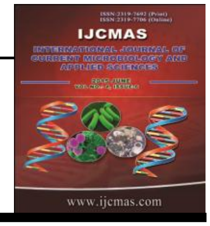

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Review Article

Inoculant / Elicitation Technology to Improve Bioactive/Phytoalexin Contents in Functional Foods

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A B S T R A C T

Plant bioactives are unique sources for pharmaceuticals, food additives, flavors, and other industrial materials. Since a great part of beneficial foods and food components are from plant origin, improving agricultural production of crops with a high bioactive content is of increasing interest. On the other hand, a great part of plant bioactives are secondary metabolites, and therefore synthesized by plants only to overcome environmental changes along the plant's biological cycle; hence, since secondary metabolism is inducible, bioactive levels change constantly on field produced foods. In view of the above, identification of biotic elicitors from microbial origin is a topic with increasing interest due to its potential application in cell and tissue culture to obtain functional ingredients, or even in fresh functional foods directly to consumers. In this sense the literature reports a number of studies in which elicitors from pathogenic microorganisms are used, but the use of beneficial microorganisms as plant growth promoting rhizobacteria (PGPR) or their metabolic elicitors are still to see an outstanding application on the field of functional foods. Two case studies are presented to illustrate the rationale of our working hypothesis, showing how the inoculants can improve contents of bioactives: one dealing with *Hypericum perforatum* hipericins, another one on *Glycine max*. with isoflavones

Keywords

Plant bioactives, Elicitation, Inoculation, Plant defense, Isoflavones, Flavonoids, Hipericins

Introduction

The primary role of diet is to provide sufficient nutrients to meet the nutritional requirements of an individual. There is now increasing scientific evidence to support the hypothesis that some foods and food components have beneficial physiological and psychological effects over and above the provision of the basic nutrients. Today, the research focus has shifted more to the identification of biologically active components in foods that have the potential to optimize physical and mental well-being and which may also reduce the risk of disease (Martin *et al.*, 2013) Many traditional food products including fruits, vegetables, soya, whole grains and milk have been found to contain components with potential health benefits. In addition to these foods, new foods are being developed to enhance or incorporate these beneficial components (bioactives) for their health benefits or desirable physiological effects (Baenas *et al.,* 2014).

Since a great part of these functional foods are from plant origin, most of these arise from agriculture, so obtaining high quality healthy foods or ingredients relays to a great extent in good agricultural practices. Furthermore a good knowledge of plant physiology and plant secondary metabolism will allow optimized agronomic production of high quality products. On the other hand, a great part of plant bioactives are secondary metabolites, and therefore synthetized by plants only to afford changes in environmental conditions along the plant's biological cycle (Capanoglu, 2010); hence, since secondary metabolism is inducible, bioactive levels change constantly on field produced foods (Burrit, 2013; Poulev *et al.,* 2013).

However, far from being an inconvenient factor, the variability of secondary metabolism due to environmental conditions appears as an excellent source of information to identify biotic or abiotic factors able to modify secondary metabolism directed to defensive compounds (Pieterse *et al.,* 2014); factors that trigger secondary metabolism are known as elicitors and the stimulated secondary metabolites are known as phytoalexins (Capanoglu, 2010; Boue *et al.,* 2009). At this point, knowledge of the plant-microorganisms interactions is of outstanding relevance to enhance those secondary metabolic pathways leading to increase target phytoalexins, contributing to naturally enriched functional foods.

In view of the above, identification of biotic elicitors from microbial origin is increasing due to its potential application in cell and tissue culture to obtain functional ingredients (Baenas 2014; Zhao *et al.,* 2005; Cai *et al.,* 2012), or even in fresh functional foods directly to consumers. So far, the literature reports a number of studies in which elicitors from pathogenic microorganisms are used (Radman *et al.,* 2003), but the use of beneficial microorganisms (Ramos Solano *et al.,* 2010a) or their metabolic elicitors are still to see an outstanding application (Gutiérrez Mañero *et al.,* 2012) on the field of functional foods.

In order to obtain the best out of elicitation, a few considerations ought to be outlined concerning the following issues: the precise nature of a functional food in the health and legal frameworks; plant secondary metabolism; physiological meaning of plant secondary metabolism and benefits for human health; beneficial microorganisms to obtain effective elicitors and finally, basic knowledge of plant-microorganism interactions to obtain the best of it. Each of these points are considered as a result of the rationale and integrated in the Results and Discussion section being the final conclusion the rationale and working procedure that is stated in this title

Functional Foods, what are they?

The concept of functional foods was born in Japan. In the 1980s, health authorities in Japan recognized that an improved quality of life must accompany increasing life expectancy for the expanding number of elderly people in the population if health care costs were to be controlled. The concept of foods that were developed specifically to promote health or reduce the risk of disease was introduced (Stein and Rodriguez-Cerezo, 2008).

Consumer interest in the relationship between diet and health has increased substantially in Europe. There is much greater recognition today that people can help themselves and their families to reduce the risk of illness and disease and to maintain their state of health and well-being through a healthy lifestyle, including the diet. Ongoing support for the important role of foods such as fruits and vegetables and wholegrain cereals in disease prevention and the latest research on dietary antioxidants and combinations of protective substances in plants has helped to provide the impetus for further developments in the functional food market in Europe.

Trends in population demographics and socio-economic changes also point to the need for foods with added health benefits. An increase in life expectancy, resulting in an increase in the number of elderly and the desire for an improved quality of life, as well as increasing costs of health care, have stimulated governments, researchers, health professionals and the food industry to see how these changes can be managed more effectively. There is already a wide range of foods available to today's consumer but now the impetus is to identify those functional foods that have the potential to improve health and well-being, reduce the risk from, or delay the onset of, major diseases such as cardiovascular disease (CVD), cancer and osteoporosis. Combined with a healthy lifestyle, functional foods can make a positive contribution to health and well-being.

Functional foods have already been defined by legislation in Europe. The European Commission Concerted Action on Functional Food Science in Europe proposed a working definition of functional food (Diplock *et al.,* 1999): a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. Functional foods must remain foods and they must demonstrate their effects in amounts that can normally be expected to be consumed in the diet: they are not pills or capsules, but part of a normal food pattern.

A functional food can be a natural food, a food to which a component has been added, or a food from which a component has been removed by technological or biotechnological means. It can also be a food where the nature of one or more components has been modified, or a food in which the bioavailability of one or more components has been modified, or any combination of these possibilities. A functional food might be functional for all members of a population or for particular groups of the population, which might be defined, for example, by age or by genetic constitution.

