Production of L-Lactic Acid from D-Xylose for the Synthesis of Biopolymers

Elzbieta Oltuszak-Walczak, and Piotr Walczak

***Abstract*—**Eight strains of *Enterococcus faecium* capable of fermenting D-xylose to L-lactic acid were isolated and characterized. The strains differed in their production dynamics, sugar utilization rate and biomass production level. Biomass concentration after 72 h of fermentation ranged between 1.05 and 1.47 g/l depending on strain. Lactic acid concentration in the growth medium after 72 h of fermentation was between 16.8 and 29.1 g/l. Strains KD31 and K4 produced more than 28 g/l of lactic acid whereas 2B2 only 16.8 g/l. Production between 22.3 and 26.4 g/l of lactic acid was characteristic for remaining strains. The yield of lactic acid calculated per consumed D-xylose ranged between 62.0 and 80.1%. Concentrations of by-products, acetic acid and ethanol, at the end of fermentation were low being 0.02 to 0.07 g/l for acetic acid and 0.01 to 0.10 g/l for ethanol. Chiral purity of produced lactic acid varied between 93.8 and 100 %.

***Keywords*—** D-xylose fermentation, *Enterococcus faecium*, L-lactic acid.

I. Introduction

HYDROLYSIS of hemicelluloses from lignocellulosic materials derived from plant biomass leads to the production of D-xylose solutions which may be used as cheap renewable substrate for the production of numerous products of microbial metabolism. Among them lactic acid is of great importance due to its high demand for the production of biodegradable polymer polylactic acid (PLA). Due to the required tacticity of synthesized polymer it is advantageous to produce L-form of lactic acid which, when processed to the polymer, gives plastic material with desired mechanical properties. Pure L stereoisomer of lactic acid can be produced by fermentation of carbohydrates with Lactic Acid Bacteria (LAB) possessing stereospecific L-lactate dehydrogenases. Among many species of lactic acid bacteria, strains belonging to the genus *Lactobacillus*, *Lactococcus* and *Enterococcus* are capable of producing L-isomer of lactic acid. The new strain of *Enterococcus mundtii* QU 25 capable of homolactic fermentation of D-xylose was isolated and characterized by Abdel-Rahman et. all. [1, 2]. Authors claimed its application for fermentation of hydrolysates made from lignocellulosic materials to optically pure L-lactic acid. Strain of *Enterococcus faecalis* RKY1 was successfully applied for L-lactic acid fermentation of wood hydrolysates supplemented with corn steep liquor and yeast extract [3]. Pessione et all. described new strain of *Enterococcus faecium* LLAA-1 actively producing L-lactic acid from hexoses however D-xylose was not fermented [4]. Strain CBRD01 of *Enterococcus faecalis* quickly produced up to 182 g/l of L-lactic acid after short fed-batch fermentation on glucose. However it was unable to ferment D-xylose and L-arabinose [5]. Efficient fermentation of D-xylose to the L-lactic acid without formation of acetate and ethanol requires new microorganisms capable of conducting of such production process. The aim of work was isolation and characterization of lactic acid bacteria fermenting D-xylose and producing lactic acid without or with minute quantities of by-products.

Elzbieta Oltuszak-Walczak, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, 90-924 Lodz, Wolczanska 171/173, Poland (corresponding author's phone: +4842 6313486; e-mail: elzbieta.oltuszak-walczak@p.lodz.pl).

Piotr Walczak, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, 90-924 Lodz, Wolczanska 171/173, Poland (e-mail: piotr.walczak@p.lodz.pl).

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II. Materials and methods

A. Strain isolation and characterization

Eight strains assigned as 1B1, 2B2, Can1, KR2, KR12, KD12, KD31, K4, were isolated from self-fermented vegetables and plant biomass. They were identified with API 20 STREP tests Biomérieux, France, as *Enterococcus faecium*. All isolates were able to grow at 44^{o}C in the Slanetz medium giving characteristic dark-pink colonies. Their biochemical profiles were evaluated with API 50CH biochemical test. Strain identification was confirmed genetically by sequencing of 16S rRNA gene.

B. Cultivation conditions

The ability of strains to ferment D-xylose were evaluated by fermentation tests carried out at 42^{o}C for 72 h with gentle mixing (100 rpm) in 250 ml Erlenmayer flasks containing 100 ml of medium and secured from oxygen access by screw caps. Fermentation medium contained in g/l: D-xylose - 50.0, $CaCO_3$ - 28.0, Yeast extract - 5.0, KH_2PO_4 - 2.6, $(NH_4)_2SO_4$ - 2.5, $MgSO_4 \cdot 7H_2O$ - 0.2, $MnSO_4 \cdot H_2O$ - 0.05, Thiamine·HCl 250 μg/l, Biotin 50 μg/l in distilled water. Calcium carbonate present in the medium served as neutralizing agent stabilizing pH at the level of 5.4 - 5.6. Flasks were inoculated using 5% inoculum prepared from 24 h culture of tested strains in modified MRS broth.

C. Analytical methods

Flasks were sampled at 24 h intervals and biomass, D-xylose, lactic acid, acetic acid, ethanol concentrations were estimated using optical density measurement and HPLC analysis. Ion exclusion chromatography (IEC) technique was used to determine concentrations of substrate and fermentation products. Samples were analysed with Surveyor Plus chromatograph on Aminex HPX 87H column (Bio-Rad). 10 µL of the sample was loaded on the column operated at 60°C. The mobile phase (5 mM sulphuric acid) flow velocity was equal to 0.6 mL/min. The Surveyor RI Plus (Thermo Scientific) detector measuring the refractive index of the eluent was used for quantitative analysis of fermentation products.

