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Short communication

The effects of elevated process temperature on the protein carbonyls in the filamentous fungus, *Aspergillus niger* B1-D

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ABSTRACT

It is now accepted that heat treatment can lead to oxidative stress events in microorganisms, but there are few detailed studies on protein oxidation and consequent recycling/elimination of oxidatively damaged proteins following such heat treatment. In batch cultures of a filamentous fungus, *Aspergillus niger* B1-D, raising culture temperature from 25 °C to 30 °C and 35 °C led to a general enhancement of intracellular metabolism, a higher specific growth rate, increased consumption of carbon and nitrogen sources, and raised intracellular ATP content in the exponential phase. By contrast, there was a transient accumulation of protein carbonyls, a widely used biomarker of protein oxidation, following such temperature increases, which could indicate that cellular antioxidant defences were being temporarily overwhelmed under these circumstances, despite the fact that enhanced activities in antioxidant enzyme activities have been reported in microorganisms during such heat treatment. Protein carbonyls can only be removed by proteolysis. The intracellular proteolytic activity in batch cultures of *A. niger* was found generally enhanced by temperature elevation, suggesting the role of proteolytic activity in protein quality control during heat treatment was dependant on the culture temperature.

1. Introduction

Raised culture temperature has been associated with oxidative stress events. It has been shown that several antioxidant enzymes, such as superoxide dismutase and catalase, play important roles in the heat shock response and development of thermotolerance in various microorganisms, from *Escherichia coli* [1,2], *Saccharomyces cerevisiae* [3,4], to filamentous fungi [5–7]. This induction of antioxidant enzymes in different strains as a result of heat treatment may indicate the over-production of reactive oxygen species (ROS), mainly produced from the respiratory chain via electron leakage, following heat treatment [8]. Although heat treatment has been found to compromise genetic stability [9] and membrane integrity [10] by oxidizing DNA and lipid, detailed investigation into protein oxidation during heat treatment in microorganisms is still scarce. It has been reported that intracellular oxidation, measured by an "oxidant-sensitive" fluorescent probe, is increased during heat treatment [4]. Though interesting, this study provides only limited information as it does not distinguish the types of oxidation. It is now clear that intracellular proteins may undergo several types of oxidation caused by ROS, depending on the type of oxidants and level of exposure [11]. More importantly, cells use different mechanisms to deal with different types of oxidation. Therefore, it would be worthwhile to examine protein oxidation and elimination of damaged proteins in microorganisms, rather than just the overall level of "oxidation" in the cells following heat treatment.

A widely used biomarker for protein oxidation is the carbonyl group, which is introduced to amino acid residues, especially proline, arginine, lysine and threonine, by oxidation [12]. Carbonyls are relatively difficult to induce and in contrast to other oxidative modifications, for example, methionine sulfoxide and cysteine disulfide formation, carbonlyation is irreversible and irreparable, so these altered proteins must be eliminated from the cell by protein degradation [13]. It is generally held that in eukaryotes, mildly oxidized proteins are recognized, and selectively degraded by the 20S proteasome, a large multi-catalytic proteinase complex, in an ATP-independent manner [14,15]. However, extensively oxidized proteins may aggregate or covalently cross-link to each other, and thus escape proteolysis.

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Submerged culture of filamentous fungi is widely used in industrial production of valuable products [16]. However, Nienow [17] pointed out that due to the highly viscous and non-Newtonian character of fungal fermentation fluids, temperature gradients inevitably form inside such bioreactors, especially at large scale. Thus, it is likely that growing fungi at production scale are frequently subjected to changes of environmental temperature, due to the inhomogeneity of the process fluid in the bioreactor. There are few studies on the effects of this uneven temperature distribution in fungal fermentations, but several heat shock genes have been found to be strongly upregulated in *E. coli* grown in a fermenter which mimics the temperature variation in production scale bioreactor [18].

Here the effects of elevated culture temperature on protein carbonylation and intracellular proteolytic activity in batch cultures of an industrially relevant filamentous fungus, *Aspergillus niger* B1-D. Nutrient consumption, specific growth rate and energy levels (ATP) in these cultures were also characterised to help elucidate the potential causes of oxidative stress during heat treatment.

2. Materials and methods

2.1. Strain and cultivation

A recombinant filamentous fungus, *A. niger* B1-D [19], has been used in present work. Aspergillus complete medium was used for the batch cultivation [20]. All fermentations were carried out in batch mode using a 15-l (total volume) stainless steel bioreactor (BIOSTAT C.-DCU, B.Braun Biotech International, Switzerland) with a working volume of 10 l. The fermentor was inoculated with 4.0% of 48-h-old shake flask culture grown at 25 °C and 200 rpm. The pH was kept at 4.0 by automatic titration addition (2 M NaOH and 1 M H_2SO_4). The temperature was kept at 25 °C throughout the control runs. In the runs with upshifted temperatures, the culture temperature was changed to 30 °C and 35 °C in early exponential phase (at 24 h). The agitation rate was set at 400 rpm. The air-flow rate of control runs was controlled at 1.0 volume of air per volume of culture per minute (vvm) for all the batch cultures. Real time values of pH, DOT, agitation speed, temperature, air-flow rate during fermentations were recorded automatically by software, MFCS DA (Sartorius, UK).

