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Abstract

Ionizing radiation has inevitably become a health concern due to exposure from natural sources like space travel and from artificial sources like diagnostic and therapeutic medical usage. By definition, radioprotectors are chemical compounds that have the ability to reduce the biological effects of ionizing radiation on normal tissues, including lethality, mutagenicity and carcinogenicity. The radioprotective agents prevent or lessen the damage and lethal effects of acute radiation exposure. Isoflavones possess a variety of biological properties namely anti-oxidant, anti-cancer, anti-inflammatory, immuno-stimulative, anti-biotic and anti-fungal. Here, we review the state of the art production technology using microbial systems as an alternative for development of radioprotective agent. Thus, this system appears as an attractive production alternative for production of radioprotectors. This issue provides promising opportunities for the development of new methodologies and technologies in biosynthetic engineering that can be utilized in several other biosynthetic engineering projects related to high-value products. It could potentially be a great strategy for overproduction of isoflavones from biosynthetic engineering using a combinatorial assembly. This artificial biosynthesis is a tool for the production of a radioprotective agent in smart microorganism.

Keywords Ionizing radiation, Radioprotective agent, Microorganism, Biosynthetic engineering

Importance & Biological effects of ortho-dihydroxyisoflavones

Daidzein (7,4'-dihydroxyisoflavone) and genistein (7,5,4'-trihydroxyisoflavone) are diphenolic phytoestrogen compounds found in numerous plants and soybeans. They have been reported to act as antioxidants, antimicrobials, free radical scavengers, metal chelators and antibacterial agents (Dixon et al., 2002). In addition, Isoflavones are known to have medicinal and chemopreventive activities in human health. Isolation and synthesis of isoflavones have become frequent research topics, due to their interesting biological activities (Foti et al., 2005). Recently, hydroxylated products of daidzein and genistein are a growing scientific interest for their health-related qualities. Hydroxylated products have potent antioxidant properties that contribute to their cholesterol lowering effect, cardiovascular protection, anti-tumor effect, and anti-carcinogenic properties (Rufer et al., 2006). Furthermore, compounds with the ortho-dihydroxyl group are known to exhibit anti-inflammatory and anti-allergenic activity (Rufer and Kulling, 2006) and to express anti-carcinogenic properties due to the inhibition of protein tyrosine kinases (Akiyama et al., 1987), as a potent tyrosinase inhibitor (Chang et al., 2005) and as active inhibitors of lipoxygenases (Voss et al., 1992). Hydroxylated isoflavones are invaluable components for lowering incidence of cancer-related diseases (Klus and Barz, 1995). Klus and Rufer reported that antioxidant capacity of hydroxylated compounds has high activity than original isoflavones, which might affect their biological properties (Klus and Barz, 1995; Rufer and Kulling, 2006). Also, Rufer group showed *ortho-dihydroxyisoflavones* such as 7,8,4'-trihydroxyisoflavone, 6,7,4'-trihydroxyisoflavone and 7,3',4'-trihydroxyisoflavone exhibited more effective in biological activity by the oxygen radical absorbance capacity (ORAC) assay as well as the oxidation of low-density lipoproteins (LDL) (Rufer and Kulling, 2006; Rufer et al., 2006).

Bottleneck & Alternative for production of ortho-dihydroxyisoflavones

Regio-specific hydroxylation of aromatic compounds by chemical synthesis is difficult and involves diverse reaction steps. The conversion of a carbon-hydrogen to a carbon-hydroxyl bond is one of the key features of the oxidative metabolism of many aromatic compounds (Holland and Weber, 2000). Regio-specific microbial hydroxylation at a non-activated carbon atom of aromatic compounds is attractive and remarkable biosynthesis. The introduction of hydroxyl groups into isoflavones by the use of microorganisms represents an attractive alternative to conventional chemical synthesis. The selective modification of isoflavones by microorganisms is a powerful approach.

