Electronic Acknowledgement Receipt

EFS ID:	1299535
Application Number:	60864798
International Application Number:	
Confirmation Number:	1654
Title of Invention:	Composition containing prebiotic mix and lactic acid producing bacterium delivering isoflavones to small intestines and producing selective estrogen receptor site modulators (SERM) including daidzein metabolites equol and O-desmethylangolensin (ODMA). The composition is effective for the prevention and alleviation of cell damage by scavenging oxygen free radicals through the up-regulation of mammalian antioxidant enzyme gene expression.
First Named Inventor/Applicant Name:	Robert G Kavanaugh
Correspondence Address:	Agrigenic Food Company Attn: Robert G. Kavanaugh 5152 Bolsa Avenue STE 101 - Huntington Beach CA 92649-1047 US 714-899-3477 robert_kavanaugh@hotmail.com
Filer:	Robert Kavanaugh
Filer Authorized By:	
Attorney Docket Number:	IsoSproutPlex-Provisional
Receipt Date:	08-NOV-2006
Filing Date:	
Time Stamp:	09:26:57
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment was successfully received in RAM	$ 100
RAM confirmation Number	908

Deposit Account	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)	Multi Part /.zip	Pages (if appl.)
1		Patent.pdf	60363	yes	3
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Abstract		1	1	
	Claims		2	3	
Warnings:					
Information:					
2	Specification	description.pdf	136127	no	4
Warnings:					
Information:					
3	Fee Worksheet (PTO-875)	fee-info.pdf	8382	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	204872		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

Abstract
The present invention provides a prebiotic medium containing as a essential ingredient thereof, dehydrated sprout powders from glycine max, zea mays and triticum.turgidum durum with a probiotic premix containing lactic acid bacteria belonging to the genus lactococcus and genus bifidobacteria and having an ability to by-pass digestive acids in the stomach and to be delivered to the small intestines, resulting in endogenous reactions with isoflavones including genistein, glycosides, daidzein, and metabolites of same including dihydrodaidzein and equol. Such a composition is effective for the prevention and alleviation of cell damage by scavenging oxygen free radicals through the up-regulation of mammalian antioxidant enzyme gene expression.

Claims

1. A prebiotic mix containing dehydrated sprout powders from glycine max, zea mays, and triticum.turgidum, cyanocobalamin, d-alpha tocopherol, and a probiotic mix described in claim 2, and prepared as described in claim 4 for delivery through the acids of the stomach and into the small intestines causing endogenous reaction of isoflavones consisting of genistein, daidzein, biochanin A, formononetin, O-desmethylangolensin, glycitin, and equol, in an amount sufficient to produce an up-regulation of mammalian superoxide dismutase and catalase gene expression endogenously. Specifically, copper zinc SOD known as cellular SOD or SOD1, and manganese superoxide dismutase or SOD2, and extra-cellular SOD or SOD3.

2. A probiotic compound, an equol-producing lactic acid bacteria-containing composition comprising, as an essential component thereof, a lactic acid bacterial strain belonging to the genus lactococcus and bifidobacteria having an ability to utilize at least one isoflavone compound selected from the group consisting of genistein, daidzein glycosides, daidzein, and dihydrodaidzein to produce the metabolites equol and O-desmethylangolensin (ODMA) by delivering and promoting the growth of the equol and ODMA producing intestinal bacteria.

.

3. The composition according to claim 2, wherein said lactic acid bacterial strain belonging to the genus Lactococcus is one or more of the following: Lactococcus Acidophilus, Lactococcus Rhamnosus, Lactococcus Salivarius, Lactococcus Plantarum; and belonging to the genus Bifidobacteria is one or more of the following: Bifidobacteria. Longum, Bifidobacteria Bifidum.

4. The administration of a therapeutically-effective concentration of the prebiotic mix described in 1 and strains of lactic acid-producing bacteria described in 2 within a pharmaceutically-acceptable carrier suitable for administration to the gastrointestinal tract of a mammal, wherein said lactic acid-producing bacteria is reacted with said prebiotic mix, resulting in increased promotion and growth of the equol and ODMA producing intestinal bacteria, in order to maximize endogenous production and absorption of selective estrogen receptor site modulators (SERM) that bind to estrogen receptor site beta (ER beta), with a higher affinity than to estrogen receptor site alpha.

