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Petit-High Pressure Carbon Dioxide stress increases synthesis of *S*-Adenosylmethionine and phosphatidylcholine in yeast *Saccharomyces cerevisiae*

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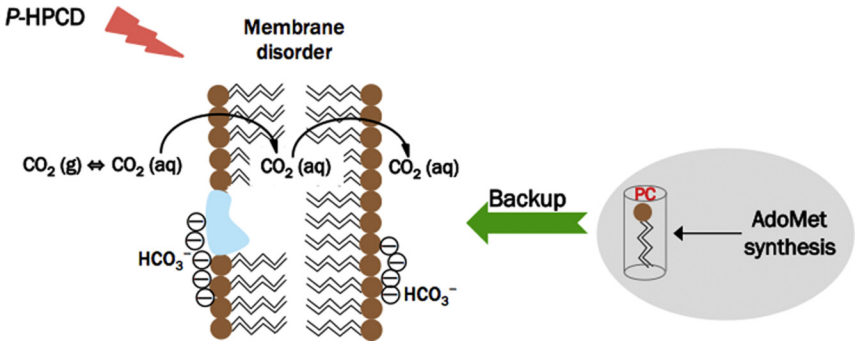
HIGHLIGHTS

- Synthesis of *S*-Adenosylmethionine and phosphatidylcholine in yeast are increased by *p*-HPCD stress.
- *p*-HPCD stress decreases amounts of most of amino acids involving protein synthesis in yeast.
- *p*-HPCD stress leads to morphological modification on yeast cell surface.
- *p*-HPCD treatment is a very promising nonthermal pasteurization technology in food processing and distribution.

GRAPHICAL ABSTRACT



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ABSTRACT

Petit-High Pressure Carbon Dioxide (*p*-HPCD) is a promising nonthermal technology for foods pasteurization. Cluster analysis of gene expression profiles of *Saccharomyces cerevisiae* exposed to various stresses exhibited that gene expression profile for *p*-HPCD stress (0.5 MPa, 25 °C) was grouped into a cluster including profiles for Sodium Dodecyl Sulfate and Roundup herbicide. Both are detergents that can disorder membrane structurally and functionally, which suggests that cell membrane may be a target of *p*-HPCD stress to cause cell growth inhibition. Through metabolomic analysis, amount of *S*-Adenosylmethionine (AdoMet) that is used as methyl donor to participate in phosphatidylcholine synthesis via phosphatidylethanolamine (PE) methylation pathway, was increased after *p*-HPCD treatment for 2 h. The key gene *OPI3* encoding phospholipid methyltransferase that catalyzes the last two steps in PE methylation pathway was confirmed significantly induced by RT-PCR. Transcriptional expression of genes (*MET13*, *MET16*, *MET10*, *MET17*, *MET6* and *SAM2*) related to AdoMet biosynthesis was also significantly induced. Choline as the PC precursor and ethanolamine as PE precursor in Kennedy pathway were also found increased under *p*-HPCD condition. We also found that amounts of most of amino acids involving protein synthesis were found decreased after *p*-HPCD treatment for 2 h. Moreover, morphological changes on cell surface were observed by scanning electron microscope. In conclusion, the effects of *p*-HPCD stress on cell

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membrane appear to be a very likely cause of yeast growth inhibition and the enhancement of PC synthesis could contribute to maintain optimum structure and functions of cell membrane and improve cell resistance to inactivation.

1. Introduction

Thermal processing is the most well-known and traditional technology for microbial inactivation in foods. Heat treatment inactivates pathogens and spoilage organisms. However, heat processing makes many side effects on foods: destruction of thermolabile vitamin, particular thiamine, vitamin C and folate and decrease of organoleptic quality [1]. To supply safe foods with high nutritional and sensory properties to consumers, nonthermal processing as alternative technologies have been proposed during the last decades and currently being explored on a global scale. High Pressure Carbon Dioxide (HPCD) has been of interest to researchers in the field of food processing since its remarkable biocidal effect was discovered in 1951 [2]. HPCD technique has many important opportunities in food processing industry: natural image; high fresh-like organoleptic quality; continuous processing for liquid foods, etc. In addition, the pressure used in HPCD (generally <20 MPa) is much lower than that employed in High Hydrostatic Pressure (300 MPa–600 MPa) [3], one of the most investigated nonthermal alternative techniques.

Although application of HPCD in food preservation makes the processing economical and easier to handle, researchers never stop exploring other possibilities. In 2008, Harada et al. [4] attempted to treat Lyceum Barbarum fruit juice harvested from China by CO_2 under mild pressures (3 atm–12 atm) at room temperature and surprisingly observed significant inactivation effects on bacteria, yeast and mold after long time treatment (1–2 weeks). Then CO_2 under mild pressure (1.5 atm–13 atm) with long period treatment was defined as *petit*-High Pressure Carbon Dioxide (*p*-HPCD) pasteurization technique. *p*-HPCD with long time treatment seems a more promising pasteurization technique. From an economical point of view, it significantly decreases both operating and investment costs. In addition to food processing, this new technique is applicable in food distribution including storage and transport. The *p*-HPCD technique is better than refrigeration in storage due to its effective bactericidal action. Moreover, because pressure level during transport is restricted to be <4 atm in Japan, it is realistic for food transport under *p*-HPCD condition [4].

