



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N		A2	(11) International Publication Number: WO 00/17319
			(43) International Publication Date: 30 March 2000 (30.03.00)
(21) International Application Number: PCT/US99/21323 (22) International Filing Date: 16 September 1999 (16.09.99) (30) Priority Data: 60/100,937 18 September 1998 (18.09.98) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/100,937 (CON) Filed on 18 September 1998 (18.09.98) (71) Applicant (for all designated States except US): BOARD OF TRUSTEES operating MICHIGAN STATE UNIVERSITY [US/US]; East Lansing, MI 48824 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): FROST, John, W. [US/US]; 1621 Dobic Circle, Okemos, MI 48864 (US). (74) Agents: GIBBS, Barbara, S. et al.; Harness, Dickey & Pierce, P.L.C., P.O. Box 828, Bloomfield Hills, MI 48303 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published Without international search report and to be republished upon receipt of that report.	

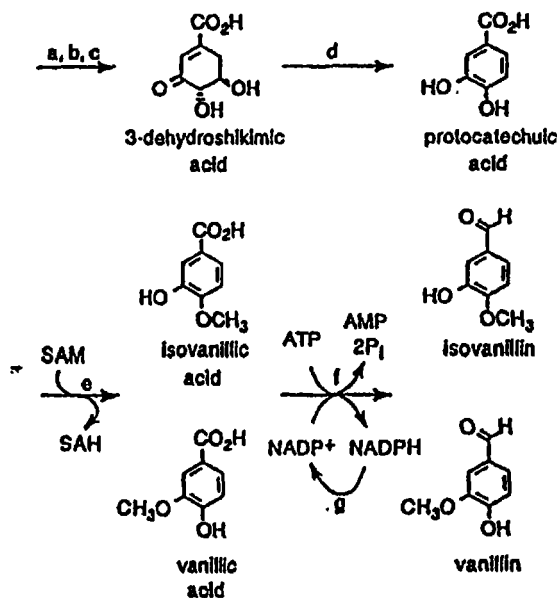


G00002

(54) Title: SYNTHESIS OF VANILLIN FROM A CARBON SOURCE

(57) Abstract

A bioengineered synthesis scheme for the production of vanillin from a carbon source is provided. The bioconversion methods of the present invention comprise the steps of microbe-catalyzed conversion of a carbon source to vanillic acid followed by enzyme-catalyzed reduction of the vanillic acid to produce vanillin. As shown in the synthesis scheme of Figure 2, the microbe-catalyzed conversion step of the present invention requires five enzymes which are provided by a recombinant microbe. In a preferred embodiment, the recombinant microbe is *Escherichia coli* designed to cause dehydration of 3-dehydroshikimic acid and regioselective methylation of the resulting protocatechuic acid. The enzyme-catalyzed reduction step of the present invention comprises the reduction of vanillic acid to vanillin by aryl-aldehyde dehydrogenase.



^a 3-deoxy-D-arabino-heptulosonic acid 7-phosphate synthase (*aroF*); ^b 3-dehydroquinate synthase (*aroB*); ^c 3-dehydroquinate dehydratase (*aroD*); ^d 3-dehydroshikimate dehydratase (*aroZ*); ^e catechol-O-methyltransferase (*COMT*); ^f aryl-aldehyde dehydrogenase; ^g D-glucose 6-phosphate dehydrogenase, D-glucose 6-phosphate; SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine.



162162