Apart from the term "functional food" there are more terms for dietary products that explicitly link nutrition with health, namely "food supplements" (or "dietary supplements") and nutraceuticals (or "nutriceuticals"). According to the DG Health and Consumer Protection of the European Commission "food supplements are concentrated sources of nutrients or other substances with a nutritional or physiological effect whose purpose is to supplement the normal diet. They are marketed 'in dose' form i.e. as pills, tablets, capsules, liquids in measured doses etc.(EC.2007a). Similarly, the US Food and Drug Administration defines a dietary supplement as "a product taken by mouth that contains a 'dietary ingredient' intended to supplement the diet. The 'dietary ingredients' in these products may include: vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes organ tissues, glandulars, and metabolites. Dietary supplements can also be extracts or concentrates, and may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders. They can also be in other forms, such as a bar, but if they are, information on their label must not represent the product as a conventional food or a sole item of a meal or diet" (FDA, 2014). Hence, the main difference between functional food and food supplements is that the former "are similar in appearance to conventional foods and are consumed as part of a normal diet" (Zeisel, 1999), whereas the latter are not considered to be proper "food".

In summary, Functional food (FuFo) is defined as food that is taken as part of the usual diet and has beneficial effects that go beyond nutritional effects. In addition to foods that are naturally in this group, functionality is created during the industrial processing of food through the addition of bioactive ingredients. Hence, obtaining bioactive ingredients from plants is a challenge for industry, especially in view of the increased market shares and the health claims made on food have recently been regulated at the EU level.

In the meantime the European Parliament and the Council have adopted a new regulation "on nutrition and health claims made on foods" (EC.2007c). This regulation is expected to ensure that consumers are not misled by unsubstantiated, exaggerated or untruthful claims about foodstuffs and to provide food producers and manufacturers with clear, harmonized rules that would ensure fair competition and help protect innovation in the food industry (EC.2007b), thus not only tying in with the EU campaign for healthier lifestyle choices and the Commission's consumer protection objectives, but also benefiting the food industry.

Types of functional foods available in the market

Classification

Dairy products and beverages have the biggest market share – followed by cereals only as a distant third. In terms of bioactive ingredients, probiotic bacteria cultures clearly dominate, followed by prebiotics (Stein and Rodriguez-Cerezo, 2008), plant extracts or foods including plant extracts or its bioactive ingredients, alone or added (fortified foods), in different foods are among the most common.

Functional food can be classified according to several principles, namely the food group it belongs to (e.g. dairy products, beverages, cereal products, confectionary, oils and fats), the diseases it is expected to prevent or alleviate (e.g. diabetes, osteoporosis, colon cancer), its physiological effects (e.g. immunology, digestibility, anti-tumor activity), the category of its specific biologically active ingredients (e.g. minerals, antioxidants, lipids, probiotics), its physico-chemical and organoleptic properties (e.g. color, solubility, texture), or the processes that are used in its production (e.g. chromatography, encapsulation, freezing) (Juvan *et al.*, 2005).

Functional food products by types of bioactive ingredients

The 385 functional food products that were identified for the European market in 2008 contained 503 different "functional" ingredients. About one third of the products were enriched with (probiotic) bacteria cultures, one sixth of the products contained functional saccharides (most of them prebiotics), plant extracts without explicit specification of their active molecules were added to one tenth of the products and terpenes to another 8 %. About one third of the products contained more than one bioactive ingredient (Stein and Rodriguez-Cerezo, 2008). The "functionality" of more and more new ingredients is continually proven and, therefore, functional food might find larger acceptance in the future, when, for instance, food may help control elevated blood pressure, reduce body fat, improve the glucose metabolism (Sloan, 2004) or may cater for a growing market of men's health (e.g. helping address benign prostate hyperplasia or erectile dysfunction) (Tallon, 2004).

Carbohydrates are the most abundant class of organic compounds found in plants, and therefore, in foods from plant origin. They are many types of carbohydrates classed according to their size in monosaccharides, oligosaccharides and polysaccharides. In plants, they are derived from glucose formed by photosynthesis. In addition to their role in energetic metabolism, they may be stored as starch, or play a structural role like cellulose, and are used for other scaffoldings needed by the plant, and they are also involved in signal recognition and transduction in the defense processes.

Apart from the use of monosaccharides and some oligosaccharides (sucrose) as sweeteners, polysaccharides are relevant for health because of their structure; cellulose is a lineal polymer that constitutes up to 80% of plant cell walls, and humans are not able to degrade it, hence it is part of the dietary fiber; the second relevant component of cell walls are branched polysaccharides, able to absorb relevant amounts of water, that also contribute to dietary fiber. Polysaccharides are relevant for the pharmaceutical and the food industry.

Plant secondary metabolism

In order to organize functional foods derived from plants by their bioactive ingredients, an outline of secondary metabolism is a must. Metabolic pathways have been split in two main groups, primary and secondary metabolism. Primary metabolism refers to metabolic pathways present in all organisms like carbohydrate, lipid and protein metabolism, that present few differences therein, and secondary metabolism refers to metabolic pathways that are present only in some groups of organisms (Romeo *et al.*, 2000; Dixon, 2001).

Because plants have evolved secondary metabolism strategies to survive the changing biotic and abiotic conditions encountered during their existence, this has also allowed them to colonize most habitats. Given the number of possibilities of changing conditions, both for biotic and abiotic factors, the array of secondary metabolites designed for each situation is enormous (Mierziak *et al.*, 2014). Secondary metabolites can be studied from different points of view, such as the above examples for agriculture, the evaluation of their role in a plant's defense against pathogens, and their effects on human health.

Secondary metabolism is developed to a greater extent in sessile organisms, among which plants are found. Sessile organisms are fixed to a substrate and have developed an array of chemical molecules to overcome changing environmental conditions throughout their lives (Yamane, 2013; Wink, 2003) and to communicate with other organisms in order to survive. Bearing this two ideas on mind, compounds for protection against excessive UV-radiation like carotenoids or flavonoids or essences for attraction of insects for pollination, are quite necessary (Wink, 2010). One common feature of these molecules is the very low presence within the plant tissues, being the general rule under 1% in the best of the occasion, with the corresponding exceptions, for example in clove (*Eugenia carryophylata*), there is 15% essential oil, and 0.0003% for the antitumoral vinblastine in *Catharantus roseus* (Bruneton, 2001). A summarized overview of plant metabolism is depicted on figure 1, and a few examples of each with the pharmacological effect or industrial application follows.

Terpenes

Terpenes are secondary metabolites made by isoprene subunits. All organisms are able to synthetize isoprene through the mevalonic acid pathway in the cytoplasm (Jansen and de Groot, 2004), and only plants and microorganisms have an alternative pathway, the DOXP pathway located in plastids (Brielman *et al.*, 2006) through which a greater diversity of terpenes of different sizes and functions are obtained.

Terpenes in plants play an ecological and physiological role (Perveen *et al.*, 2015). On one hand, allelopathic substances that inhibit growth of other plants may be found, as well as insecticidal (piretryns) or insect attractants (essential oils). From the physiological point of view, plant growth regulators like gibberellins and abscisic acid belong to this group, and also, photosynthetic pigments like carotenoids are included in this category (Brielman *et al.*, 2006).

