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CENTRE WALLON DE BIOLOGIE INDUSTRIELLE

Faculty of Sciences-University of Liège

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Fundamental and Applied Studies on Freeze-dried Vinegar Starter

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Acetic acid bacteria (AAB) are used industrially to produce different kinds of bioproducts. AAB encounter very aggressive conditions during acetous fermentation (AF) including high acid and ethanol concentrations, low pH and also abrupt increases in temperature. In subtropics such as central and southern parts of Iran, fruits and also the by-products of the fruit processing industries are used to produce different kinds of foods. However, because of high temperature and deficiency in water resource, fermentation industries face many restrictions during spring and summer. One of the main problematic restrictions is the low productivity or the ceases of fermentation due to the use of non-thermotolerant microorganisms. In this case, initiation of a new fermentation run needs efficient starter.

In previous studies in Walloon Center of Industrial Biology, *Acetobacter senegalensis*, a novel thermo-tolerant bacterium, was isolated and used to produce vinegar starter and acetic acid at high temperature. However, in those studies, the viability and vitality of the starter were not evaluated under stress conditions. In addition, since most kinds of industrial vinegars have low prices, the use of high-priced nutrients for the production of low quantity of starter is not commercially cost-effective.

In the present study, with a deep look to the acetous fermentation requirements, we analyzed the fundamental and applied aspects of *A. senegalenisis* resistance to stress inducers. Proteomics-based techniques and flow cytometry methods in combination with different biomass production techniques were used to develop a fermentation process improving cell viability and vitality during freeze-drying process and revitalization procedure. In addition, the trend of cell senescence during storage of starter and its effect on some bio-molecules were studied.

In the first part of the study, the quality of the produced biomass was improved in order to achieve an acetic acid tolerant biomass. Adaptive laboratory evolution technique (ALE) enabled cells to grow rapidly in higher concentration of acetic acid. The results of 2D-DiGE on the produced biomass revealed that structural and regulatory proteins were expressed differently under various conditions (Chapter II and III). Use of acetic acid in combination with glucose in a fed-batch fermentation mode could induce a physiological condition in *A. senegalensis* which was close to the physiological state of cells oxidizing ethanol. In addition, the presence of acetic acid in fermentation media could cause a cross-adaptation and improved the tolerance of cells to stressors (ethanol, low pH and acetic acid). Interestingly, by using this method for production of biomass, the rate of growth on ethanol improved significantly.

In parallel to the first part of the study, we exhibited the influence of different stress on the produced biomass. Cell envelope integrity and respiration (dehydrogenase activity) were the two important targets for adverse effects of the stress. Assessment of the cell envelope integrity and respiration system of produced biomass by Multiparametric Flow Cytometry (MFC) method (Chapter II) demonstrated that the detrimental effects of ethanol and acetic acid depended on the carbon sources and fermentation conditions used for pre-adaptation. Respiration system and cell envelope integrity of cross-adapted

cells were not compromised after exposure to different concentrations of ethanol and acetic acid. Thus, according to the obtained results, by

using a mixture of acetic acid and glucose as carbon sources, it is possible to enhance not only the viability of cells but also induce tolerance to physicochemical stress during downstream process.

Our investigation about the freeze-drying process provided a better understanding of lethal and sub-lethal damage to cells (Chapter IV). The results showed that drying process had the greatest effect on the viability and vitality of *A. senegalensis* especially by affecting on the cell envelope. In addition, entrance into viable but non-culturable state (VBNC) was initiated during the drying process and enhanced during storage period.

Analysis of the stored cell proteome by 2D-DiGE and western blotting (Chapter V) revealed that high storage temperature could induce a kind of senescence in the cells by different modifications in cellular proteome such as insolubility, degradation and carbonylation of cellular proteins and shift of isoelectric point. Carbonylation of the proteins involved in transcriptional and translational process could cause cell death whereas VBNC formation at low storage temperature seemed to be due to other deteriorative reactions such as fatty acid peroxidation.

At the end of this dissertation, the discussion (Chapter VI) provides a general overview of the results and compares our findings with earlier studies. Potential industrial applications are reviewed and suggestions for further research are made.

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