.

5. The composition according to claim 4 of the SERM consist of the isoflavones: genistein, daidzein, biochanin A, formononetin, O-desmethylangolensin, glycitin, and the endogenously produced isoflavone metabolites equol and ODMA.

6. The composition of the pharmaceutically-acceptable carrier in claim 4 is a cellulose coating selected from the group consisting of carboxymethylcellulose sodium methylcellulose, ethylcellulose, ethylmethylcellulose, hydroxy-ethylcellulose, or microcrystalline cellulose; and a pharmaceutical lubricant from the group consisting of stearic acid, magnesium stearate, aluminum magnesium silicate, sodium silicate, silicon dioxide and colloidal silicon dioxide.

7. A method of producing equol and O-desmethylangolensin (ODMA) endogenously comprising of the steps of delivering the lactic acid bacterial strains belonging to the genus Lactococcus and genus Bifidobacteria described in 3 and the prebiotic mix described in 1 by using the pharmaceutically-acceptable carrier defined in claim 6.

8. A method according to claim 7, wherein the premix compound is defatted, granulated soybean meal, defatted soybean flour or defatted soy sprout powder.

9. A method according to claim 4, wherein the SERM absorption results in up-regulation of superoxide dismutase gene expression.

10. The composition of the superoxide dismutase in claim 9 is the manganese superoxide dismutase (Mitochondrial SOD or SOD2) and Extra-Cellular superoxide dismutase (EC SOD or SOD3).

11. A method according to claim 1, wherein the premix in claim 8 blocks the absorption of iron (Fe) resulting increased cellular superoxide dismutase (cu/zn SOD or SOD1).

Description

FIELD OF THE INVENTION

[0001] The present invention provides a prebiotic medium containing as a essential ingredient thereof, dehydrated sprout powders from glycine max, zea mays and triticum.turgidum durum with a probiotic premix containing lactic acid bacteria belonging to the genus lactococcus and genus bifidobacteria and having an ability to by-pass digestive acids in the stomach and to be delivered to the small intestines, resulting in endogenous reactions with isoflavones including genistein, glycosides, daidzein, and metabolites of same including dihydrodaidzein and equol. Such a composition is effective for the prevention and alleviation of cell damage by scavenging oxygen free radicals through the up-regulation of mammalian antioxidant enzyme gene expression

BACKGROUND OF THE INVENTION

[0002] Studies with phytoestrogens, in particular soy-derived isoflavones, indicate that a number of health benefits may be derived from naturally occurring plant components with estrogenic properties. Among the noted benefits are reductions in the incidence of breast and prostate cancer as well as the reduction in cardiovascular disease incidence [*Guarner F, Malagelada JR. Gut flora in health and disease. Lancet 361:512–519, 2003*.]. The cardiovascular protective effect of isoflavones has been linked to their antioxidant activity based, in part, on the oxidative resistance of low-density lipoprotein (LDL) obtained from subjects consuming these products [*Puupponen-Pimia R, Aura AM, Karppinen S, Oksman-Caldentey, KM, Poutanen K. Interactions between plant bioactive food ingredients and intestinal flora—effects on human health. Biosci Microflora 23:67–80, 2004*]. However, the mechanism by which isoflavones exert their antioxidant action remains open to debate for several reasons. Among these is the relatively weak radical quenching action of these agents, making then far weaker antioxidants in vivo than compounds such as vitamin E.