Concentration of D-xylose was measured with chemical method using 3,5-dinitrosalicylic acid reagent. Concentration of lactic acid was estimated by indirect method using complexometric titration with EDTA of calcium ions released into the medium from insoluble $CaCO_3$ by the reaction with formed acid. Chiral purity of lactic acid was evaluated with D-/L-Lactic Acid Assay Kit "K-DLATE" (Megazyme International Ireland).

III. Results

Fermentation profiles of isolated strains evaluated with API 50CH test were very wide and differed between each other. The individual strains were able to ferment glycerol, three pentoses, six hexoses, two polyols, two methylated hexoses, N-acetylglucosamine, four beta-glucosides, nine disaccharides and gluconate. All strains fermented D-xylose, L-arabinose, glucose and celobiose most abundant sugars present in hydrolysates of lignocellulosic materials.

Figure I shows dynamics of lactic acid fermentation by eight strains of *Enterococcus faecium* in the fermentation medium containing 50 g/l of D-xylose. Seven strains were actively grown from the beginning of the fermentation process whereas one strain KR2 was characterized with delayed growth.

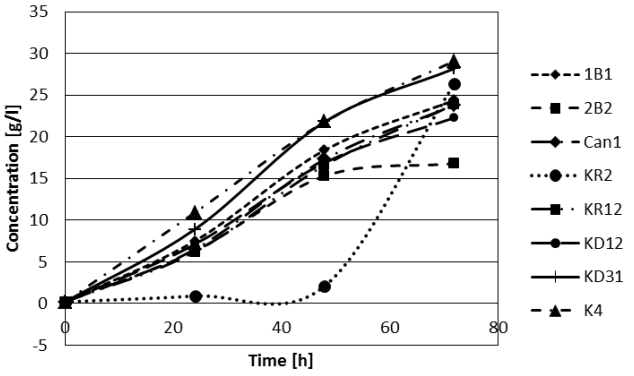

Fig. 1 Dynamics of lactic acid production by strains of *Enterococcus faecium*

Lactic acid concentration in the growth medium after 72 h of fermentation was between 16.8 and 29.1 g/l. Strains KD31 and K4 produced more than 28 g/l of lactic acid whereas 2B2 only 16.8 g/l. Production between 22.3 and 26.4 g/l of lactic acid was characteristic for remaining strains.

Table I shows values of final concentration of lactic acid, biomass and D-xylose after 72 h of fermentation as well as the yield of product calculated for the utilized substrate. The yield of lactic acid varied between 53.7 to 70.5 %. Biomass concentration after 72 h of fermentation ranged between 1.05 and 1.47 g/l depending on strain. The relatively low concentration of biomass in the fermentation medium and presence of substantial amounts of unused substrate was due to the limited amount of yeast extract in the fermentation medium. This phenomenon may be possibly overcome by increasing concentration of yeast extract. All strains mainly produced L-form of lactic acid being 93.8% for KR2 and almost 100% for strains Can1, KR12, KD31 and K4.

Figure 2 shows concentration of by-products acetic acid and ethanol in the fermentation medium after 72 h of cultivation. The range of acetic acid production was between 0.02 and 0.07 g/l being highest for strain KD31. Ethanol concentration at the end of fermentation varied between 0.01 and 0.1 g/l. Both metabolites were produced in relatively small quantities and from the point of view of product recovery they may be neglected. Production of L-lactic acid from lignocellulosic materials is of great interest since this substrate is not competing with food and feed resources and is widely available for low cost.

TABLE I
CONCENTRATION OF LACTIC ACID, D-XYLOSE, BIOMASS, CHIRAL PURITY OF LACTIC ACID AND YIELD OF PRODUCT CALCULATED FOR UTILIZED SUBSTRATE AFTER 72 H OF FERMENTATION

Strain	Lactic acid [g/l]	D-xylose [g/l]	Chiral purity [% L-form]	Yield [%]	Biomass [g/l]
1B1	24,4	11.0	98.0	62.6	1.15
2B2B	16.8	18.7	96.7	53.7	1.05
Can1	23.8	13.4	100	65.1	1.21
KR2	26.4	9.8	93.8	65.7	1.40
KR12	23.9	14.4	100	67.0	1.15
KD12	22,3	16.5	98,7	66.6	1.38
KD31	28.2	10.2	100	70.1	1.47
K4	29,1	10.4	100	70.5	1.29

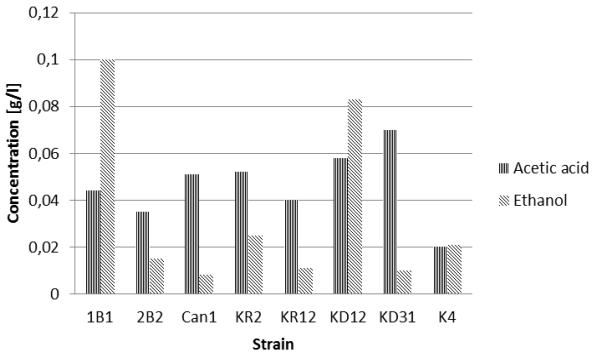

Fig. 2 Production of by-products, acetic acid and ethanol after 72 h of fermentation

IV. Conclusion

Comparative analysis of fermentation results showed that *Enterococcus faecium* strains KD31 and K4 were most suitable for

D-xylose fermentation to L-lactic acid. Further optimization of growth medium composition may increase fermentation dynamic, allow for complete utilization of substrate and shorten fermentation time.

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