In addition to the traditional use of teas, like mint (*Mentha piperita*) of chamomile (*Matricharia camomila*) for stomachake, effect that relies on the essential oils contained therein, in the context of functional food the terpenes sub-category of carotenoids is relevant. This family includes beta-carotene, lycopene, astaxanthin and lutein – which are also widely used for animal feed, as food colouring, in cosmetics, in pharmaceuticals and in dietary supplements. In 2008, the Western European carotenoids market was worth EUR 308 million; with betacarotene having the largest share and is usually synthetic. However, the use of naturally-derived carotenoid is increasing, particularly in functional food and health food applications

Phenols

Phenolic compounds from plant origin derive mainly from the shikimic acid pathway; this pathway leads to aromatic amino acids phenylalanine and tyrosine, which undergo a deamination yielding cinnamic acid, from which all phenylpropanoids derive. They can exist free or combined; they frequently acylate, may cyclize (coumarins), dimerize (lignans), polymerize (lignins) or undergo side chain shortening (phenolic acids, benzoic acids) or elongation (stilbenes and flavonoids). Flavonoids may further evolve by cyclization (isoflavonoids, neoflavonoids…) and or polymerizing through different carbons, in variable numbers turning into tannins, proanthocyanidins, anthocyanidins present in variable amounts in the different plant species (Bruneton, 2001; Tomas Laursen *et al.*, 2014).

The variability within this group of compounds is even greater than in terpenoids. The physiological role of these compounds ranges from communication signals with microorganisms (isoflavones with rhizobia in legumes), to nutritional deterrents for herbivores (condensed tannins) or protection to UV irradiation (flavonoids and antocyanins), which in turn are responsible for fruit and flower colors.

Polyphenols, being traditional food colorants, are increasingly marketed as antioxidants in functional food. Research findings suggest that polyphenols can protect against cancer and cardiovascular diseases, as well as increase anti-inflammatory activity and immune function, targeting both specific and nonspecific mechanisms. Furthermore, some of the structures like isoflavones exert an estrogenic activity and are used to palliate hot flushes in postmenopausal women. The market for polyphenols in Western Europe has grown to an estimated EUR 78 million in 2003, but analysts question its further growth once food manufacturers have to validate the health claims they make (as this may involve costly clinical trials and regulatory burdens (Nutra.2004).

Alcaloids

Alcaloids are aminoacid derived secondary metabolites present in a limited group of plants. They are not included within the functional food market due to the narrow pharmacological window, but they are relevant for the pharmaceutical industry and for our health. Here very useful molecules targeting a number of different physiological targets are found, for example the phenylalanine derived morphine, with the strongest analgesic effect, or the tryptophan derived antitumorals vinblastine or vincristine, or anasethetic curares… to name a few.

The diversity of this group overcomes the already large variability of the other two groups, with even larger chemical complexity since molecules show a high number of chiral centers that turn chemical synthesis into a complex and expensive activity. Therefore, most molecules are obtained from plants and any strategy to increase contents is welcome. However, their role in plant physiology is not yet clear, and still, their production can be increased considering they are secondary metabolism and hence, it is inducible.

Plant extracts

Beyond the groups that have been depicted above, as individual molecules, there is a different product, the plant extracts. Extracts have a more complex composition which is often not well defined due to analytical limitations, and often have a better effect on health than the individual components. Among these extracts, antiinflamatory effects from *Harpagofitum procumbens* or memory improvement by EGB760 from *Ginkgo biloba* achieve better effects than their individual components.

The key point in this type of extracts is reproducibility of the effects that may be a problem since plant metabolism changes according to environmental conditions. In order to establish a proper relation, these extracts are usually standardized to one of its components. This is also the problem on field produced functional foods and its derived extracts, especially in fruits.

Plant extracts (which include herbal extracts, oleoresins, essential oils and fruit and vegetable extracts) had a market value of over EUR 1 billion in Western Europe in 2002. The market share for herbal extracts (e.g. *Ginkgo biloba*, ginseng, green tea or St. Johns Wort) was about EUR 340 million and the corresponding US market share was worth EUR 315 million. The market share for fruit and vegetable extracts and powders was about EUR €410 million and the corresponding US market amounted to about EUR 600 million. However, these ingredients are not only used because of their potential health benefits but also as colorants and flavors. As ingredients for functional foods and food supplements, during the preparatory work for this report the market revenue of fruit and vegetable extracts and powders was only EUR 169 million for Western Europe and EUR 286 million for the USA [13].

Plant secondary metabolism and defense

Once the outline of plant secondary metabolism has been presented, the variability of secondary metabolism due to environmental conditions needs to be recalled in order to introduce elicitors as a tool to trigger secondary metabolism.

At the beginning of the 1990's, Van Peer *et al.* (1991) (Van Peer *et al.*, 1991) and Wei *et al.* (1991) (Wei *et al.*, 1991) made an important discovery about plant defense mechanisms and productivity. These investigators found that certain non-pathogenic bacteria were able to prevent a pathogen attack before the pathogen reached the plant. The difference with biocontrol is that the beneficial bacteria do not interact physically with the pathogen, but instead, trigger a response in the plant which is effective against subsequent attack by a pathogen. This response is systemic; that is, the bacteria interact with the plant in a restricted area, but the response is extended to the whole plant. This response is mediated by metabolic changes that are not evident at first glance. As a matter of fact Priming or biopriming is the physiological state of a plant that is systemically induced by non-pathogenic bacteria against subsequent pathogen attack, but the effect is not detected until pathogen challenge (Conrath *et al.*, 2002); since energetic metabolism is detoured to secondary metabolism, this physiological state is usually coupled to lower growth rates as compared to non-primed controls (Van Hulten *et al.*, 2006). For the protection to be effective, an interval is necessary between the PGPR-plant contact and the pathogen attack in order for the expression of the plant genes involved in the defence. This mechanism was first known as "rhizobacteria-mediated induced systemic resistance" (Liu *et al.*, 1995) but it is now termed "induced systemic resistance" (ISR) (Van Loon *et al.*, 1998). ISR was reported in the plant-pathogen-beneficial bacteria model, *Arabidopsis thaliana-Pseudomonas syringae* DC3000-*Pseudomonas fluorescens* WSC417r as the defensive response induced by *P. fluorescens* WSC417r in *A. thaliana* against *P. syringae* DC3000, mediated by JA (jasmonic acid) and ethylene. Since then, it has been described in many plant species, including bean, tobacco, tomato and radish, with different PGPRs and pathogens, and an increasing number of signal transduction pathways. This finding is fundamental because it proposes an "immune" response in the plant, raising the possibility of "vaccination" for the plant.

The plant can also acquire immunity after a pathogen attack. This response has been described before the ISR. The acquisition of resistance by the plant after a pathogen attack, causing little damage or localized necrosis in response to a further pathogen attack has been known for many years. The phenomenon is called systemic acquired resistance (SAR) (Ryals *et al.*, 1996). During a pathogen attack, reactive oxygen species (ROS) are produced in necrotic areas, causing tissue death. If the plant survives the challenge, it remains protected for life.

SAR and ISR responses lead to plant protection against different pathogen spectra, but there are spectra which overlap.

However, both responses can coexist in the same plant at the same time (Van Wees *et al.,* 2008) and contribute to plant fitness, ensuring plant survival (Beckers and Conrath, 2007).

