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VANILLIN PRODUCTION BY RECOMBINANT STRAINS OF *ESCHERICHIA COLI*

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ABSTRACT

Vanillin production from ferulate was studied using different recombinant strains of *Escherichia coli*. To prevent the occurrence of aerobic conditions and then possible product oxidation, tests were performed in Erlenmeyer flasks under mild mixing (150 rpm). Among other transformants, *E. coli* JM109(pBB1) appeared to be the best vanillin producer, being able to convert no less than 95% of starting ferulate to the product within 1h. This yield decreased down to 72% after 72h, likely because of a non-specific oxidase activity responsible for vanillin oxidation to vanillate.

Key words: vanillin, ferulate, *Escherichia coli* JM109, recombinant strains.

INTRODUCTION

The increasing interest of the food industry for the use of biocatalysts, as an answer to the increased demand of natural products (1), has recently been stimulating the search for alternative ways to produce vanillin (2). Seeing that the use of a natural substrate or catalyst would allow the product itself to be classified as natural (3), we hope for a biotechnological production of this compound, which is at present synthesized chemically and mainly utilized as flavor in food industry (4). One of the most interesting biotransformations to produce vanillin is the microbial conversion of ferulate (5).

Such a bioconversion has been tested in this work using five vanillate-producing mutants of *Escherichia coli*, previously made unable to oxidize vanillin through deletion of the gene codifying for vanillin dehydrogenase. Among these, *E. coli* JM109(pBB1) was shown to be the best vanillin producer.

MATERIALS AND METHODS

Microorganisms growth and bioconversion conditions

Tests were carried out using different recombinant strains of *Escherichia coli* (DH5α, JM109, Novablu, SureII and XL10gold), belonging to the collection of the Agrobiology and Agrochemistry Department, Tuscia University, Viterbo, Italy. Cells were maintained at 4ºC on Petri plates containing LB medium and 0.1 g/L ampicillin. The pre-culture was prepared in 5 mL of the same medium without agar. The inoculum was cultivated over-night at 150 rpm and 37ºC. Then, 30 mL of fresh LB containing 0.1 g/L ampicillin were transferred into 300 mL Erlenmeyer flask and inoculated with 0.6 mL of the pre-culture. Cells were collected at the end of the log phase, at which O.D. (λ = 600 nm) and cell concentration were 2.3-2.7 and 1.15-1.35 g_{DW}/L, respectively, harvested by sterile centrifugation (6000 rpm, 10 min) and washed twice with a salt solution (6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 1 g/L NH_4Cl, 0.5 g/L NaCl). The

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bioconversion medium was constituted by this solution supplemented with ferulate (0.3-0.4 g/L) and YE (50 mg/L) as a co-substrate. All experiments were carried out at 30ºC in triplicate using an inoculum of about 5 g/L.

Analytical determinations

Biomass concentration was determined by optical density measurements at 600 nm. Vanillate, vanillin and ferulate concentrations were determined by HPLC 1100, Hewlett Packard (Palo Alto, CA) provided with a Vydac 201TP54 C18 reverse-phase column (Hesperia, CA), maintained at 35ºC, using an UV/Vis detector, Hewlett Packard (Palo Alto, CA), set at $\lambda = 254$ nm. The mobile phase, used at 0.5 mL/min flow rate, consisted of 40% methanol and 60% of a solution prepared adding 6.8 g/L KH_2PO_4 in water and adjusting the pH with H_3PO_4 up to 2.5. Data acquisition and processing were controlled by the Rev. A.09.03 ChemStation Software, Agilent Technologies (Palo Alto, CA).

RESULTS AND DISCUSSION

The experimental data of *E. coli* JM109(pBB1) growth were used to estimate the growth kinetic parameters of this recombinant strain, referring either to the overall growth curve ($dX/dt = 1.2$ g/Lh; $\mu = 0.29$ h^{-1}) or only the exponential phase ($dX/dt = 2.2$ g/Lh; $\mu_{max} = 0.60$ h^{-1}; $t_d = 1.1$ h). Comparison with literature data demonstrates that the LB medium allowed for notably slower cell growth with respect to that in simple carbon sources. For example, a duplication time of 0.5 h or even less is reported for *E. coli* growth on glucose under optimum conditions (6).

