2017).

It is worthy to note that at all the tested times, grapes that were treated with the *H. uvarum* T and *H. uvarum* alone were significantly different from the control. Similar reports (Rühmann et al., 2013) indicated that *Aureobasidium pullulans* culture filtrate induced stilbene synthase level in grape with the expression of the gene encoding *StSy* and a higher enzyme activity. The involvement of *StSy* in the defense of plants through the reinforcement of cell walls and phytoalexins has previously been investigated (Rühmann et al., 2013).

MS-MS proteomics and functional genomics, can be used to examine the physiological state of biological control agents as well as the effect imposed by environmental stress (Droby, Wisniewski, Teixidó, Spadaro, & Jijakli, 2016). It was observed that all the protein spots excised from the pretreated *H. uvarum* T gel were significantly ($p < 0.05$) up-regulated compared to those of the control. The improvements noted in this study may be due to the pretreatment of the *H. uvarum* with 2 w/v trehalose in NYDB for 24 h. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (spots 1 and 2) is a crucial protein in glycolysis that catalyzes the initial step of the pathway by transforming D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate. GAPDH is a high suppressor of lethality caused by Sir2 overexpression in yeast cells (Ringel et al., 2013). Sir2 is an NAD^+-dependent histone deacetylase required to mediate transcriptional silencing and suppress rDNA recombination in budding yeast. Therefore the up-regulation of GAPDH in this findings is an indication that the trehalose significantly impacted on *H. uvarum.*

Moreover, enolase 1 which was observed to be up-regulated is one

Table 1
Two-dimensional gel electrophoresis analysis of differentially expressed intracellular proteins in *H. uvarum* pretreated with 2% w/v trehalose in NYDB and analyzed through MS-MS. Protein spots were excised from 2D PAGE gel and digested with trypsin.

Protein spot	Protein name	NCBI accession	Mass	PI	Species	Score	Protein Matches
Carbohydrate and energy metabolism							
1	glyceraldehyde-3-phosphate dehydrogenase	gi|146419367	35,831	6.61	*Meyerozyma guilliermondii* ATCC 6260	209	3(1)
2	glyceraldehyde-3-phosphate dehydrogenase	gi|146419367	35,831	6.61	*Meyerozyma guilliermondii* ATCC 6260	348	3(3)
7	DEHA2F09570p	gi|50424381	44,438	5.48	*Debaryomyces hansenii* CBS767	523	6(2)
8	pyruvate decarboxylase 1	gi|149288859	65,273	9.57	*Scheffersomyces stipitis*	77	1(1)
14	phosphomannomutase	gi|146423739	28,678	6.61	*Meyerozyma guilliermondii* ATCC 6260	342	4(2)
15	enolase 1	gi|146415384	46,951	5.02	*Meyerozyma guilliermondii* ATCC 6260	393	4(3)
17	Fructose-bisphosphate aldolase	gi|459370126	39,329	6.61	*Candida maltosa* Xu316	190	2(1)
23	GDP-mannose pyrophosphorylase	gi|3777501	40,102	5.48	*Candida albicans*	232	4(2)
Cell structure and signal transduction							
11	tryptophan-tRNA ligase	gi|754845100	37,720	5.86	*Paenibacillus durus*	66	1(1)
18	40S ribosomal protein S3	gi|126132980	26,224	9.72	*Scheffersomyces stipitis* CBS 6054	450	7(3)
19	polyubiquitin-like protein	gi|224503947	25,804	5.49	*Piriformospora indica*	459	6(5)
24	40S ribosomal protein S19-B	gi|146415016	19,551	8.78	*Meyerozyma guilliermondii* ATCC 6260	156	2(1)
25	60S ribosomal protein L31	gi|302308129	13,196	10.23	Ashbya gossypii ATCC 10895	104	2(0)
29	40S ribosomal protein S0	gi|146417344	28,665	4.77	Meyerozyma guilliermondii ATCC 6260	204	4(1)
Defense response							
13	peroxiredoxin TSA1	gi|146418172	21,833	4.96	*Meyerozyma guilliermondii* ATCC 6260	177	3(2)
20	superoxide dismutase	gi|385303182	15,833	6.57	*Brettanomyces bruxellensis* AWRI1499	77	1(1)
Protein biosynthesis and degradation							
26	cell division control protein 48	gi|146415458	91,010	4.84	*Meyerozyma guilliermondii* ATCC 6260	355	7(3
Amino acid and fatty acid metabolism							
9	saccharopine dehydrogenase	gi|241954848	41,811	6.77	*Candida dubliniensis* CD36	85	1(1)
Unclassified							
3	DEHA2F04796p	gi|146416269	35,926	6.30	*Debaryomyces hansenii* CBS767	271	3(2)
4	hypothetical protein PGUG_03485	gi|146416269	46,767	6.31	*Meyerozyma guilliermondii* ATCC 6260	195	3(1)
5	DEHA2F04796p	gi|50423973	35,926	6.30	*Debaryomyces hansenii* CBS767	275	3(2)
6	hypothetical protein TRIATDRAFT_300064	gi|358396039	37,870	6.31	*Trichoderma atroviride* IMI 206040	107	1(1)
10	hypothetical protein PGUG_04223	gi|190347786	28,436	6.32	*Meyerozyma guilliermondii* ATCC 6260	200	3(2)
12	DEHA2B13090p	gi|50416024	32,348	5.28	*Debaryomyces hansenii* CBS767	167	2(1)
16	hypothetical protein PGUG_05691	gi|146411913	33,902	6.33	*Meyerozyma guilliermondii* ATCC 6260	67	1(1)
21	hypothetical protein CANTEDRAFT_104120	gi|575512947	10,607	6.34	*Candida tenuis* ATCC 10573	69	1(1)
22	Piso0_000062	gi|448123918	85,125	5.89	*Millerozyma farinosa* CBS 7064	87	2(0)
27	hypothetical protein PGUG_05425	gi|146412868	17,763	6.32	*Meyerozyma guilliermondii* ATCC 6260	92	2(0)
28	conserved hypothetical protein	gi|146417765	34,973	5.34	*Meyerozyma guilliermondii* ATCC 6260	78	1(1)