Elicitation to improve bioactive/phytoalexin contents in functional foods

The induction of defensive metabolism, in fact, involves an induction of secondary metabolism. While using a pathogenic agent involves lowering crop yields, the role of beneficial agents to trigger secondary metabolism appears as a promising alternative to increase levels of phytopharmaceuticals. Some PGPR may trigger secondary metabolism against pathogens that may also be effective against biotic stress, such as saline conditions in soils, a frequent situation in agriculture. Furthermore, when a medicinal plant is used, phytopharmaceutical levels may be increased, or even new molecules may appear after treatment with several elicitors, as SAR and ISR are often associated with priming for enhanced mobilization of defense responses after subsequent exposure of the plants to stress (Conrath *et al.,* 2002; Beckers and Conrath, 2007) (Figure 2). New molecules that might appear are called phytoalexins and were first defined as plant secondary metabolites with antimicrobial activity that were synthesized de novo and functioned as the basis of a disease resistance mechanism (Müller and Börger, 1940). Pre-existing compounds levels may also be increased, J.W. Mansfield coined the term phytoanticipins for these pre-existing compounds and defined them as low molecular weight, antimicrobial compounds that are present in plants before challenge by microorganisms, or are released after infection solely from preexisting constituents (VanEtten *et al.,* 2013). The differences between phytoanticipins and phytoalexins are based solely on how these compounds are produced. Therefore, resveratrol in grapes and daidzein in soy would be both phytoanticipins and phytoalexins depending on how they were produced (Boue *et al.,* 2009).

One common feature of these molecules is the very low presence within the plant tissues, being the general rule under 1% in the best of the occasion, with the corresponding exceptions, for example in cloves (*Eugenia carryophylata*), there is 15% essential oil, while the antitumoral vinblastine in *Catharantus roseus* is 0.0003% (Bruneton, 2001).

The variability of secondary metabolism is a problem for the pharmaceutical industry since field production is uncertain and may condition availability of final products. Sometimes, the problem may be solved by chemical synthesis, but it is not always possible, or at least it usually lacks economic feasibility. Another alternative is cell culture, but some species are not amenable to this type of culture, or yields achieved are too low because secondary metabolism is not necessary under such controlled and undifferentiated conditions. Therefore, one of the main goals in industry now is obtaining reproducible extracts of plants in field production, or even more challenging, in plant cell cultures. For this purpose, the use of elicitors appears to be an encouraging alternative (Radman *et al.,* 2003). In support of this last statement, (Poulev *et al.,* 2003) reported the potential of elicitation to discover new molecules with pharmacological interest. But this study not only reports the presence of new molecules, but also, the use of elicitors has been able to duplicate the presence of these molecules and to increase the concentration of known compounds. A number of studies have shown that elicitors from fungi or pathogenic bacteria or other natural elicitors (VanEtten *et al.,* 1994) are able to trigger secondary metabolites with pharmacological activity (Cai *et al.,* 2012; Gutiérrez Mañero *et al.,* 2012;Ruiz-García and Gomez-Plaza, 2013; Lu *et al.,* 2001;Korsangruang *et al.,* 2010;Coste *et al.,* 2011; Pawar *et al.,* 2011). Hence, unravelling the nature of elicitors and the elicited pathways remains an exciting challenge for the pharmaceutical industry.

As previously mentioned, beneficial bacterial strains or derived elicitors are confirming their ability to upregulate secondary metabolism, increasing phytoalexin levels, to be used for human health. Among the terpenes, cardenolides in high-yielding varieties of *D. lanata* grown under controlled conditions have been increased by some strains isolated from the rhizosphere of wild populations of *Digitalis* (Gutiérrez Mañero *et al.,* 2003). Also, strains isolated from wild populations of *Nicotiana glauca*, a solanaceous native in the Iberian peninsula with an active secondary metabolism, are able to trigger secondary metabolism involved in defense in tomato (Ramos Solano *et al.,* 2010b), *Arabidopsis thaliana* (Domenech *et al.,* 2007) and soybean (Algar *et al.,* 2014); the same strains are also able to trigger secondary metabolism leading to metabolites of interest for human health as isoflavones in soybean (Ramos Solano *et al.,* 2010a) and hypericines from *Hypericum perforatum* seedlings and shoot cultures (Gutiérrez Mañero, 2012). Furthermore, *C. balustinum* Aur9, a PGPR from different origin, triggers defensive metabolism in *A. thaliana* and its structural elicitors provide the same protection (Ramos Solano *et al.,* 2008). Following this rationale, metabolic elicitors from *P. fluorescens* N21.4 trigger isoflavone metabolism in soybean cell cultures in a dose dependent manner (Algar *et al.,* 2012), inducing isoflavone biosynthesis de novo, achieving interesting increases in the most effective isoflavone in clinical studies, genistein (Pilsakova *et al.,* 2010). These elicitors have also demonstrated their effectiveness in soybean sprouts where they trigger isoflavone metabolism, inducing de novo biosynthesis within 3 days (Algar *et al.,* 2013).

The effects of secondary metabolites on human health may vary depending on several factors. One of them is how they are delivered and incorporated into the human body, either in a pharmaceutical formulation, as food supplements, or in the diet. A pharmaceutical formulation will provide known concentrations of well-characterized and identified compounds, while a food supplement will provide an extract of the plant that possesses variable concentrations of known and unknown compounds. The most variable input is seen through the diet. Some edible plants, such as soybeans or berries, contain bioactive compounds that provide more benefits than is attributed to their simple nutritional value. Levels of bioactive compounds do change depending on environmental conditions; hence, the lack of an effect on health may be due to the lack of reproducibility of bioactive content. This lack of reproducibility may be overcome by elicitation with biotic agents. Consistent with the above hypothesis, two case studies using elicitation to improve bioactive contents in functional foods follow.

Case study: The following case study (Gutiérrez Mañero *et al.,* 2012) illustrates how 6 PGPR isolated from the rhizosphere of wild populations of *Nicotiana glauca* (Ramos Solano *et al.,* 2010b) are able to enhance levels of hypericin and pseudohypericin in *Hypericum perforatum* L. (Guttiferae) seedlings. Furthermore, metabolic elicitors from the most effective strain (*Stenotrophomonas maltophilia* N5.18), were also effective in shoot cultures (Gutiérrez Mañero *et al.,* 2012).

H. perforatum (St. John's Wort), appears as an alternative treatment to mild and moderate depression and its use has become prominent in the last years (Erdelmeier *et al.*; Di Carlo *et al.,* 2001; Butterweck, 2003; Silva *et al.,* 2005; Lozano-Hernández *et al.,* 2010; Ruedeberg *et al.,* 2010) for its application as a health enhancer, a wound healer (Suntar *et al.,* 2010), antibacterial (Saddiqe *et al.,* 2010) anti-viral (Pang *et al.,* 2010), anti-retroviral (Chisembu and Hedimbi, 2010) activities and antitumor effect (Medina *et al.,* 2006). Phytochemical characterization reports hyperforin and hypericin, as the main chemicals responsible for effects on health, although other biologically active constituents, e.g. flavonoids, tannins, are also present (Barnes *et al.,* 2001).

