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The limited deglucosylation process of β-glucosidase in *Bacillus cereus* H62L for biotransforming secoisolariciresinol diglucoside into mammalian lignans

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ABSTRACT

The amount of major lignans present in the alkaline extract of the defatted flaxseed (in mg/g) is as follows: secoisolariciresinol diglucoside (SDG, 109.80), *p*-coumaric acid glucoside (CAG, 70.21), ferulic acid glucoside (FAG, 68.81), secoisolariciresinol (SECO, 0.49), coumaric acid (CA, 0.37), and ferulic acid (FA, 0.85). When biotransformed, SDG yields two valuable mammalian lignans (MLG): enterodiol (END) and enterolactone (ENL). *Bacillus cereus* strain H62-L1 was isolated and tested for the process of bioconversion. Kinetic analysis revealed the sequential reaction SDG (A) → CAG (B) → END (C) → ENL (D) to be reversible at the first step with the generalized reaction type $A \underset{k_2}{\overset{k_1}{\rightleftarrows}} B \xrightarrow{k_3} C \xrightarrow{k_4} D$.

The obtained rate coefficients were as follows: $k_1 = 0.001–0.028\ h^{-1}$, $k_2 = 0.006–0.077\ h^{-1}$, $k_3 = 0.013\ h^{-1}$, and $k_4 = 0.002\ h^{-1}$, and the overall kinetic parameter $k_1/(k_2 + k_3)$ varied from 0.05 to 1.32, mostly not favoring the forward deglucosylation reaction. SDG with higher purity favored the forward reaction. In conclusion, to facilitate the production rate, we propose (i) using substrate SDG with high purity at a higher concentration; (ii) using an allosteric inhibitor to block the reverse reaction from SECO to SDG; or (iii) genetically modifying *Bacillus cereus* H62-L1.

1. Introduction

Lignans are produced via the shikimic acid pathway and are categorized as secondary plant metabolites [1,2]. Most of the lignans occur in form of glycosidic conjugates. The total lignan content in flaxseeds constitutes approximately 1.8% (w/w) [3]. Secoisolariciresinol diglucoside (SDG, $C_{32}H_{46}O_{16}$, MW: 686.7) (**I**), *p*-coumaric acid glucoside (CAG) (**V**), and ferulic acid glucoside (FAG) (**VI**) are the major lignans. Upon ingestion, SDG is deglucosylated by the β-glucosidase present in the intestinal microflora and yields free lignan secoisolariciresinol (SECO, $C_{20}H_{26}O_6$, MW: 362.4) (**II**), which is further consecutively dehydoxylated and demethylated to yield enterodiol (END, $C_{18}H_{22}O_4$, MW: 302.1) (**III**). On dehydration, Compound **III** further produces enterolactone (ENL, $C_{18}H_{18}O_4$, MW: 298.3) (**IV**) [4–6] (Figs. 1 and 2).

Type 2 diabetes is associated with an increase in oxidative stress, and SDG is effective in delaying the development of diabetes [7–9]. The downstream products of SDG, free END and ENL together with their corresponding glucosides, form a category of phenolics popularly named the mammalian lignans (MLG). MLG inhibit the aromatase responsible for the biotransformation of testosterone into estrogens [10]. When bound to estrogen receptors α (ERα) and β (ERβ), MLG evoke a strong antagonistic effect on estrogen [11,12]. MLG exhibit a diversity of bioactivities involving antioxidative, anti-atherosclerotic, anticancer, and anti-diabetes mellitus effects [8,9,13]. MLG stimulate the production of human sex hormone binding globulin (*h*-SHBG). The latter is able to entrap the