of the main proteins involved in the glycolytic pathway converts 2-phosphoglyceric into 2-phosphoenolpyruvic (Zhang et al., 2011). Zheng et al. (2016) reported that phosphomannomutase catalysis mannose-6-phosphate and mannose-1-phosphate which is an enzyme in eukaryotic organisms. Mannose-1-phosphate is an important conserved enzyme that synthetize GDP-mannose, which is crucial in the formation of polysaccharide needed for glycosylation of lipid molecules and protein (Moremen, Tiemeyer, & Nairn, 2012). This indicated that *H. uvarum* T had a positive effect in the enhancement of carbohydrate and energy metabolic related proteins. This is primarily due to the fact that cells generally metabolize trehalose over other fuels. During quiescence or stationary phase, trehalose reserves are normally maintained in order to fulfill its stress-protectants function (Shi, Sutter, Ye, & Tu, 2010). The results suggest that trehalose provides a source of energy that aids to drive cellular activities leading to their up-regulation. For example, GDP-mannose pyrophosphorylase (spot 23) as found in *H. uvarum* has been reported to be essential in maintaining cell wall integrity, viability and morphogenesis in *Aspergillus fumigatus* (Jiang, Ouyang, Zhou, & Jin, 2008).

It was also observed that SOD, one of the differentially up-regulated proteins is a well-known antioxidant defense against $O_2^{\cdot -}$ radical. SOD has the ability to catalyze dismutation $O_2^{\cdot -}$ into ordinary H_2O_2 or oxygen molecule (O_2) (Gill & Tuteja, 2010). Thus, SOD is a crucial antioxidant defense in most live cells exposed to oxygen. The up-regulation of this antioxidant protein in *H. uvarum* T suggests that the trehalose has the ability to induce its rate. Besides, SOD has been reported to play critical role in preventing oxidative inactivation of nitric oxide (NO), there by inhibiting endothelial, peroxynitrite formation and mitochondrial dysfunction (Fukai & Ushio-Fukai, 2011).

Similarly, the results also revealed that peroxiredoxin TSA1, a defense related protein was observed to be significantly up-regulated. Generally, peroxiredoxins are thiol-specific proteins with multiple functions in stress protection, such as protection against oxidative stress (Weids & Grant, 2014). Reactive oxygen species (ROS) are produced in the mitochondria, and aggregation of proteins adjacent it causes active mitochondria to fragment triggering the release of $O_2^{\cdot -}$. TSA1 has the ability to localize at the site (misfolded and aggregated proteins) and acts as antioxidants to scavenge ROS caused by misfolded and aggregated proteins. Therefore, the up-regulation of SOD and peroxiredoxin TSA1 in this study demonstrated the potential of using trehalose to enhance *H. uvarum* as a promising antagonist against postharvest diseases. This may explained the reason why the *H. uvarum* T significantly inhibited the lesion diameter and disease severity in the potato dextrose agar.