The six bacterial strains were tested in *Hypericum perforatum* seedlings for their ability to enhance seedlings growth. Strain N5.18 showed a significant positive effect on plant growth. Only two strains, N21.4 and N5.18, caused a three-fold significant increase in hypericins and a moderate but significant increase in pseudohypericin.

Among the effective strains to increase hypericins in seedlings, N5.18 was selected for elicitation assays in shoot cultures. Three fractions containing putative molecular elicitors from N5.18 were assayed at three different concentrations. In shoot cultures, only pseudohypericin was detected and significant increases were observed under the different elicitors, reaching values 30 fold higher with one of the fractions in the middle concentration.

Irrespective of the eliciting fraction, these are encouraging results for several reasons. First there seems to be at least three different elicitors that are effective triggering pseudohypericin levels, and secondly, low concentrations are effective which is an asset to develop a profitable comercial product.

Case study: The aim of this case study involving four separate studies (Ramos Solano *et al.,* 2010a; Ramos Solano *et al.,* 2008; Domenech *et al.,* 2007) was to study effects of bioeffectors on isoflavone metabolism and defense in soybean (*Glycine max* (L.) Merr.).

Research with plant growth promoting bacteria (PGPR) includes both the approach to crop production for food and biomass production and the approach oriented to pharmaceutical purposes. An interesting goal is to deliver PGPR to increase crop production in low production areas aiming to alter not only biomass production, but also levels of secondary metabolites involved in defense and which, in turn, can be considered as bioactives, i.e., with an important pharmacological effect (Pawar *et al.,* 2011; Al-Tawaha *et al.,* 2005). In this sense, the results evidenced the direct relationship between defense pathways and pharmacologically active metabolites such as flavonoids, defensive metabolites against abiotic stress and antioxidant effects, with regenerative properties of great importance for human health through the diet (Al-Tawaha *et al.,* 2005). The variation of isoflavone (IF) content in soybean can provide multiple benefits to human health and for the pharmaceutical and food industries. The specific objectives that arise in this case study are defined as a biotechnological approach in elicitation processes that relate plant defensive metabolism with metabolite production with pharmacological and /or food interest.

Figure.1 Overview of plant metabolism

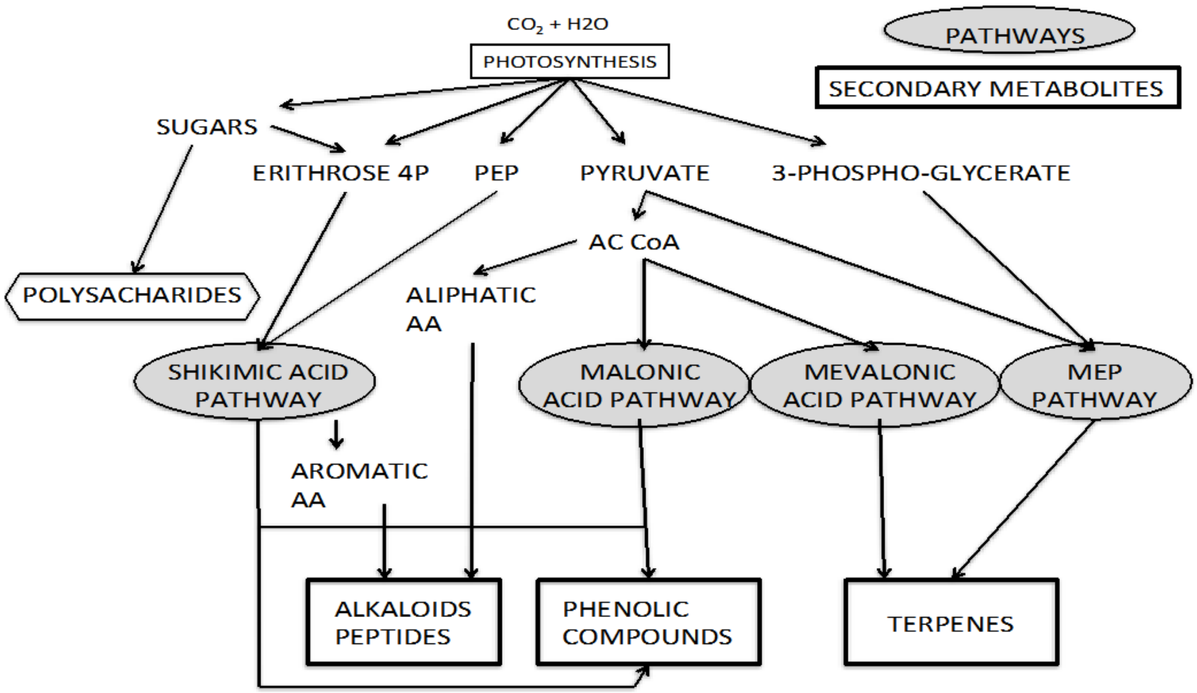
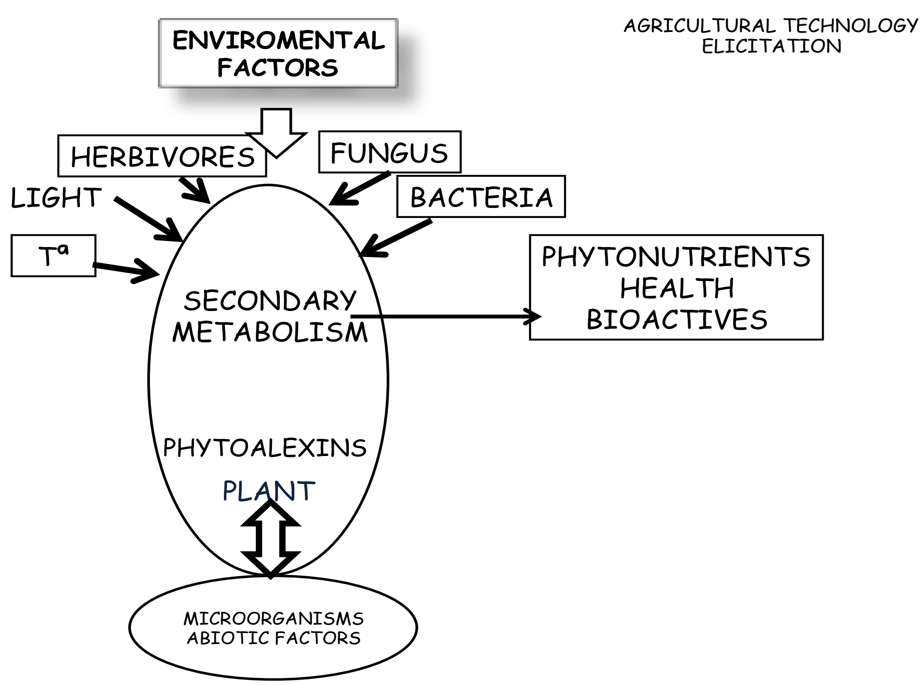


Figure.2 Systemic induction of plant defensive metabolism for phytoalexin production



First, 9 PGPR strains who had previously demonstrated their ability to stimulate growth and metabolism in various plant defenses were tested (Paredes-López *et al.*, 2010; Ramos *et al.*, 2003; Domenech *et al.*, 2007; Lucas García *et al.*, 2003; Barriuso *et al.*, 2005; Barriuso *et al.*, 2008) and their potential to stimulate IF metabolism in soybean seedlings was evaluated. Results of these experiments revealed that the 9 strains tested were able to alter IF metabolism differentially with four different behaviors based on the changes in daidzein and genistein IF families. Among these groups, strains N21.4 and M84 were outstanding, the first increased the two IF families and the second decreased them (Ramos Solano *et al.*, 2010a).