2.2. Biochemical assays

Biomass was estimated by the method of Bai et al. [6], starch and ammonium concentrations in the culture filtrates were measured by enzymatic essay kits (r-biopharm, Germany) as described previously [21]. Intracellular protein carbonyl contents, proteolytic activity and ATP content were measured by the methods described previously [21].

3. Results

Fig. 1 shows the effects of elevated temperature on growth as measured by dry cell weight (DCW), dissolved oxygen tension (DOT) and nutrient consumption in batch cultures of *A. niger*. In the control where culture temperature was controlled at 25 ± 0.1 °C throughout the process, *A. niger* grew exponentially from 24 h until 36 h when the culture became oxygen-limited, as indicated by the

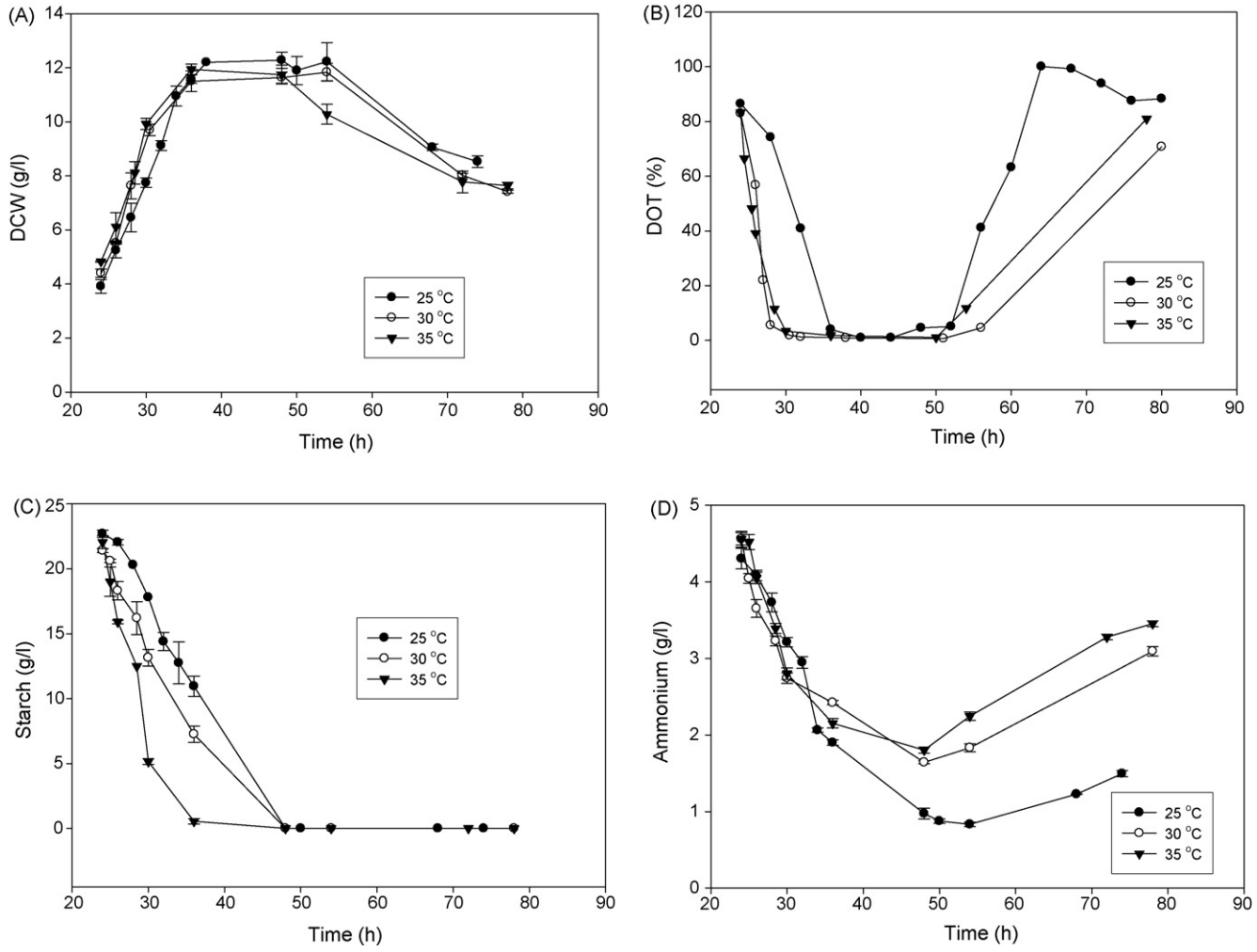

Fig. 1. Effects of elevated temperature on DCW (A), DOT (B), starch (C) and ammonium (D) concentrations in batch cultures of *Aspergillus niger* B1-D. Cultivation conditions: 1 vvm, 400 rpm, pH 4.0 and 10 l. Culture temperature was elevated to 30 °C and 35 °C in early exponential phase (24 h). In the control, culture temperature was kept at 25 °C.