Review of: "Metabolomics and transcriptomics unravel the mechanism of browning resistance in Agaricus bisporus"

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Review of Metabolomics and transcriptomics unravel the mechanism of browning resistance in Agaricus bisporus.

Cai et al (2022) have carried out metabolomic and transcriptomic analysis on button mushrooms (*Agaricus bisporus*) to unravel the mechanism of browning resistance.

Browning of the mushroom cap is indeed an important phenomenon that lowers the quality of mushrooms and causes substantial economic damage to the mushroom industry. Unraveling the pathway that is involved in browning mushroom caps and study DEGs in sensitive and tolerant strains can contribute to our knowledge of genes that should be down or up-regulated to inhibit browning during shelf life of mushrooms. It might generate important information for breeders too.

Studies of DEGs is often complex and the generated data do not always give clear clues what pathways are involved and certainly not how that data can be used in practice. It is, therefore, that first of all the research should be done accurate and precise to generate reliable data. And some comments on the methodology is certainly appropriate. First of all, no variety names were mentioned, nor what the difference in genetic background is between the BS and BT strain. A reproducible induction of browning by damaging the mushroom caps depend on the time, the pressure applied and also on the developmental stage of the mushroom. That is all described in detail in Weijn et al. 2012 who developed a reproducible way to apply bruising to button mushroom caps (no reference to this paper, surprisingly). The authors do not describe their induction of browning in detail and it cannot be seen if this as done in a reproducible way. It is also unclear what mushroom developmental stage was used. In conclusion, no one could repeat these experiments.

The gene transcriptional analysis was subsequently done by taking “..2 samples randomly selected as biological repeats in each group”. First of all, 2 biological samples is usually not enough to do a reliable DEG study (see Schurch et al. 2016). The authors state that they extract total cellular RNA from fibroblasts. Why using the word “fibroblasts”. Did the authors take whole mushrooms or only the cap? If so, why not the skin of the mushroom where the browning takes place? This section of M&M does also not state if mushrooms were first bruised and then analyzed. So it is unclear how this was done, while an accurate description is needed to reproduce this.

The sequence reads were subsequently aligned to a reference genome mentioned in database http://fungi.ensembl.org/Agaricus_bisporus_var_bisporus_h97/Info/Index). I do not know this database well and could not fined the genome of H97. Why not using the most complete and most recent published genome of A. bisporus as cited in Sonnenberg et al. 2020 (https:// fungalgenomics. science. uu. nl/.)?

In total, 522 genes were up-regulated and 611 down regulated in BT compared to BS. It is not clear that the DEGs presented in Figure 2 are indeed the 100 most up/down regulated genes of all DEGs detected. There is also no

supplemental data where all DEGs are listed. In figure 3, the authors list the 40 pathway in which genes are significantly differential regulated. The prominent pathways mentioned in the text (melanogenesis, tryptophane, Taurine and hypotaurine melanogenisis..) cannot be found in Figure 3 and a number of pathways mentioned are irrelevant (Human diseases).

The authors also suggest that the higher concentrations of organic acids in the tolerant strain lowers the pH in cells and might, therefore, inhibit PPO activities. That is highly speculative. First of all, there are no data on how different the organic acid levels are between the BS and BT strains. Second, there is no evidence that intracellular pH levels in the BT strain is lower than in the BS strain. In addition, why is one of the 4 PPOs upregulated and the other down regulated in the BS strain if organic acids negatively influence PPO activities? The argument that Liu et al reported that with an increasing citric acid concentration, the activity of PPO decreases gradually, is an incorrect comparison. The citric acid was applied to mushrooms and not produced by the mushroom.

It is also surprising to see that the authors did not refer to Weijs et al, 2013; Melanin biosynthesis pathway in Agaricus bisporus mushrooms). These authors describe all putative genes involved in melanin biosynthesis in the button mushroom and they also measured RNA expression of the genes in different tissues and at different developmental stages.

In the abstract the authors state “..breeding is also a reliable way..” to indicate the usefulness of breeding to decrease browning of mushrooms. In my opinion, "reliable" is not adequate here since breeding in general for the button mushroom is not easy and certainly not for a complex trait such as shelf life (see Gao et al 2013, 2015).

In conclusion, it seems to me that the methodology is suboptimal and it is unclear how reliable the data are that are generated, and the conclusions drawn are not always sound.