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Comparative study of *Monascus sanguineus* and *Monascus purpureus* for red pigment production under stress condition

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Abstract

Monascus sp. is known for pigment production. *Monascus* sp. synthesized pigment has been used as food colorants for quite a long time. According to literature, many strains have been isolated from *Monascus*, which are internationally acknowledged and there are many studies on *Monascus purpureus*, *Monascus anka* or other species. In the present study, *Monascus* strain was isolated and identified as Monascus sanguineus on the molecular basis. This strain was then compared with a reference strain *Monascus purpureus* MTCC 410 for red pigment production under stress condition. Both strains were treated with different stress conditions viz. different concentration of glycerol, NaCl, peptone and also with the spores treated at different temperatures. Both strains had shown increased pigmentation under stressed condition. Maximum pigment yield was observed with 0.5M glycerol concentration for both strains (*Monascus sanguineus* 33.4 color value units (CVU)/ml, *Monascus purpureu*s 36.7 CVU/ml). For salt stress, both strains produced maximum pigment with 3% NaCl concentration. At 12% NaCl concentration, both the strains showed very slow growth and almost no pigment yield. When spores were treated with different temperatures, *Monascus sanguineus* produced maximum pigment with spores treated at 90°C, whereas *Monascus purpureus* lost viability at this range.

Introduction

Monascus sp., a filamentous fungus has been used to make rice wine, soybean cheese and anka (red rice) in many Asian countries (especially Japan and China) for centuries. The interest in red pigments produced by *Monascus* sp. in the food industry has been growing because of their wide applications (meat, fish, ketchup, liquor, etc.). Also some synthetic colorants show carcinogenic and teratogenic effects, e.g. the nitrosamines formed from nitrites and nitrates in cured meats (Hamano and Kilikian, 2006) has diverted the attention towards the natural colorants. Strains of *Monascus* species are known to produce several polyketide bio-pigments (Watanabe *et al*., 1997). Six pigments are considered most important. These are Anka flavin, Monascine (for yellow color), Rubrapunctatine, Monascorubrine (for orange color) Rubrapunctamine and Monascorubramine (for purple color) (Wild *et al.*, 2003).

Environmental abiotic and biotic stress factors have been proven to effect variety of responses in microbes. Some microorganisms develop systems to counteract the effect of osmotic stress such as salt stress (NaCl). This stress results in two different phenomena: ion toxicity and osmotic stress. Defense responses to salt stress are based on osmotic adjustments by osmolyte synthesis and cation transport systems for sodium exclusion. In osmo-stressed *S. cerevisiae*, polyols (glycerol in particular) are the major osmolytes produce accumulated by cells (Blomberg, 2000; Blomberg and Adler, 1992).

The aim of present study was to investigate the effect of stress on pigment yield and biomass growth from Monascus sanguineus & Monascus purpureus. Stress was induced with different concentration of glycerol, NaCl and peptone whereas; heat stress was induced by treating spores with different range of temperatures. Both strains were able to tolerate stress condition and in certain conditions they had produced more pigment as compared to the control.

Materials and Methods

Culture

Wild strain of Monascus sanguineus was isolated from pomegranate. Both the strains isolated *Monascus sanguineus* as well as reference strain *Monascus*

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purpureus were maintained on Potato Dextrose Agar (PDA) medium and incubated at 28-30°C for 7 days, preserved at 4°C, and sub-cultured once every 4 weeks (Rashmi and Padmavathi, 2011).

Source of reference culture of Monascus *sp.*

The reference culture *Monascus purpureus* MTCC 410 was procured from the Microbial Type Culture Collection, IMTECH, Chandigarh, India.

Inoculum preparation

Sporulated (5-day old) culture was diluted in distilled water. The spores were scraped off under aseptic conditions to produce a spore suspension. 15% spore suspension was inoculated into conical flasks containing 50 ml. of potato dextrose medium (PDB). This culture was incubated at 30°C for 5 days in a shaker incubator at 110 rpm (Ahmad *et al*., 2009).

Submerged fermentation

Influence of stress condition on mycelial growth and red pigment yield from *M. sanguineus* and *Monascus purpureus* was investigated in potato dextrose broth (PDB) fungal media. 100 ml flasks were taken and 50 ml media were prepared, autoclaved at 121°C for 20 min for each strain. Medium pH was adjusted to 5.5. After cooling, this media was inoculated with 0.5 ml of *M. sanguineus* and *Monascus purpureus* culture separately and incubated for 16 days in static condition (Ahmad *et al*., 2009).