Based on the above results, one representatives of each of the different behaviors were selected: N21.4 (*Pseudomonas fluorescens*), N5.18 (*Stenotrophomonas maltophila*) Aur9 (*Chryseobacterium balustinum*) and M84 (*Curtobacterium* sp.). The ability of these strains to stimulate IF metabolism soybean plants and to confer protection against foliar pathogen *Xanthomonas axonopodis* pv. glycines was assessed. These experiments confirmed that the four strains altered IF metabolism differentially also on mature plants. Furthermore, it was observed that all four were able to induce systemic resistance in soybean plants since in all cases a reduction of disease symptoms were found. In addition, a relationship between IF and defense in plants treated with N5.18 and M84 strains was established. In the case of plants treated with M84, IF increased in the presence of the pathogen, which may be engaged directly as defense compounds. By contrast, on N5.18-treated plants, IF decreased in the presence of the pathogen, so in this case a role as precursors of other compounds of defense, like glyceollins, was proposed (Algar *et al.*, 2014).

Having established the effects on IF metabolism in soybean seedlings and plants, we proceeded to study whether elicitors released into the culture medium by strains Aur9 and N21.4 were able to stimulate IF metabolism on soybean cell lines. In these experiments we used three cell lines that showed IF different content. The IF content analysis of elicited cell lines indicated that elicitors from N21.4 produced significant increases in all cell lines tested and that the effective dose was different in each cell line (Algar *et al.*, 2012).

Finally, elicitation soybean seeds experiments were performed to assess whether elicitors from N21.4 and N21.4 strain directly, were capable to trigger IF biosynthesis during the germination process as biotechnological tool for obtaining sprouts with high isoflavone content. These experiments again confirmed the potential of N21.4 strain to stimulate the IF biosynthesis (Algar *et al.*, 2013).

Based on the above, it´s evidenced that elicitation with some specific beneficial strains, or bacterial derived molecules, either structural or bacterial metabolites, is an effective tool to increase bioactive/phytoalexin contents in plants of interest, either in plants or in cell or organ cultures. Plant based products with improved quality can be used directly as foods, or as a source of bioactive ingredients. On the other hand, bacterial elicitors are excellent tools for different plant materials and may trigger plant metabolism in different ways. Hence, elicitation is an excellent tool for high quality functional foods.

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Author Contributions

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References

Algar, E., Gutierrez-Manero, F. J., Garcia-Villaraco, A., Garcia-Seco, D., Lucas, J. A., Ramos-Solano, B. 2014. The role of isoflavone metabolism in plant protection depends on the rhizobacterial MAMP that triggers systemic resistance against Xanthomonas axonopodis pv. glycines in Glycine max (L.) Merr. cv. Osumi. *Plant Physiol. Biochem.*, 82: 9–16.

Algar, E., Gutierrez-Mañero, F.J., Bonilla, A., Lucas, J.A., Radzki, W., Ramos-Solano, B. 2012. Pseudomonas fluorescens N21.4 Metabolites Enhance Secondary Metabolism Isoflavones in Soybean (Glycine max) Calli Cultures. *J. Plant Physiol.*, 60: 11080–r11087.

Algar, E., Ramos-Solano, B., Garcia-Villaraco, A., Sierra, M.D.S., Gomez, M.S.M., Gutierrez-Manero, F. 2013. Bacterial bioeffectors modify bioactive profile and increase isoflavone content in soybean sprouts (*Glycine max var Osumi*). *Plant foods Hum. Nutr.*, 68: 299–r305.

Al-Tawaha, A.M., Seguin, P., Smith, D.L., Beaulieu, C. 2005. Biotic elicitors as a means of increasing isoflavone concentration of soybean seeds. *Ann. Appl. Biol.*, 146: 303–r310.

Baenas, N., Garcia-Viguera, C., Moreno, D. A. 2014. Elicitation: A tool for enriching the bioactive composition of foods. *Molecules*, 19: 13541–r13563.

Bakker, P.A.H.M., Pieterse, C.M.J., van Loon, L.C. 2007. Induced systemic resistance by fluorescent Pseudomonas spp. *Phytopathology*, 97: 239–r243.

Barnes, J., Anderson, L.A., Phillipson, J.D. 2001. St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. *J. Pharm. Pharmacol.*, 53: 583–r600.

Barriuso, J., Pereyra, M.T., Lucas García, J.A., Megías, M., Gutiérrez Mañero, F.J., Ramos, B. 2005. Screening for putative PGPR to improve establishment of the symbiosis *Lactarius deliciosus-pinus*. *Microb. Ecol.*, 50: 82–r89.

Barriuso, J., Ramos Solano, B., Gutiérrez Mañero, F.J. 2008. Protection against pathogen and salt stress by four PGPR isolated from *Pinus sp*. on *Arabidopsis thaliana*. *Phytopathology*, 98: 666–r672.

Beckers, G.J.M., Conrath, U. 2007. Priming for stress resistance: from lab to the field. *Curr. Opin. Plant Biol.*, 10: 425–r431.

Boue, S.M., Cleveland, T.E., Carter-Wientjes, C., Shih, B.Y., Bhatnagar, D., Mclachlan, J.M., Burow, M.E. 2009. Phytoalexin-enriched functional foods. *J. Agric. Food Chem.*, 57: 2614–2622.

Brielmann, H.J., Setzer, W.N., Kaufman, P.B., Kirakosyan, A., Cseke, L.J. 2006. Phytochemicals: The chemical components of plants. In: Brielmann, H.L., Kaufman, P.B., Duke, J.A., Cseke, L.J., Warber, S.L., Kirakosyan, A. (Eds). Natural products from plants, 2nd edn. CRC Press. Pp. 1–r49.

Bruneton, J. 2001. Farmacognosia, Fitoquímica, Plantas Medicinales. 2ª edición; Acribia, S.A., Zaragoza. pp. 1099.

Burrit, D.J. 2013. The influence of pre- and postharvest environmental stress on fruit bioactives. Bioactives in Fruit: Health Benefits and Functional Foods. Pp. 409–r428.

Butterweck, V. 2003. Mechanism of action of St. John's wort in depression: What is known? *CNS Drugs,* 17: 539–r562.