Under high-pressure condition, inactivation effectiveness of CO_2 was compared to that of other gases; HPCD in general is the most effective method [3]. Dillow et al. [5] have pointed out the importance of proximity to the critical points and chemical properties of gases. Nitrogen ($T_c = -147$ °C; $P_c = 3.39$ MPa) and argon ($T_c = -122.28$ °C; $P_c = 4.90$ MPa) are nontoxic and inert similar to CO_2, but both gases didn't exhibit the special gas-like mass transport properties and liquid-like densities of a supercritical fluid because the experimental conditions were far away from their critical points. While tetrafluoroethane ($T_c = 55$ °C; $P_c = 4.06$ MPa) has similar critical points as CO_2 ($T_c = 30.98$ °C; $P_c = 7.37$ MPa), pressurization of it was less effective than CO_2 owing to different chemical properties they possess (dipole moment, $D_{CO2} = 0$, $D_{TFE} = 1.80 \pm 0.22$ D; solubility parameter, $\delta_{CO2} = 12.3$, $\delta_{TFE} = 13.6$). Another investigated gas is nitrous oxide (N_2O) that has critical points ($T_c = 36.5$ °C; $P_c = 7.25$ MPa) similar to CO_2 and high solubility in water, which was found effective bactericidal action, but not as great as CO_2 [6].

In recent years, many investigations have focused on the mechanism of CO_2 bactericidal action. Based on physical and chemical properties of CO_2, there are two general mechanisms presumed and discussed: mechanical cell rupture and physiological deactivation. Physical cell rupture was the earliest proposed mechanism. It was presumed that the explosive expansion of high-pressure CO_2 led to cell rupture [2,6,7,8]. Burst cells, wrinkles and holes on the cell surface have been observed using scanning electron microscope [8]. The physiological deactivation mechanism involves several theories, such as (1) decrease of extracellular and intracellular pH (pH_i), (2) cell membrane modification, (3) decrease of key enzyme activity due to pH_i lowering, (4) direct (indirect) effect of molecular HCO_3^- and CO_3^{2-} on carboxylation and decarboxylation reactions, (5) extraction of cell contents [3,9]. These hypotheses were proposed under conditions of high-pressure even supercritical treatment, however pressure employed in *p*-HPCD is well removed from critical point of CO_2. Thus, the concentration of CO_2 in *p*-HPCD treatment is far smaller than that in HPCD treatment. We have analyzed biological effects of *p*-HPCD stress on the transcriptional level of yeast *Saccharomyces cerevisiae* through genomics approach and found that the most highly induced function urea cycle metabolism plays an important role in cell survival [10]. While mechanism of *p*-HPCD pasteurization must be complicated, within the mass of data produced by genomics approach, other biologically meaningful information needs to be explored. Therefore, in this study we conducted hierarchical clustering, one of conventional clustering methods, for further analysis of gene expression data. According to the similarity in expression profiles of yeast cell, conditions with similar patterns are grouped together and connected by a series of branches. The co-regulated and functionally related genes in response to various conditions can be discovered by clustering of genes expression profiles rows. While mRNA gene expression data analysis does not tell the whole story of what might be happening in a cell, metabolic profiling can help us to observe the physiological changes of a cell under stress conditions [11]. In this study, we used capillary electrophoresis-time-of flight mass spectrometry (CE-TOFMS) to analyze the metabolites responses to *p*-HPCD stress in yeast cell. The

Table 1
Primers used in this study.

Gene	Forward primer	Reverse primer
ACT1	5′-ATTGCCGAAAGAATGCAAAAGG-3′	5′-CGCACAAAAGCAGA GATTAGAAACA-3′
OPI3	5′-TGGGGCCAGAAAGGGCTGTT-3′	5′-AGCCCCGCAGGCTTTCCTTT-3′
MET3	5′-GCCCTTTTCCAAGATGATGA-3′	5′-CTGGATGTTCTGGGTCACCT-3′
MET16	5′-ATTTGGTTTGACTGGCTTGG-3′	5′-ATCTGCCTCCGATTCACATC-3′
MET10	5′-TACCACCATCTCCAAAGCAAC-3′	5′-CCAAGTAGGGCCACACAAGTA-3′
MET17	5′-CGCTCAAACCCTTGCCATCCA-3′	5′-TGACAGAAGTAAC CACCGGCACCA-3′
MET6	5′-CGGCCAAAAGCCAGTTGACGA-3′	5′-GCAACTGGCAAGCCCTTGATGG-3′
SAM2	5′-CAGATATCGCTCAAGGTCTGC-3′	5′-GGTAACCCTTCTGGAGTTTCG-3′

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