The influence of glycerol, peptone and NaCl stress was investigated by adding glycerol at different concentrations namely 0.25, 0.5, 0.75, 1 and 1.25M peptone and NaCl at concentrations namely 0.25, 0.5, 0.75, 1 and 1.25% (w/v) to the PDB media prior to autoclaving for both the strains (Sumathy *et al*., 2007).

To investigate the effect of thermal stress on spores, spore suspension for both the strains was subjected to various temperatures (40, 50, 60, 70, 80 and 90ºC) for one minute before inoculation. These spores were then used as inoculum (Sumathy *et al*., 2007).

Dry cell weight

The mycelia separated from broth by filtration (Whatmann No. 1) was weighed on an analytical scale, vacuum filtered through pre-weighed membrane filters, washed with distilled water, dried in an oven at 50°C. The results were expressed in grams per liter (Mukherjee and Singh, 2010).

Pigment estimation

Filtrate was centrifuged at 10000x g for 15 minutes. Pigment concentration was determined by colorimeter at 510 nm. The absorbance values were converted into pigment units using by the following formula:

Color value = O.D. × dilution × volume of extracts / Amount of sample (ml) (Ratana and Toshima, 1987).

Results

Effect of thermal stress on pigment yield & biomass

Thermal stress was induced by incubating the spores at different temperatures. Both strains were able to tolerate thermal stress and the production of pigment started after 4th day of incubation. The growth was slow and biomass production decreased at elevated temperatures. For *Monascus sanguineus* maximum pigment yield was observed when spores were treated at 80°C and 90°C (17.08 CVU/ml, 17.87 CVU/ml) and for biomass at 40°C and 50°C. In comparison M*onascus purpureus* showed maximum pigment yield and biomass which was observed at 40°C (32.05 CVU/ml, 12.04 g/l), and there was no growth observed with spores treated at 90°C (Figure 1).

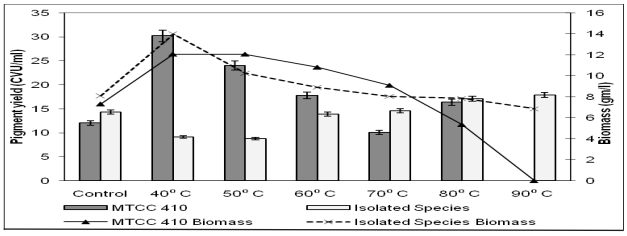

Figure 1. Effect of thermal stress on pigment yield and biomass for *M. purpureus* MTCC 410 and isolated *M. sanguineus*

Effect of glycerol stress on pigment yield & biomass

Both strains were able to tolerate glycerol stress and produced maximum pigment and biomass under glycerol stress. For both the strains, optimum glycerol concentration was 0.25 and 0.5M, beyond which reduction in pigment yield and biomass was observed. At these concentrations glycerol was utilized as carbon source which resulted in maximum pigment yield and biomass. The observed values were 34.5 CVU/ml and 14.6 g/l respectively for Monascus sanguineus and 36.7 CVU/ml and 16.1 g/l respectively for Monascus purpureus (Figure 2).

Effect of salt stress on pigment yield and biomass

When strains were exposed to different concentrations of NaCl, not much pigment yield was observed as compared to control. Maximum red

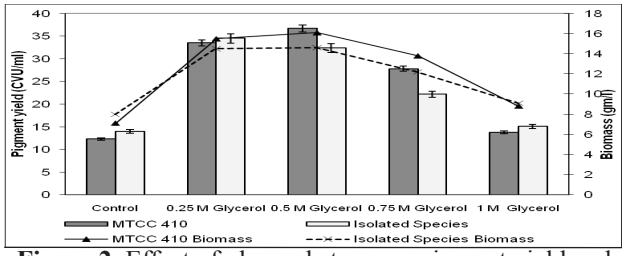

Figure 2. Effect of glycerol stress on pigment yield and biomass for *M. purpureus* MTCC 410 and isolated *M. sanguineus*

pigment was observed at 3% salt stress and beyond that reduction in yield was observed for both the strains. Both strains lost their viability at 12% salt stress. For Monascus sanguineus maximum pigment yield and biomass were observed as 16.45 CVU/ml and 10 g/l respectively whereas for Monascus purpureus the values observed were 18.5 CVU/ml and 12.5 g/l respectively (Figure 3).

