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PRODUCTION OF PETROSELINIC ACID FROM CELL SUSPENSION CULTURES OF *CORIANDRUM SATIVUM*

SUK-WEON KIM, MI-KYUNG PARK, KYUNG-SOOK BAE, MOON-SOO RHEE and JANG-RYOL LIU*

Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology, KIST, P.O. Box 115, Yusung, Taejon, 305-600, Korea

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Key Word Index—*Coriandrum sativum*; Umbelliferae; petroselinic acid; cell suspension culture.

Abstract—The fatty acid pattern and petroselinic acid content in calli, somatic embryos and cell suspension cultures of *Coriander sativum* are described. The petroselinic acid content was 0.15–0.23 mg g^{-1} fresh wt of calli, somatic embryo and cell suspension cultures and varied little with callus origin and culture conditions. The ratio of unsaturated:saturated fatty acid was *ca* 4:1 in calli. However, the unsaturated fatty acid content was greater in somatic embryos and cell suspension cultures. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Petroselinic acid is an unusual fatty acid that occurs primarily in seeds of some species of the Umbelliferae, Araliaceae and Garryaceae [1]. The structure of petroselinic acid differs from that of oleic acid, a common plant fatty acid, in the position of its double bond. Petroselinic acid is of potential industrial significance because of the unsaturation at C-6. Through chemical cleavage at its double bond, petroselinic acid can be used as a precursor of both lauric acid, which is a component of detergents and surfactants, and adipic acid, which is the monomeric component of nylon 66. Monounsaturated fatty acids of plants are typically derived from desaturation of C_{16} and C_{18} saturated fatty acids bound either to the acyl carrier protein or to glycerolipids [2, 3].

Coriander (*Coriandrum sativum*) is an herbaceous annual plant but despite its commercial importance only a few *in vitro* culture studies have been published. These studies include a report of successful micropropagation through shoot-tip cultures [4] and plant regeneration via somatic embryogenesis [5]. The present study describes the production of petroselinic acid from calli, somatic embryos and cell suspension cultures of coriander.

RESULTS AND DISCUSSION

Fatty acid composition and petroselinic acid contents are shown in Table 1. The major fatty acids produced in coriander were palmitic, oleic and α- and γ-linoleic acid. The patterns of fatty acid production in calli derived from various explants, including cotyledon, hypocotyl, root and zygotic embryo, were all similar. However, the pattern of fatty acid production in cell suspension cultures was slightly different from that of calli. Petroseliadic acid, elaidic acid and *cis*-vaccenic acid, which were the common unsaturated fatty acids produced in calli, were not detected in cell suspension cultures. Instead, 16:0 *anteiso*, 16:1w9c and an unindentified fatty acid were produced. Furthermore, the unsaturated:saturated fatty acid ratio was *ca* 4:1 in calli. However, the unsaturated fatty acid content was greater in cell suspension cultures and somatic embryos. The petroselinic acid content was 0.15–0.23 mg g^{-1} fresh wt in calli, somatic, embryo, and cell suspension cultures. The petroselinic acid content did not alter significantly, regardless of callus origin or culture conditions.

Petroselinic acid comprises as much as 85% of the total fatty acid content of Umbelliferae seeds, but it is virtually absent from leaves and other tissues of these plants [1]. Petroselinic acid is metabolized and accumulated in the developing endosperm of some Umbelliferae species, including coriander and carrot [6]. Petroselinic acid is the product of acyl–acyl carrier protein (ACP) desaturase. This polypeptide is highly expressed in seed. However, it is absent in tissues that do not synthesize petroselinic acid, including leaves and roots of coriander [7]. In this study, however, a small amount (*ca* 0.20 mg g^{-1} fresh wt) of petroselinic acid was produced in calli, somatic embryos and cell suspension cultures. The yield of petroselinic acid in calli was much lower than that of seed endosperm in coriander [6]. This result indicates that petroselinic acid can be continuously synthesized at a low level in cultured cells.

Recent increased interest in plant fatty acid metabo-

*Author to whom correspondence should be addressed.

Table 1. Yield of petroselinic acid produced in calli and cell suspension cultures of coriander and total content of saturated and unsaturated fatty acids. Origin of callus: zygotic embryo (ZE), root (R), hypocotyl (H), cotyledon (C), somatic embryo (SE) and cell suspension cultures (S)

Callus origin	% Saturated fatty acid	% Unsaturated fatty acid	% Petroselinic acid	Yield (mg g^{-1}) of petroselinic acid
ZE	19.0	81.0	0.29	0.20
R	18.2	81.8	0.33	0.19
H	21.4	78.6	0.46	0.26
C	20.0	80.0	0.25	0.15
SE	13.9	86.1	0.32	0.23
S	13.3	86.7	0.3	0.23

lism has been stimulated by the potential to design new oilseed crops which produce higher-value oils. Modification of the fatty acid composition of oil seeds of transgenic plants has been accomplished in soybean, canola and other species by introduction of new genes, and by suppressing existing activities with antisense technology [7, 8]. Coriander cell suspension cultures developed in this study can be used for cloning cDNAs relevant to the biosynthesis of petroselinic acid.

EXPERIMENTAL

Plant materials. Seeds of coriander (*C. sativum* L.) were surface-sterilized with 70% EtOH for 1 min and immersed in a 0.4% NaOCl soln or 10 min. They were then rinsed ×4 with sterile dist. H_2O. Transversely sliced cotyledon, hypocotyl and root segments (*ca* 2.5 mm long) of 2-week-old seedlings and intact zygotic embryos (*ca* 1.5 mm long) were placed onto Murashige and Skoog's [9] medium containing 100 mg l^{-1} myo-inositol, 0.4 mg l^{-1} thiamine · HCl, 3% sucrose, 0.4% Gelrite and 1 mg l^{-1} 2,4-D (MS1D). After 4 weeks' culture, calli which formed on cut surfaces of explants were subcultured on MS medium with 1 mg l^{-1} 2,4-D (MS1D) every 4 weeks. To establish cell suspension cultures, subcultured hypocotyl-derived embroygenic calli (*ca* 1 g) were placed in a 300 ml Erlenmeyer flask containing 50 ml of liquid MS1D medium [5]. Cell suspension cultures were maintained on a gyratory shaker (100 rpm) and subcultured every 2 weeks. All cultures were maintained at 25° in the dark.

Analysis of fatty acids. Fatty acid patterns and the petroselinic acid contents in calli derived from various explants, including cotyledon, hypocotyl, root, zygotic embryo and cell suspension cultures, were analysed by the method of ref. [10]. Fatty acid Me esters were prepared from calli and somatic embryos after 4 weeks' culture, on MS1D medium, and from 2-week-old cell suspension cultures (fresh wt 200 mg), by heating at 100° for 30 min in a saponification soln (15% NaOH in 50% MeOH). Me ester formation of fatty acids was performed by addition of methanolic HCl (6 N HCl–MeOH), followed by heating at 80° for 10 min. Resulting Me esters were transferred from the aq. phase to an organic phase by solvent (hexane–MTBE) extraction, washed with a dilute NaOH soln and analysed by GC using a 25 m × 0.2 mm Ultra 2 silica capillary column and oven temp. programming from 170° to 270° at 5° min^{-1}. Quantitative analysis of petroselinic acid was carried out by comparing the peak areas of the samples with those of the authentic compound.

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