Cai, Z., Kastel,l A., Mewis, I., Knorr, D., Smetanska, I. 2012. Polysaccharide elicitors enhance anthocyanin and phenolic acid accumulation in cell suspension cultures of *Vitis vinifera*. *Plant Cell Tiss. Org.,* 108: 401–409.

Capanoglu, E. 2010. The potential of priming in food production. *Trends Food Sci. Technol.,* 21: 399–r407.

Chinsembu, K.C., Hedimbi, M. 2010. Ethanomedical plants and other natural products with anti-HIV active compounds and their putative modes of action. *IJBMBR,* 1: 74–r91.

Conrath, U., Pieterse, C.M.J., Mauch-Mani, B. 2002. Priming in plant–pathogen interactions. *Trends Plant Sci.,* 7: 210–r216.

Coste, A., Vlase, L., Halmagyi, A., Deliu, C., Coldea, G. 2011. Effects of plant growth regulators and elicitors on production of secondary metabolites in shoot cultures of Hypericum hirsutum and Hypericum maculatum. *Plant Cell Tiss. Org.,* 106: 279–r288.

Di Carlo, G., Borrelli, F., Ernst, E., Izzo A.A. 2001. St John's wort: Prozac from the plant kingdom. *Trends Pharmacol. Sci.,* 22: 292–r297.

Diplock, A.T., Aggett, P.J., Ashwell, M., Bornet, F., Fern, E.B., Roberfroid, M.B. 1999 Scientific concepts of functional foods in Europe Consensus document. *Br. J. Nutr.,* 81(Suppl 1): S1–S27.

Dixon, R.A. 2001. Natural products and plant disease resistance. *Nature,* 411: 843–r847.

Domenech, J., Ramos, B., Probanza, A., Lucas, J.A., Gutiérrez, F.J. 2007. Elicitation of systemic resistance and growth promotion of *Arabidopsis thaliana* by PGPRs from *Nicotiana galuca*. A study of the putative induction pathway. *Plant Soil,* 290: 43–r50.

EC. 2007a. Food supplements. Website, 19 October. Brussels: DG Health and Consumer Protection, European Commission.

EC. 2007b. Questions and Answers on health and nutrition claims. MEMO/07/267. Brussels: European Communities. Available at: http: //europa.eu/rapid/pressReleasesAction.do?reference=MEMO/07/267

EC. 2007c. Regulation (EC) No 1924/2006 of the European Parliament and the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal L 12: 3–r18. Available at: http: //eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX: 32006R1924: EN: NOT

Erdelmeier, C.A.J., Koch, E., Hoerr R. Hypericum perforatum – St. John's wort chemical, pharmacological and clinical aspects. In: Studies in natural products chemistry – bioactive natural products (Part C); Atta-ur-Rahman, (Ed). Elsevier Direct, New York. 200, 22, 643–r716 pp.

FDA. 2004. Dietary supplements guidance documents & regulatory information.

Gutiérrez Mañero, F., Algar, E., Martín Gómez, M.S., Saco Sierra, M.D., Ramos Solano, B. 2012. Elicitation of secondary metabolism in Hypericum perforatum by rhizosphere bacteria and derived elicitors in seedlings and shoot cultures. *Pharm. Biol.,* 50(10): 1201–9.

Gutiérrez Mañero, F.J., Ramos, B., Lucas García, J.A., Probanza, A., Barrientos Casero, M.L. 2003. Systemic induction of terpenic compounds in D. lanata. *J. Plant Physiol.*, 160: 105–r130.

Jansen, B.J.M., de Groot, A. 2004. Occurrence, biological activity and synthesis of drimane sesquiterpenoids. *Nat. Prod. Rep.*, 21: 449–r477.

Juvan, S., Bartol, T., Boh, B. 2005. Data structuring and classification in newly-emerging scientific fields. *Online Inform. Rev.*, 29(5): 483 –r498.

Korsangruang, S., Soonthornchareonnon, N., Chintapakorn, Y., Saralamp, P., Prathanturarug, S. 2010. Effects of abiotic and biotic elicitors on growth and isoflavonoid accumulation in *Pueraria candollei var. candollei* and *P. candollei var. mirifica* cell suspension cultures. *Plant Cell Tiss. Org.*, 103: 333–342.

Liu, L., Kloepper J.W., Tuzun S. 1998. Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth-promoting rhizobacteria. *Phytopathology*, 85: 843–r847.

Lozano-Hernández, R., Rodríguez-Landa, J.F., Hernández-Figueroa, J.D., Saavedra, M., Ramos-Morales, F.R., Cruz-Sánchez, J.S. 2010. Antidepressant-like effects of two commercially available products of Hypericum perforatum in the forced swim test: A long-term study. *J. Med. Plant Res.*, 4: 131–r137.

Lu, M.B., Wong, H.L., Teng, W.L. 2001. Effects of elicitation on the production of saponin in cell culture of Panax ginseng. *Plant Cell Rep.*, 20: 674–677.

Lucas García, J.A., Probanza, A., Ramos, B., Gutiérrez Mañero, F.J. 2003. Effects of three plant growth promoting rhizobacteria on the growth of seedlings of tomato and pepper in two different sterilized and nonsterilized peats. *Arch. Agron. Soil Sci.*, 49: 119–r127.

Martin, C., Zhang, Y.; Tonelli, C., Petroni, K. 2013. Plants, diet, and health. *Plant Biol.*, 64: 19–r46.

Medina, M.A., Martínez-Poveda, B., Amores-Sánchez, M.I., Quesada, A.R. 2006. Hyperforin: More than an antidepressant bioactive compound? *Life Sci.*, 79: 105–r111.

Müller, K. O., Börger, H. 1940. Experimentelle untersuchungen über die Phytopthora-resistenz der kartoffel. *Arb. Biol. Reichsasnstalt. Landw. Forstw. Berlin.*, 23: 189–231.

Nutra, 2004. EU laws set to hamper growth in polyphenols market. Breaking News on Supplements & Nutrition – Europe, 6 January. Montpellier: Decision News Media. Available at: http: //www.nutraingredients.com/news/ng.asp?id=48812

Pang, R., Tao, J., Zhang, S., Zhu, J., Yue, X., Zhao, L., Ye, P., Zhu, Y. 2010. In vitro anti-hepatitis B virus effect of *Hypericum perforatum L. J. Huazhong Univ. Sci. Technol. Med. Sci.*, 30: 98–r102.

Paredes-López, O., Cervantes-Ceja, M.L., Vigna-Pérez, M., Hernández-Pérez, T. 2010. Berries: Improving human health and healthy aging, and promoting quality life-A Review. *Plant Foods Hum. Nutr.*, 65: 299–308.

Pawar, K.D., Yadav, A.V., Shouche, Y.S., Thengane, S.R. 2011. Influence of endophytic fungal elicitation on production of inophyllum in suspension cultures of *Calophyllum inophyllum L. Plant Cell Tiss. Org.*, 106: 345–r352.

