**Role of L-Phenylalanine in 2-Phenylethanol Synthesis by *Kluyveromyces marxianus* ATCC 36907 using *Anacardium occidentale*.**

André C. Macedo1, Fernando K. C. Costa2, M. Valderez P. Rocha1, Luciana R. B. Gonçalves1

*1Departamento de Engenharia Química, Universidade Federal do Ceará, Campus do Pici, CEP: 60455-760. Fortaleza - CE, Brazil. 2Universidade Federal de Santa Catarina,* *CEP: 88040-970. Florianópolis – SC, Brazil.*

*\*Corresponding author: acasimiro@gmail.com*

**Highlights**

* Alternative biotech-process to provide a high-purity 2-PE.
* Possibility and benefits of *CAJB* use as microbial cultivation broth.
* Synergistic effect between sugar concentration and L-Phe/precursor.

**1. Introduction**

2-Phenylethanol (2-PE) is an aromatic compound with rose-like odours used as a food, cosmetics and perfumery additive. Currently, this aroma is produced from natural rose extracts and chemical synthesis in high-cost processes. Due to this problem, the biotechnological pathway has been described in current literature as a promising alternative process to provide a high-purity 2-PE in an environmentally friendly process (**Etschmann** *et al.* 2002). Significant reports have described the use of *Kluyveromyces marxianus* as the best 2-PE producers. In fact, some yeast strains produce 2-PE by the Ehrlich pathway (**Fabre** *et al.* 1997; **Wittmann** *et al.*, 2002; **Schrader** *et al.* 2004). In this context, a biotech-system has been investigated for the 2-PE production by *K. marxianus* using cashew apple (*Anacardium occidentale L., CAJB*) as a nutritional substrate for microbial cultivation. *CAJB* is a native fruit from Tropical America discarded as an agricultural by-product. Considering the rich composition, CAJB can be used as a low-cost carbon source and other media components, that greatly influence the 2-PE productivity. This study not only explored the possibility and benefits of *CAJB* use, but also describes the role of L-phenylalanine (L-Phe) as a metabolic-key in bioreactions-chain which leads to 2-PE synthesis.

**2. Methods**

**Microorganism:** *Kluyveromyces marxianus* ATCC 36907 was used for 2-PE production. **Cultivation Broth**: CAJB (133.4 ± 2.4 g/L total sugar) was used as microbial cultivation broth. Additional L-Phe concentrations (1.0; 3.0; 4.0 and 10.0 g/L) were used in CAJB. All the assays were conducted using a Tec-Bio bioreactor (performed at 35°C, 250 rpm, 2 L/min air, using 750 mL/75 mL of inoculum). **Analytical Methods**: Cell concentration was measured by gravimetric method and 600nm optical density using a spectrophotometer. Sugar concentration measurement were performed using HPLC/RID and Aminex® column (150 mm x 7.8 mm) at 65°C. 1 mmol/L H2SO4 was used as eluent at a 0.4 mL/min flow rate. 2-PE concentration was also determined by HPLC/UV (at 254 nm) using Nova-Pak® C18 (5μ, 4.6mm x 150mm) at 35 °C and. Acetonitrile/water (40:60 v/v) was used as eluent at 0.8 mL/min flow rate.

**3. Results and discussion**

2-PE is synthesized *via* L-Phe transamination to phenylpyruvate, which is decarboxylated to phenylacetaldehyde and finally reduced to 2-PE. This classical Ehrlich pathway (EP) description suggests the important role of L-Phe and thus, it can be predicted that the synthesis of 2-PE is highly influenced by L-Phe in media composition. In fact, the results for cell (X) growth and product (P) accumulation with different concentrations of L-Phe (illustrated by Table 1) confirmed this hypothesis when we evaluated 1.0-3.0 g/L L-Phe, although the similar results for the concentrations of 3.0; 4.0 and 10.0 g/L showed no significant differences in the metabolite production.

**Table 1**- Description of yield (Y), kinetic () and volumetric productivity (QX and QP) parameters obtained for *K. marxianus* ATCC 36907 cultivation using CAJB.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **L-Phe Concentration (g/L)** | | | |
| **1.0 (g/L)** | **3.0 (g/L)** | **4.0 (g/L)** | **10.0 (g/L)** |
| *𝑌 𝑋/𝑆* (g/g) | 0.115 | 0.128 | 0.133 | 0.135 |
| *𝑌 𝑃/𝑆* (g/g) | 0.004 | 0.006 | 0.006 | 0.006 |
| *Y P/X* (g/g) | 0.035 | 0.051 | 0.046 | 0.049 |
| *QP* (g/L.h) | 0.007 | 0.011 | 0.011 | 0.011 |
| *Qx* (g/L.h) | 0.205 | 0.244 | 0.245 | 0.254 |
| *µXmáx* (1/h) | 0.511 | 0.342 | 0.863 | 0,600 |

This observation might also reflect the effects of the CAJB complex composition, interfering in the 2-PE bioconversion. Parallel experiments using synthetic broth (10 g/L glucose and 3 g/L L-Phe) pointed 0,028 g/L.h for *QP*. Thus, we can suppose the important metabolic role of L-Phe in 2-PE synthesis when we use nutritionally-poor broths. When we use CAJB the synergistic effect between sugar concentration and L-Phe is more evident. The accelerated cell growth () indicates intensive C-N-energy deviation to the biomass synthesis and limiting EP. EP is still limited by 2-PE inhibition. 2-PE at even low-concentrations could start biological *sensing* mechanisms, limiting EP and 2-PE productivity even when we use appropriate broths or L-Phe high-concentrations.

**4. Conclusions**

These results suggest that CAJB is an appropriate, unconventional medium for bioproduction of 2-PE by *K. marxianus* ATCC 36907. Also, these results pointed out the synergistic effect between sugar/L-Phe concentration and 2-PE inhibition as an important mechanism for the process optimization.

**References**

1. M.M.W. Etschmann, W. Bluemke, D. Sell, J. Schrader, Appl. Microbiol. Biotechnol. 59 (2002) 1–8.
2. C.E. Fabre, P.J. Blanc, G. Goma, Biotechnol. Tech. 11 (1997) 523–525.
3. J. Schrader, M.M.W. Etschmann, D. Sell, J.M. Hilmer, J. Rabenhorst, Biotechnol. Lett. 26 (2004) 463–472.
4. C. Wittmann, M. Hans, W. Bluemke,. Yeast 19 (2002) 1351–1363.