[0003] The mechanism by which isoflavones exert antioxidant effects in vivo have been compared to estrogen; however, it is unclear if similar receptor-mediated pathways are involved or if other cell-mediated events are needed to exert antioxidant activity. Among the phytoestrogens, the isoflavones, coumestans and lignans [3] have received much attention with many studies investigating isoflavone-rich soy protein. Soy protein is mainly composed of genistein and daidzein, however, a metabolic derivatives of daidzein—equol and O-desmethylangolensin (ODMA)—are particularly potent antioxidants. Equol, an isoflavan, is an isoflavone metabolite that is formed in the intestinal tract in only a subset of the population [4,5]. This may be an important determinant for the response of subjects to the protective effects of soy protein. It has been proposed that the ability to form and excrete equol is linked to the beneficial effects of isoflavone intake based on a regulatory effect on endogenous hormones [6]. Thus, lowered breast cancer risk was evident largely in equol excretors who had a more favorable hormonal profile, as opposed to merely reflecting increased isoflavone intake. In addition to estrogenic effects of isoflavones and their metabolites on the estrogen

receptor system [7], compounds like equol also behave as potent LDL antioxidants [8], and a stronger inhibitory effect on LDL oxidation than genistein or daidzein may contribute significantly to atherosclerotic effect of phytoestrogens [9]. Increasing evidence suggests that oxidative modification of LDL is an important contributing factor to the progression of atherosclerosis [10]. There remains, however, uncertainty about the manner by which LDL is oxidized in vivo. The various forms of modified LDL that have been identified and the heterogeneity of LDL particles found in plasma suggests that multiple mechanisms for modification may exist. Lipoxygenase [11], myeloperoxidase [12], and nitric oxide synthase (NOS) [13] represent three enzymes that mediate LDL oxidation by vascular cells, but the contributions of transition metals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are also notable [14]. Nitric oxide (NO) is also a potent LDL antioxidant [15] but the amount of free NO produced, relative to its reaction fates with other agents (notably ROS), influences its anti- vs. pro-oxidant actions [16]. The role of RNS in conferring antioxidant or pro-oxidant effects has received much attention because NO is a potentially reactive radical species that can be produced in variable amounts under pathological conditions. Combined with its vasoactive and signal-transducing properties, NO or derived RNS are candidate oxidants that can modify LDL as determined by the extent of interaction with ROS. In this report, we describe the oxidation of LDL by cultured monocyte/macrophages and the effects of equol (an isoflavan metabolite with high antioxidant activity) on the production of electronegative LDL (LDL_{-}), a marker of cell-mediated LDL modification. Because the vessel wall generates modified LDL and cell-mediated LDL oxidation is widely used as an in vivo surrogate for oxidation by vascular tissues, we investigated the effects of equol on ROS and RNS generation by J774 monocyte/ macrophages. This investigation focused on NO formation by inducible NOS (iNOS) and O2 production via reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and related enzymes in J774 macrophages, and the consequences of altered ROS/RNS formation on LDL modification.

References: [3] Kurzer, M. S.; Xu, X. Dietary phytoestrogen. Annu. Rev. Nutr. 17:353–381; 1997. [4] Morton, M. S.; Wilcox, G.; Wahlqvist, M. L.; Griffiths, K. Determination of lignans and isoflavonoids in human female plasma following dietary supplementation. J. Endocrinol. 142:251–259;1994. [5] Kelley, G. E.; Joannou, G. E.; Reeder, A. Y.; Nelson, C.; Waring, M. A. The variable metabolic response to dietary isoflavones in humans. Proc. Soc. Exp. Biol. Med. 208:40–43; 1995. [6] Duncan, A. M.; Merz-Demlow, B. E.; Xu, X.; Phipps, W. R.; Kurzer, M. S. Premenopausal equol excretors show plasma hormone profiles associated with lowered risk of breast cancer. Cancer Epidemiol. Biomarkers Prev. 9:581–586; 2000. [7] Kuiper, G. G.; Carlsson, B.; Grandien, K.; Enmark, E.; Haggblad, J.; Nilsson, S.; Gustafsson, J. A. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138:863–870; 1997. [8] Hwang, J.; Sevanian, A.; Hodis, N. H.; Ursini, F. Synergistic inhibition of LDL oxidation by phytoestrogens and ascorbic acid. Free Radic. Biol. Med. 29:79–89; 2000. [9] Yamakoshi, J.; Piskula, M. K.; Izumi, T.; Tobe, K.; Saito, M.; Kataoka, S.; Obata, A.; Kikuchi, M. Isoflavone aglycone-rich extract without soy protein attenuates atherosclerosis development in

