ABSTRACT The present study was designed to isolate new yeast strains with significant pyruvate decarboxylase PDC activity for their use in the pharmaceutical industry for the production of L-phenyacetyl carbinol L-PAC. Two hundred fifteen yeast strains isolated from fruits, soil and water were screened for pyruvate decarboxylase PDC production using Brady's reagent. Thirty isolates were selected after qualitative screening for L-phenyacetyl carbinol L-PAC production, were studied for all products of pyruvate decarboxylase PDC through gas chromatographic analysis. Among these isolates, eight were selected and screened for temperature tolerance, solvent stress and crabtree effect. Two yeast isolates were finally selected and identified as *Pichia cecembensis* and *Torulaspora delbrueckii*.Random mutagenesis was employed to enhance L-phenyacetyl carbinol L-PAC production. Nitrous acid mutant of *Pichia cecembensis* produced maximum L-PAC (1.55 g/L) with 39.6 % improvement as compared to wild-type strain. Ultra violet UV mutagenesis improved L-phenyacetyl carbinol L-PAC production from *Torulaspora delbrueckii* up to 21.3%. Two selected mutants were optimized for further studies.

Ten culture media with different combinations of four substrates (whole cheese whey, pure cheese whey, corn steep liquor and molasses) were screened for L-phenyacetyl carbinol L-PAC biosynthesis. *Pichia cecembensis* under optimized incubation conditions i.e., incubation temperature of 34°C, 8% inoculum size, pH 6.0, sugar concentration 16% and 24hrs of incubation time produced 8.88 g/L L-phenyacetyl carbinol L-PAC. While *Torulaspora delbrueckii* produced 7.3 g/L at 30°C, 10% inoculum size, pH 5.5 with sugar concentration 17% and 24hrs of incubation time.

Bioprocess engineering and metabolic engineering techniques were employed for further rise in L-phenyacetyl carbinol (L-PAC) production. The effect of cofactors of pyruvate decarboxylase PDC (Mg^{2+} and thiamine pyrophosphate TPP), effect of inhibitors of alcohol dehydrogenase ADH (1, 10 phenanthroline, boric acid, and sodium metaborate, dimethyl sulfoxide DMSO and benzyl alcohol) and impact of acetaldehyed and benzaldehyde at different temperatures were also studied. Six different dose patterns in ascending and descending mode were screened for L-phenyacetyl carbinol L-PAC production. Descending dose pattern was better (A 125,180, 155, 155, 280, 315, 345) for wild-type strain of *Pichia cecembensis* and F; 125, 155, 155, 185, 185, 205, 230, 305, 320 for *Pichia cecembensis* (mutant) producing 10.44 and 5.99 g/L L-PAC, respectively. Time lap between doses was also studied to minimize the yield of side products. Delay in dosing, up to five min have no effect on L-phenyacetyl carbinol L-PAC yield. The *Pichia cecembensis* (wild-type type strain) have reduced L-PAC after five min to 5.99 g/L sugar addition with benzaldehyde produced L-phenyacetyl carbinol L-PAC 76.89 % and effect of L-alanine raised yield of L-phenyacetyl carbinol L-PAC to 77%. Growth of yeast cells was inhibited by boric acid when used as inhibitor.

The process of production was optimized using response surface method. Pyruvate decarboxylase PDC activity and L-phenyacetyl carbinol L-PAC yield during present studies were 56.27 U/ml and 13.55 g/L, respectively, after statistical method development. The yield of L-phenyacetyl carbinol L-PAC (12.88 to 13.55, g/L) was increased by 5.2% after process optimization through response surface methodology RSM with incubation time of 13hrs, temperature of 33°C and total sugar at 18 % as significant factors (p-values, 0.902, 0.260 and 0.247, respectively). Process design had Adjusted R^2 value of 0.562, R-squared as 0.770 and Adequate precision as 4.888 with a uniformly distributed standard error. Pyruvate decarboxylase PDC used in the form of *Pichia cecembensis* whole cells revealed higher stability towards benzaldehyde and elevated temperature as compared to partially purified pyruvate decarboxylase PDC. Whole cells and partially purified pyruvate decarboxylase PDC showed half-lives of 240hrs and 72hrs at 4°C whereas 33hrs and 28.5hrs at 25°C. High performance liquid chromatography HPLC was used to purify L-PAC with purification level of 76.18%.

In benchtop fermenter the yield of L-phenyacetyl carbinol L-PAC was increased by 29%. The activity of pyruvate decarboxylase PDC was 60.7 U/L which produced 15.33 g/L of L-phenyacetyl carbinol L-PAC. Pyruvate decarboxylase PDC was purified through salt precipitation, active pyruvate decarboxylase PDC was obtained at 60% with molecular weight 240kDa determined through western blotting after purification. Purified pyruvate decarboxylase PDC was sequenced.