Biosynthetic Engineering

Biosynthetic engineering using combinatorial assembly

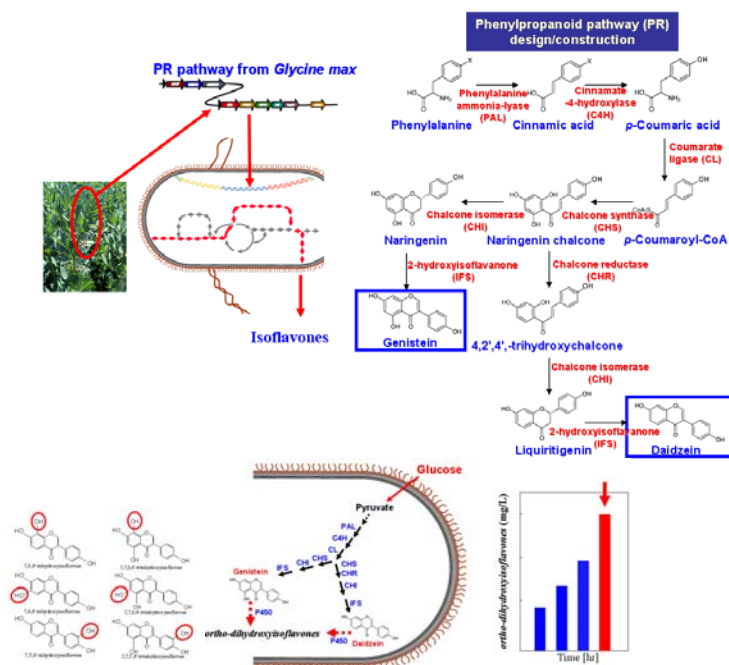


Fig. 2 Biosynthetic engineering for production of *ortho-dihydroxyisoflavones*

Current state & Perspectives

Klus and Barz reported five tempe-derived bacterial strains identified as *Micrococcus* or *Arthrobacter* species were shown to transform the soybean isoflavone daidzein to *ortho-dihydroxyisoflavones* (6,7,4'-trihydroxyisoflavone and 7,8,4'-trihydroxyisoflavone) by regio-specific hydroxylation reactions (Klus and Barz 1995).

Roh reported the screening of bacterial whole cells was performed for regio-specific hydroxylation of daidzein and genistein. Among the strains examined, *Streptomyces avermitilis* MA-4680 showed high ortho-hydroxylation activity to produce 7,3',4'-trihydroxyisoflavone and 5,7,3',4'-tetrahydroxyisoflavone from daidzein and genistein, respectively. Roh showed using 100 g/L of *S. avermitilis* wet cell mass, 7,3',4'-trihydroxyisoflavone and 5,7,3',4'-tetrahydroxyisoflavone were produced to the level of 2.03 mg/L in batch system with 0.5 mM of daidzein and genistein, respectively. This biotransformation result demonstrates that ortho-hydroxylated isoflavones exhibiting more potent antioxidant activity can be produced on a large scale using microbial biotransformation (Roh et al., 2009).

Pandey and Roh showed an example for the overproduction of 7,3',4'-trihydroxyisoflavone with identification of the P450 gene responsible for the daidzein biotransformation in *S. avermitilis* strain. In a 7 L (w.v. 3 L) jar fermentor, the recombinant *S. avermitilis* in batch system produced 112.5 mg of 7,3',4'-trihydroxyisoflavone (i.e., 29.5%, yield) from 381 mg of daidzein in 15 h (Pandey and Roh, 2010).

It might be great strategy for overproduction of *ortho-dihydroxyisoflavones* from biosynthetic engineering using **combinatorial assembly by PR design/construction**.

Thus, this system appears as attractive production alternatives for commercial production of these high-value chemicals. This issue provides a golden opportunity for the development of new methodology and technology in biosynthetic engineering that can be utilized in several other biosynthetic engineering projects related to high-value product such as radioprotective agents.