

ABSTRACT

The present investigation aims at the increase in L-phenylacetylcarbinol (L-PAC) production through improvement of a yeast strain. Two hundred and fifty yeast strains were isolated from samples of different sources such as cane molasses, decaying fruits, wet bagasse, distilleries' soil, decaying vegetables, sugar Mills' effluent and decaying rice hulls. Amongst the isolates, 106 could not grow on acetaldehyde (1.0 g/l) added to yeast extract/peptone/dextrose (YPD) plates during qualitative screening. Out of remaining 144 isolates (acetaldehyde tolerants), 64 showed negligible L-PAC productions (≤ 0.5 g/l) in glucose/peptone medium using shake flasks during quantitative screening. The eighty best isolates produced from 0.58-2.58 g/l of L-PAC. The highest L-PAC production of 2.35 and 2.58 g/l was found in isolates GCU-2 and GCU-36, respectively. Time course analysis showed isolate GCU-36 was the most efficient because it had lower biomass formation and higher L-PAC production than GCU-2. The isolate GCU-36 was identified as *Saccharomyces cerevisiae* and recoded as *S. cerevisiae* GCU-36.

The isolate, *S. cerevisiae* GCU-36 was mutagenized using ultraviolet (UV) radiations, nitrous acid (HNO_2) or ethylmethane sulfonate (EMS) to improve L-PAC production. Significant ($p < 0.05$) increases of 1.61, 1.76 or 1.79 fold L-PAC production was found compared to wild strain when cells of *S. cerevisiae* were exposed to UV, HNO_2 or EMS, respectively. Out of 18 double mutants screened after reciprocal treatments, only 2 (1 UV/EMS and 1 HNO_2 /UV sensitive) were found to have significantly higher L-PAC production (~ 2.0 fold). In the subsequent 1st step of alternate treatment (HNO_2 exposure to HNO_2 /UV sensitive double mutant), the production increased to 2.11 fold. It declined to 2.11 fold (a sign of cessation of mutagenesis) after

the 2nd step of alternate treatment (UV exposure to HNO₂/UV/HNO₂ sensitive mutant). The selected *S. cerevisiae* mutant GCUN/V/N-1 was found to be a better L-PAC producer (5.61 g/l) with better L-PAC fermentation kinetics than the wild type strain. During physiological characterization, it utilized ethanol as a sole carbon source.

The optimal values of specific growth rate (0.24 h⁻¹) and dry biomass (13.20 g/l) of *S. cerevisiae* GCUN/V/N-1 were achieved using a simple growth medium urea molasses broth (UMB; urea 1.25 g/l; molasses sugars 150 g/l; pH 5.0) at 30°C. Seed culture was developed in the UMB medium using 10% (v/v) inoculum. Maximal pyruvate decarboxylase (PDC; *EC 4.1.1.1*) activity (0.70 U/mg proteins) was measured in the cell extracts before fermentation. L-PAC production increased only slightly to 5.67 g/l after the addition of an aldehyde mixture (acetaldehyde + benzaldehyde) at 1 h intervals. Since no positive impact of acetaldehyde addition was noted. It was dropped from the protocol for biotransformation. In contrast, descending dosages (gradual decreases in volume of dose) of benzaldehyde over time (0, 20, 30, 40, 50 min) enhanced L-PAC production (8.55 g/l). The values of optimized incubation temperature and initial pH of the medium were found to be 30°C and 5.0, respectively.

The scales up studies were investigated using a stirred fermentor (5 L). Maximal PDC activity (0.80 U/mg protein) in minimal time (9.30 h) was observed in the seed culture (working volume 3.5 L) developed at aeration rate of 4.0 l/min (250 rpm). Dissolved oxygen (% of air saturation) increased with increase in agitation (100-300 rpm) or aeration rates (1-5 l/min). Subsequently, a significant enhancement in L-PAC production (12.4 g/l) was achieved at reduced aeration rate of 2.5 l/min (200 rpm) for 3.2 litres (working volume). L-PAC production increased gradually to 19.5 g/l, became stable at the point for 20 times using growth-recycled seed. However, consistent L-PAC production (~12.40 g/l) was found for 6 times after the reuse of biomass (recovered from

fermented broth). Silicone oil (8% v/v) proved to be a better antifoaming agent for both, seed culture and biotransformation broth. Toluene facilitated maximum extraction of L-PAC from fermented broth (solvent to broth ratio 2:1).

The improved L-PAC productivity showed concomitant improvement in benzaldehyde biotransforming ability of endogenous metabolism under the optimal process parameters. Non-utility of added acetaldehyde, lower time course of fermentation and elevated L-PAC production made the present work cost-effective.