cholesterol-fed rabbits. J. Nutr. 130:1887–1893; 2000. [10] Lusis, A. J. Atherosclerosis. Nature 407:233–241; 2000. [11] Mehrabian, M.; Allayee, H.; Wong, J.; Shih, W.; Wang, X. P.; Shaposhnik, Z.; Funk, C. D.; Lusis, A. J. Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. Circ. Res. 91:120–126; 2002.
[12] Carr, A. C.; McCall, M. R.; Frei, B. Oxidation of LDL bymyeloperoxidase and reactive nitrogen species: reaction pathways and antioxidant protection. Arterioscler. Thromb. Vasc. Biol. 20: 1716–1723; 2000. [13] Rubbo, H.; Trostchansky, A.; Botti, H.; Batthyany, C. Interactions of nitric oxide and peroxynitrite with low-density lipoprotein. Biol. Chem. 383:547–552; 2002. [14] Napoli, C.; de Nigris, F.; Palinski, W. Multiple role of reactive oxygen species in the arterial wall. J. Cell. Biochem. 82:674–682; 2001. [15] Mashima, R.; Witting, P. K.; Stocker, R. Oxidants and antioxidants in atherosclerosis. Curr. Opin. Lipidol. 12:411–418; 2001.

[0004] Recently, however, doubts have been cast on the clinical efficacy of soy isoflavone and, instead, it is reported that equol as the active metabolite of soy isoflavone is a key factor in the expected efficacies in clinical application. Thus, several reports are available arguing that in breast cancer, carcinoma of the prostate, and climacteric and postmenopausal osteoporosis, the efficacy of soy isoflavone is surpassed by that of equol, the metabolite of soy isoflavone (D. Ingram et al., (1997) Lancet, 350, 990-994; A. M. Duncan et al., (2000) Cancer Epidemiology, Biomarkers & Prevention, 9, 581-586; C. Atkinson et al., (2002) J. Nutr., 32(3), 595S; H. Akaza et al., (2002) Jpn. J. Clin. Oncol., 32(8), 296-300; and S. Uchiyama et al., (2001) Ann. Nutr. Metab., 45, 113(abs)).

[0005] Moreover, many lectures were delivered on the subject of equol in the 4th International Symposium on the Role of Soy in Preventing and Treating Chronic Disease (San Diego, USA, 2001), and in December 2002 a comprehensive review of studies on equol was also reported. Thus, it is getting more or more accepted in academic circles that equol is the very entity of efficacies of soy isoflavone (K. D. R. Settchell et al., (2002) J. Nutr., 132, 3577-3584).

[0006] Furthermore, compared with soy isoflavone, equol is delivered to tissues such as the breast tissue and prostatic tissue with by far greater efficiency and, from this fact, the physiological significance of equol is endorsed (J. Maubach et al., (2003) J. Chromatography B., 784, 137-144; T. E. Hedlund et al., (2003) The Prostate, 154, 68-78).

[0007] Equol is produced by the intestinal flora and the involvement of individual difference in its production has been reported. It is also reported that equol producers among the Japanese account for about 50% (S. Uchiyama et al., (2001) Ann. Nutr. Metab., 45, 113 (abs)). Individuals who cannot produce equol are suspected to be lacking in equol-producing bacteria in their intestine. In such individuals, it is suspected that the expected antiestrogen and estrogenic-like effects may not be expected even if processed soybean foods are ingested. In order that the expected effects may be expressed in such

individuals, it seems to be a reasonable course of action to have them ingest equol-producing bacteria or equol as such.

[0008] Based on the above idea, the inventors had conducted intensive investigations on premixes and probiotic equol producing-bacteria suitable for the endogenous expression of said estrogenic-like effects, resulting in better absorption of selective estrogen receptor site modulators (SERM) and enhanced pathways to up-regulating antioxidant enzyme gene expression endogenously. The inventor applied for a provisional patent claiming inventions concerning these premixes, probiotic equol-producing strains of microorganisms and utilization of the microorganisms endogenously to up-regulate superoxide dismutase and catalase production via enhanced SERM production and absorption.