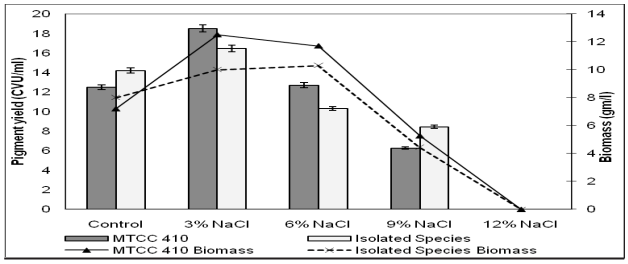

Figure 3. Effect of salt stress on pigment yield and biomass for *M. purpureus* MTCC 410 and isolated *M. sanguineus*

Effect of peptone on pigment yield & biomass

Peptone serves as nitrogen source which is essential for red pigment synthesis. With peptone, there was steady increase in pigment yield as well as biomass for both the strains. However a drastic reduction was observed in pigment yield at 12% concentration of peptone. *Monascus sanguineus* exhibited maximum pigment yield and biomass of 25.3 CVU/ml and 12.3 g/l respectively whereas for *Monascus purpureus* these values were 27.87 CVU/ml and 13.87 g/l respectively (Figure 4).

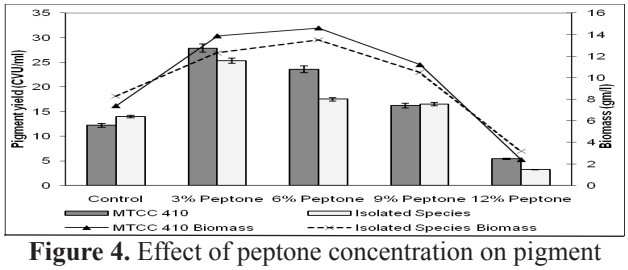

Figure 4. Effect of peptone concentration on pigment yield and biomass for *M. purpureus* MTCC 410 and isolated *M. sanguineus*

Discussion

Both strains had shown maximum pigment yield as well as biomass with 0.5M glycerol concentration. At this concentration glycerol had served as carbon source though it can induce an osmotic stress also. At 1M concentration of glycerol, a reduction in pigment yield was observed. Sumathy *et al.* (2007) reported an increase in pigment yield as well as biomass with glycerol stress from *Monascus* sp. Under salt stress condition when compared to control there was an increase in pigment yield at 3% NaCl concentration and thereafter reduction was observed. There were no traces observed at 12% NaCl concentration for both the strains. This may be due to the fact that in saline environments, cells encounter stress due to increased electrolyte concentrations that tend to inhibit metabolic functions (Adler *et al.*, 1982).

There was an enhanced pigment yield and biomass observed with peptone concentration for both the strains though at 12% peptone concentration a major decrease was observed. The formation of water soluble red pigment was strongly regulated by the amino acid used as nitrogen source. Amino acid acts as side chain precursor for the production of soluble red pigment (Lin and Demain, 1994). For thermal stress conditions, interesting results were obtained. *M. sanguineus* has shown enhanced pigment yield when the spores were subjected to elevated temperatures i.e. 80-90^{o}C whereas biomass showed decreasing trend. Similar results were observed for *M. purpureus* also but the viability was lost at 90^{o}C.

Both prokaryotic as well as eukaryotic cells are capable of responding to heat shock by undergoing rapid and massive synthesis of a subset of cellular proteins commonly referred to as heat shock proteins (HSP) (Lindquist, 1986). Heat shock proteins have been shown to impart thermo tolerance to subsequent lethal exposures at elevated temperatures (Managbanag and Torzilli, 2002). It can be concluded that both these strains had shown appreciable pigment yield till 0.5M glycerol concentration. For salt stress 3% NaCl concentration had shown maximum pigment yield for both the strains. With peptone, pigment yield showed an increase as compared to control for both the strains with peak reaching at 9% peptone concentration. For thermal stress *M. purpureus* had produced maximum pigment for spores treated at 40^{o}C with steady decrease thereafter. *M. purpureus* had not shown any pigmentation when spores treated at 90^{o}C whereas *M. sanguineus* had shown maximum pigment yield at elevated temperatures.

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