Perveen, Rashida, Suleria, Hafiz Ansar Rasul, Anjum, Faqir Muhammad, Butt, Masood Sadiq, Pasha, Imran and Ahmad, Sarfraz. 2015. Tomato (*Solanum lycopersicum*) carotenoids and lycopenes chemistry; metabolism, absorption, nutrition, and allied health claims. *Compr. Rev.*, 55: 919–r929.

Pieterse, Corne M.J., Zamioudis, Christos, Berendsen, Roeland, L., Weller, David, M., Van Wees, Saskia C.M., Bakker, Peter, A.H.M. 2014. Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.*, 52: 347–r75.

Pilsakova, L., Riecansky, I., Jagla, F. 2010. The physiological actions of isoflavone phytoestrogens. *Physiol. Res.*, 59: 651–r664.

Poulev, A., O´Neal, J.M., Logendra, S., Pouleva, R.B., Timeva, V., Garvey, A.S., Gleba, D., Jenkins, I.S., Halpern, B., Kneer, R., Cragg, G.M., Raskin, I. 2003. Elicitation, a new window into plant chemodiversity and phytochemical drug discovery. *J. Med. Chem.*, 46 (12): 2542–r2547.

Radman, R., Saez, T., Bucke, C., Keshavaraz, T. 2003. Elicitation of plants and microbial cell systems. *Biotechnol. Appl. Biochem.*, 37: 91–r102.

Ramos Solano, B., Algar, E., García-Villaraco, A., García Cristóbal, J., Lucas García, J., Gutiérrez Mañero, F.J. 2010a. Biotic elicitation of isoflavone metabolism with plant growth promoting rhizobacteria in early stages of development in *Glycine max* var. Osumi. *J. Agric. Food Chem.*, 58: 1484–r1492.

Ramos Solano, B., Barriuso Maicas, J, Pereyra de la Iglesia, M.T., Domenech, J., Gutiérrez Mañero, F.J. 2008. Systemic disease protection elicited by plant growth promoting rhizobacteria strains: relationship between metabolic responses, systemic disease protection and biotic elicitors. *Phytopathology*, 98: 451–r457.

Ramos Solano, B., Lucas García, J., García-Villaraco, A., Algar, E., García Cristóbal, J., Gutiérrez Mañero, F.J. 2010b. Siderophore and chitinase producing isolates from the rhizosphere of *Nicotiana glauca* Graham enhance growth and induce systemic resistance in *Solanum lycopersicum L. Plant Soil*, 334: 189–r197.

Ramos, B., Lucas García, J.A., Probanza, A., Domenech, J., Gutiérrez Mañero, F.J. 2003. Influence of an indigenous European alder (*Alnus glutinosa L. Gaertn*) rhizobacterium (*Bacillus pumilus*) on the growth of alder and its rhizosphere microbial community structure in two soils. *New Forest*, 25: 149–r159.

Romeo, J.T., Ibrahim, R., Varin, L., De Luca, V. 2000. Evolution of metabolic pathways. *Recent Adv. Phytochem.*, 34: 1–r467.

Ruedeberg, C., Wiesmann, U.N., Brattstroem, A., Honegger, U.E. 2010. *Hypericum perforatum L.* (St John's wort) extract Ze 117 inhibits dopamine re-uptake in rat striatal brain slices. An implication for use in smoking cessation treatment? *Phytother. Res.*, 24: 249–r251.

Ruiz-García, Y., Gómez-Plaza, E. 2013. Elicitors: A tool for improving fruit phenolic content. *Agriculture*, 3: 33–r52.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y., Hunt, M.D. 1996. Systemic acquired resistance. *Plant Cell*, 8: 1809–r1819.

Saddiqe, Z., Naeem, I., Maimoona, A. 2010. A review of the antibacterial activity of *Hypericum perforatum L. J. Ethnopharmacol.*, 131: 511–r21.

Seco de Herrera, D. G., Bonilla, A., Algar, E., García-Villaraco, A., Gutierrez Mañero, F.J., Ramos-Solano, B. 2014.Enhanced blackberry production using *Pseudomonas fluorescens* as elicitor. *Agron. Sustainable Dev.,* 33: 385–r392.

Silva, B.A., Ferreres, F., Malva, J.O., Dias, A.C.P. 2005. Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chem.,* 90: 157–r167.

Sloan, E. 2004. Fortified foods get functional. *Funct. Foods Nutraceuticals,* 11: 18–r22.

Stein, A.J., Rodríguez-Cerezo, E. 2008. Functional food in the European Union. Technical Report by the Joint Research Centre of the European Commission, EUR 23380 EN. Luxemburg, European Communities. Pp. 74.

Suntar, I.P., Akkol, E.K., Yilmazer, D., Baykal, T., Kirmizibekmez, H., Alper, M., Yesilada, E. 2010. Investigations on the in vivo wound healing potential of *Hypericum perforatum L. J. Ethnopharmacol.,* 127: 468–r477.

Tallon, M. 2004. The expanding market for men's health ingredients. *Funct. Foods Nutraceuticals,* 11: 54–r59.

Tomas Laursen, T., Lindberg Moller, B., Bassard, J-E. 2014. Plasticity of specialized metabolism as mediated by dynamic metabolons. *Trends Plant Sci.,* 20: 20–r32.

Van Hulten, M., Pelser, M., van Loon, L.C., Corné, M.J.P., Ton, J. 2006. Cost and benefits of priming for defense in Arabidopsis. *Proc. Natl. Acad. Sci. USA,* 103: 5602–r5607.

Van Loon, L.C., Bakker, P.A.H.M., Pieterse, C.M.J. 1998. Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol.,* 36: 453–r483.

Van Peer, R., Niemann, G.J., Schippers, B. 1991. Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by Pseudomonas sp. strain WCS417r. *Phytopathology,* 91: 728–r734.

Van Wees, S.C.M., van der Ent, S., Pieterse, C.M.J. 2008. Plant immune responses triggered by beneficial microbes. *Curr. Opin. Plant Biol.,* 11: 443–448.

VanEtten, H., Mansfield, J.W., Bailey, J.A., Farmer, E.E. 1994. Two classes of plant antibiotics: phytoalexins versus "phytoanticipins". *Plant Cell,* 6: 1191–r1192.

Wei, G., Kloepper, J.W., Tuzun, S. 1991. Induction of systemic resistance of cucumber to Colletotrichum orbiculare by select strains of plant-growth promoting rhizobacteria. *Phytopathology,* 81: 1508–r1512.

Wink, M. 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry,* 64: 3–19.

Wink, M. 2010. Biochemistry of plant secondary metabolism, 2nd Rev. edn. Wiley–Blackwell. Pp. 464.

Yamane, Hisakazu. 2013. Biosynthesis of phytoalexins and regulatory mechanisms of it in rice. *Biosci. Biotechnol. Biochem.,* 77: 1141–r1148.

Zeisel, S.H. 1999. Regulation of "nutraceuticals". *Science,* 285: 1853–r1855.

Zhao. J., Davis, L.C., Verpoorte, R. 2005. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol. Adv.,* 23: 283–r333.