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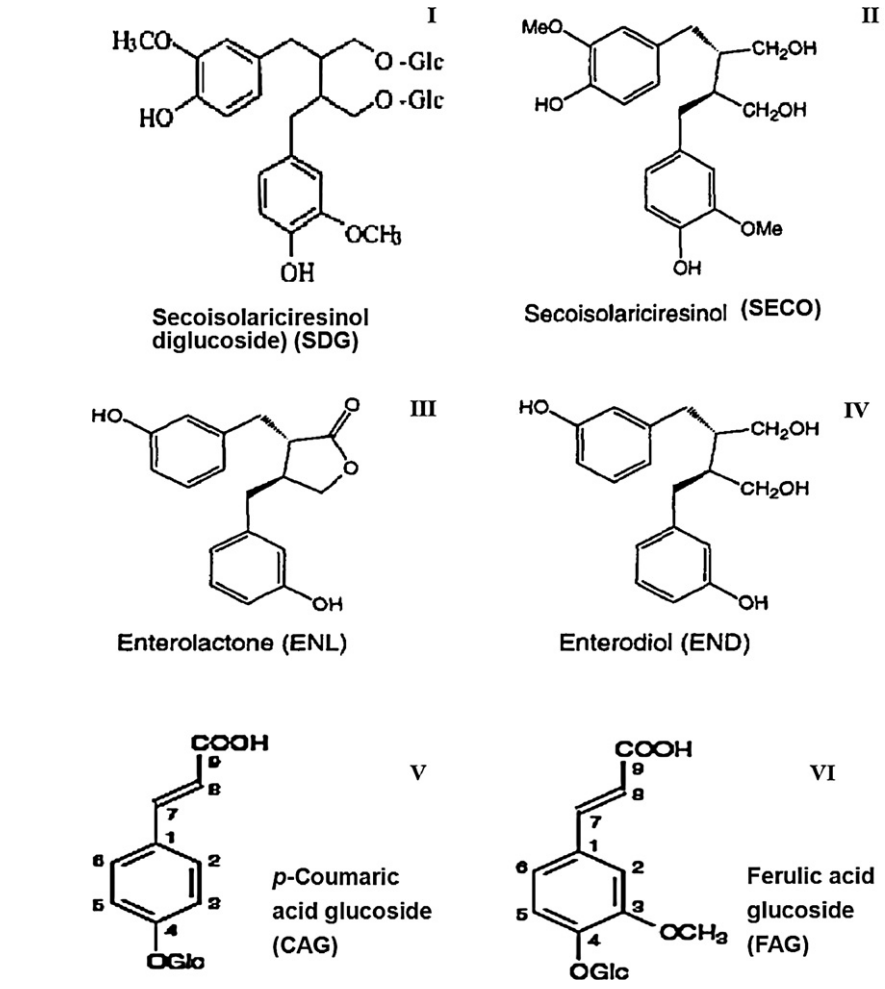

Fig. 1. The structure of main polyphenolics present in the desiccated defatted flaxseeds.

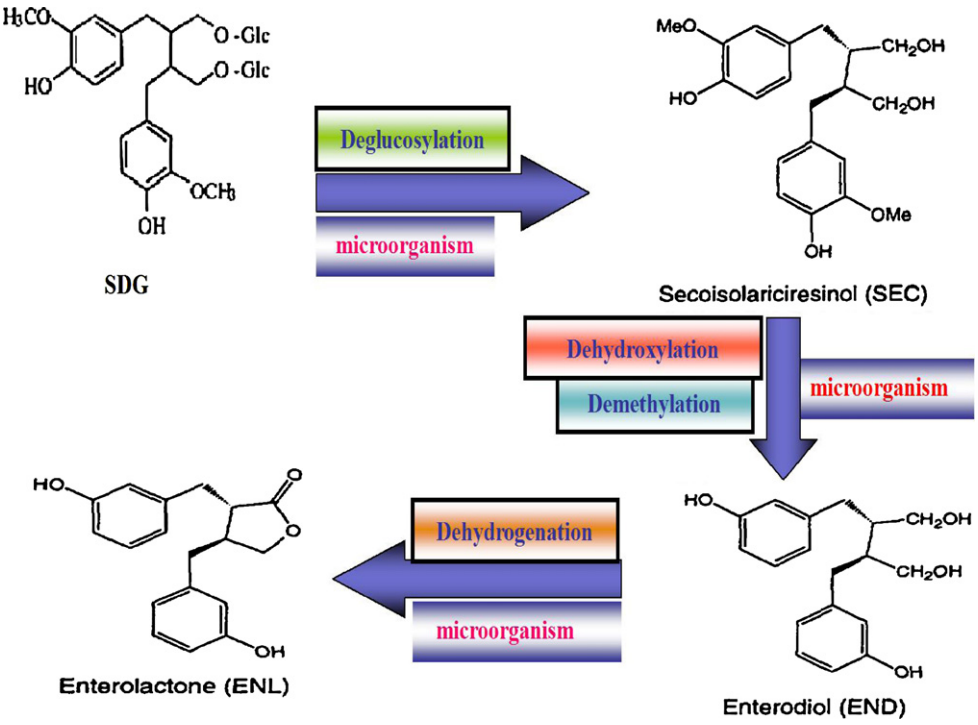

Fig. 2. The microbial biotransformation pathway from SDG to END and ENL.

Source: Modified from Clavel et al. [4].