Monascus ruber ICMP 15220 fermentation for the production of pigments

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Abstract

Edible pigments of *Monascus* species have an established history of use in certain Asiatic foods. This work focused on development and optimization of production of the red pigments of *Monascus ruber* ICMP 15220. This particular *Monascus* strain had not been previously investigated.

Suitable media compositions for growth and pigment production were first evaluated on agar plates. Media that contained glucose and monosodium glutamate (MSG) as carbon and nitrogen sources, respectively, were found to be most suitable for red colour production and became the basis for further studies. Both submerged culture and solid-state fermentations were assessed for pigment production.

In submerged culture, a fully defined medium with 10 g L^{-1} glucose and a carbon to nitrogen mass ratio of 9 : 1 at 30^{o}C proved to be the most productive for the target red pigments. This medium used MSG as the nitrogen source. Attempts were made to replace MSG with less expensive inorganic nitrogen sources, but no suitable replacement was found. Biomass and pigment productivity were evaluated on a variety of carbon sources, but ethanol was confirmed to be best.

Solid-state fermentation on steamed rice proved to be remarkably more productive for the target pigment than the submerged fermentation. Solid-state fermentation did not require supplemental nitrogen to attain a high productivity. The C : N ratio for attaining the peak pigment productivity in solid-state culture proved to be entirely different from the value that had been found to be optimal in submerged culture. In view of its superior characteristics, the solid-state fermentation was optimized in packed-bed bioreactors. A central composite experimental design was used for the optimization that focused on the initial moisture content of the substrate and aeration rate in the bed as the main operation parameters. In 18 cm deep packed beds of steamed rice, the optimal fermentation conditions were at 30^{o}C, an initial moisture content of 70%, and aeration with humidified air (97 – 99% relative humidity) at a flow rate of 0.14 L/min.

In all cases, the production of pigments was growth associated. Under optimal conditions in the packed-bed bioreactor, the red pigment productivity was nearly 3.4×10^{4}-fold greater than in the best case submerged culture. The stability of the pigments produced under the various conditions was characterized with respect to ambient light, pH, and heat.

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Abbreviations

sp.	Species
Kr	Radial growth rate
M.	*Monascus*
L*	Lightness
a*	+a* = red; -a* = green
b*	+b* = yellow; -b* = blue
h^O	Hue angle
C*	Chroma
C : N	Carbon to nitrogen ratio
Rpm	Rotation per minute
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
NMR	Nuclear magnetic resonance
IMC	Initial moisture content
TLC	Thin layer chromatography
UV	Ultraviolet
R_f	Retention factor
Aw	Water activity
SSC	Solid-state cultivation
$Y_{P/X}$	Yield of product on biomass
$Y_{X/S}$	Yield of biomass on substrate
Cf	Conversion factor
Df	Dilution factor
μ_{max}	Maximum specific growth rate
T	Time
AU	Absorbance unit
X_{max}	Maximum biomass concnetration
r_{BM}	Rate of biomass production
r_P	Specific rate of product formation
Cdw	Cell dry weight

Pr	Productivity
w/w	Weight per weight
vol/vol	Volume per volume
SS	Sum of squares
DF	Degrees of freedom
MS	Mean square
CV	Coefficient of variation
R^2	Coefficient of determination