Production of pigments by *Monascus purpureus* in solid culture

Michael R. Johns and Deidre M. Stuart

Department of Chemical Engineering, The University of Queensland, Brisbane, Australia

(Received 22 August 1990; revised 6 February 1991; accepted 11 February 1991)

Key words: Pigment; Solid culture; *Monascus purpureus*

SUMMARY

The effect of physical and nutritional factors on the production of pigments by *Monascus purpureus* FRR 2190 was studied using cultures grown on both rice and a synthetic medium that was solidified with carrageenan and extruded into rice-like particles. Pigment yield was highly sensitive to physical parameters. Optimal pigment formation in rice cultures occurred at an initial pH of 6 and an initial moisture content of 56%. Lower moisture contents led to a large decrease in pigment concentration. Red and yellow pigment production on solidified gel media was increased up to three-fold compared to that of liquid cultures of the same medium composition, particularly when peptone was used as the sole nitrogen source. Solid state rice cultures gave the highest pigment yields.

INTRODUCTION

The fungus *Monascus purpureus* produces six, closely-related polyketide pigments ranging in colour from bright yellow to deep red. The organism has attracted continuing attention as a microbial source of natural red pigment to replace synthetic dyes, particularly erythrosine (FD&C Red No.3) [12,17]. The pigments and the fungus have been used to colour food in Asia for several centuries, but have yet to gain regulatory approval in the West.

Solid culture of *M. purpureus* on steamed rice, bread, brans or cereal meals is the traditional method of pigment production [9,13]. Some authors have described the development of mutant strains of *M. purpureus* giving high pigment yields on solid culture [9,13], but the effect of cultural parameters on yields has not been reported.

In contrast, extensive submerged culture studies of red pigment synthesis by various *M. purpureus* strains have revealed that the yield is affected markedly by many factors including medium composition, pH and agitation [2-5,12,14,18]. Comparisons between the red pigment yield of submerged and solid cultures have suggested that the yields are superior in the latter [7,13], although the conditions and the media used have differed. The fundamental reasons for the better results observed in solid culture have been explored [7], but the results were equivocal.

This work examined the effect of pH and the initial moisture content of rice on red pigment production in solid culture by *M. purpureus* and used an artificial gel medium to compare pigment productivity in solid culture with that of submerged cultures grown on medium of identical composition.

MATERIALS AND METHODS

Organism and media

*Monascus purpureus* FRR 2190 was obtained from CSIRO Food Research Laboratories, North Ryde, Sydney and maintained on potato dextrose agar (PDA) slopes (Difco Lab., Detroit, U.S.A.) at 4 °C. A distilled water suspension from a 6-day old PDA slope of *M. purpureus* grown at 30 °C was used for inoculation.

The glucose-peptone medium of Johns et al. [10] was used with a glucose concentration of 50 g/l. Where required, maltose (50 g/l) or sodium nitrate (3 g/l) was substituted for glucose and peptone respectively. To achieve solidification, 50 g/l of carrageenan (Sigma, St.Louis, U.S.A.) was added, the pH was adjusted to 6 and the medium was boiled. On cooling, the medium was extruded through a sterile, 10 ml syringe into a sterile petri-dish to form solid, rice-like pellets.

Calrose short-grain white rice (Sunrice, Australia) was used as substrate where indicated after sterilisation at 121 °C for 15 min. For use in pH experiments, 40 g lots of...
121 °C for 15 min. For use in pH experiments, 40 g lots or rice were soaked in distilled water (20 g) for 5 h, the pH was adjusted using 1 M HCl or 1 M NaOH and the rice dispensed as 8 g lots into conical flasks. After sterilisation, each flask was inoculated with 2.5 ml of a spore suspension and incubated at 30 °C.

**Controlled humidity experiments**

Sealed glass bottles (560 ml) containing 25 ml of solution and a stainless steel support (Fig. 1) for three rice cultures were used to provide environments in which the relative humidity of the gaseous phase was controlled during growth. Filter paper was included to increase the surface area between the solution and the atmosphere.

The bottles contained one of nine salt solutions, chosen to provide a range of equilibrium relative humidities between 0.751 and 1.00 at 30 °C according to Rao and Rizvi [15]. These were added aseptically to the bottles, which had been sterilised at 121 °C for 15 min. Solutions of water activity below 0.950 were not sterilised [11], whereas solutions with higher water activity were either filtered through a sterile 0.2 μm filter (NaCl solutions) or, the salt was added to sterile distilled water (saturated K2SO4).

Rice samples (1 g dry rice soaked in 1 ml water) were sterilised as above and were added to the bottles, which were incubated at 30 °C for 3 days. The incubation resulted in a gradation of the water contents of the rice samples, according to the water activity of the solution present in the chamber (Table 1). Inoculation of the rice was performed using fungal spores taken from a 6-day old

**TABLE 1**

Solutions used to control equilibrium relative humidity (ERH) and corresponding rice moisture content after 3 days incubation at 30 °C

<table>
<thead>
<tr>
<th>Solution</th>
<th>ERH</th>
<th>Rice moisture content</th>
<th>Rice moisture contenta w.b. (%)</th>
<th>S.E. (%)</th>
<th>(w.b.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1.00</td>
<td>56.7</td>
<td>0.4</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>1.7% (w/w) NaCl</td>
<td>0.99</td>
<td>51.8</td>
<td>0.7</td>
<td>55.0</td>
<td></td>
</tr>
<tr>
<td>Satd K2SO4</td>
<td>0.970</td>
<td>45.8</td>
<td>2.3</td>
<td>41.2</td>
<td></td>
</tr>
<tr>
<td>8.0% (w/w) NaCl</td>
<td>0.950</td>
<td>48.2</td>
<td>1.4</td>
<td>35.0</td>
<td></td>
</tr>
<tr>
<td>Satd KNO3</td>
<td>0.923</td>
<td>39.5</td>
<td>0.5</td>
<td>30.1</td>
<td></td>
</tr>
<tr>
<td>17.0% (w/w) NaCl</td>
<td>0.873</td>
<td>38.0</td>
<td>3.0</td>
<td>24.8</td>
<td></td>
</tr>
<tr>
<td>Satd KCl</td>
<td>0.836</td>
<td>19.0</td>
<td>4.0</td>
<td>22.3</td>
<td></td>
</tr>
<tr>
<td>Satd (NH4)2SO4</td>
<td>0.806</td>
<td>20.0</td>
<td>5.0</td>
<td>20.7</td>
<td></td>
</tr>
<tr>
<td>Satd NaCl</td>
<td>0.751</td>
<td>15.0</td>
<td>2.0</td>
<td>18.4</td>
<td></td>
</tr>
</tbody>
</table>

Satd, saturated; S.E., standard error.

*a Values calculated from a correlation given by Bason and Gras [1]. The correlation is strictly valid only between 40–90% d.b., but has been extrapolated for comparison.