The results further revealed that most of the differentially expressed proteins were noticed to be involved in cellular metabolic related activities based on the GO classification analysis. Metabolism is vital to microbial life, which fuels all cellular activities and acts as building blocks and energy (Chubukov, Gerosa, Kochanowski, & Sauer, 2014). The up-regulation of the differentially expressed proteins (DEPs) therefore implied that trehalose a disaccharide supplied the yeast with sugars needed for energy. Likewise, the proteome results showed that majority of the proteins were actively involved in oxidation-reduction process. Although our hypotheses were supported statistically, some of the DEPs could not be identified, it was difficult to classify all the proteins through the data base query hence such proteins were

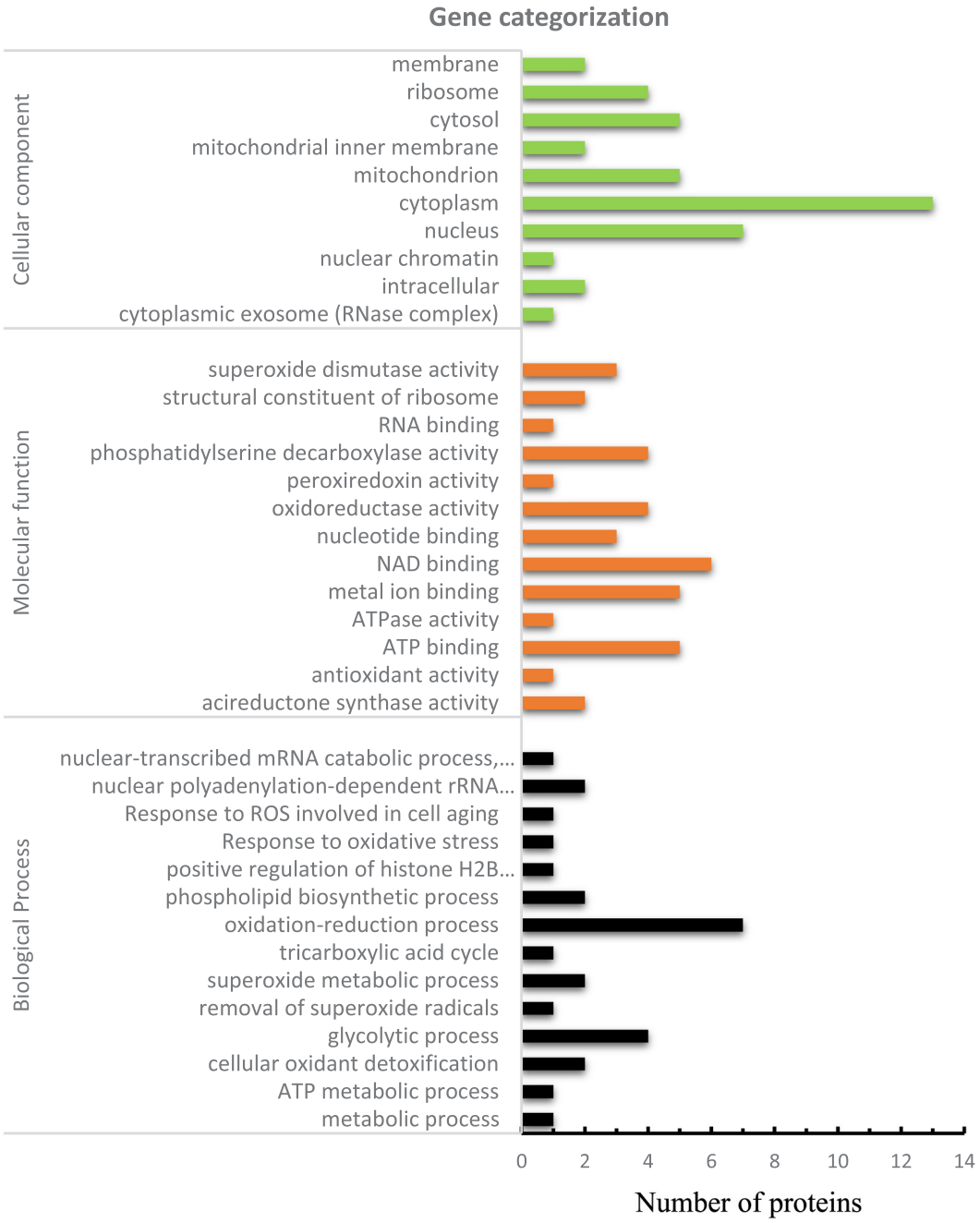

Fig. 4. Categorization of differentially expressed proteins of *H. uvarum* pretreated with 2% w/v trehalose in NYDB through Gene Ontology (GO) analysis.

considered as unclassified.

In conclusion, there was a desirable outcome of the POD and SOD levels in the table grapes as the *H. uvarum* that was pretreated with 2% w/v trehalose significantly impacted on their outburst. The study further revealed the functional characterization of the DEPs of *H. uvarum* which included carbohydrate and energy metabolism, cell structure and signal transduction, defense response, protein biosynthesis and degradation, amino acid and fatty acid metabolism. The functional classification and GO analyses showed that most of the DEPs, were involved in oxidation-reduction, glycolytic process, NAD and ATP binding. They were classified under biological process, molecular function and cellular component. Further research is required with the use of bioinformatics tools to determine the actual genes involved in the biocontrol.

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