The ability of 4 additional strains of *E. coli* transformed with the pBB1 plasmid, namely DH5α, Novablu, SureII and XL10gold, was tested for vanillin production, following the same protocol as that used for *E. coli* JM109(pBB1). The data collected after different bioconversion times, in terms of product concentration, are illustrated in Fig. 1, while Table 1 shows the values of the kinetic parameters as well as the bioconversion yields. *E. coli* JM109 ensured the highest yields at the start (1h), whereas *E. coli* DH5α and XL10gold were the most effective at the end of bioconversion (72h).

These results on the whole suggest that most of the tested recombinant strains, but *E. coli* SureII and XL10 gold, may possess a non-specific oxidase activity able to oxidize vanillin and to produce $NADH_2^+$, in order to sustain their own energetic metabolism. Conversely, *E. coli* JM109 showed the highest volumetric and specific productivities and ferulate conversion rates.

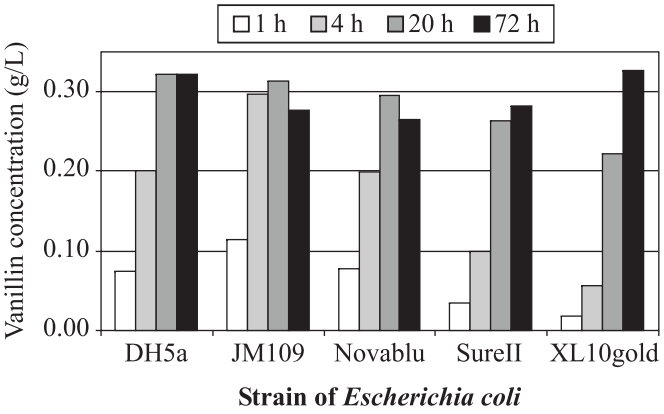

Figure 1. Vanillin production from ferulate by different recombinant strains of *E. coli*.

Table 1. Parameters of ferulate bioconversion to vanillin by different strains of *E. coli* transformed with the pBB1 plasmid.

T (ºC) XL10gold	DH5α	JM109	Novablu	SureII	
$Y_{P/S}$ (-)	0.903	0.959	0.905	0.720	0.591
$Y^f_{P/S}$ (-)	0.844	0.726	0.701	0.739	0.875
Q_p^o (g_V/Lh)	0.075	0.115	0.078	0.035	0.019
q_p^o ($g_V/g_{DM}h$)	0.013	0.022	0.017	0.008	0.004
Q_s^o (g_F/Lh)	0.106	0.153	0.110	0.062	0.041
q_s^o ($g_F/g_{DM}h$)	0.018	0.029	0.024	0.013	0.008

$Y^o_{P/S}$ = starting yield of vanillin on consumed ferulate; $Y^f_{P/S}$ = final yield of vanillin on consumed ferulate; Q_p^o = starting volumetric productivity; q_p^o = starting specific productivity; Q_s^o = volumetric rate of ferulate consumption; q_s^o = specific rate of ferulate consumption.

CONCLUSIONS

With the aim of minimizing vanillin oxidation and obtaining the highest productivity, the results of this work point out *E. coli* JM109 (pBB1) as the best recombinant strain, among others, for vanillin production from ferulate. Under aerobic conditions, a non-specific oxidase activity may be responsible for an increased production of vanillate from ferulate to the detriment of vanillin formation. Therefore, optimization of the aeration conditions is needed to improve the vanillin yield as well as to exploit this bioconversion in continuous system.

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RESUMO

Produção de vanilina por linhagens recombinantes de *Escherichia coli*

A produção de vanilina a partir de ácido ferúlico foi estudada utilizando-se diferentes linhagens recombinantes de *Escherichia coli*. Para prevenir a ocorrência de condições de aerobiose e a possível oxidação do produto, os ensaios foram realizados em frascos Erlenmeyer sob agitação moderada (150 rpm). *E. coli* JM109 (pBBI) mostrou-se o melhor produtor de vanilina entre os demais agentes transformantes, sendo capaz de converter 95% do ácido ferúlico inicial em produto após 1h, rendimento este que decresceu para 72% após 72h, provavelmente devido à atividade de uma oxidase não-específica responsável pela oxidação de vanilina a ácido vanílico.

Palavras-chave: vanilina, ácido ferúlico, *Escherichia coli* JM109, linhagens recombinantes.

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