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Interaction between Human Pathogenic Bacteria and Plants: Possibilities of New Antimicrobial Drugs

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ABSTRACT

Diverse metabolites are elaborated by plant roots which are being used as pharmacophores or drugs in treating cardiovascular diseases, as general tonics, anti-hypertensive, psychotropic agents, etc. These belong to different chemical classes from simple sugars to complex-like sesquiterpenes and alkaloids. The nature of exudates elaborated by the root or metabolites accumulated entirely depends upon the biotic and abiotic stresses faced during acquisition of nutrients and flourishing within the soil. With increasing incidence of human pathogens in the soil and in particular in the rhizosphere it becomes imperative to establish an interaction between the two under *in vitro* conditions for exploring the chemical diversity produced by roots as a defense method. These molecules could serve as novel antimicrobial agents to combat human pathogens or serve as templates for designing superior antimicrobial agents to overcome the problem of antimicrobial drug resistance in human pathogenic bacteria.

Keywords: antimicrobials, elicitors, rhizosphere, roots, secondary metabolites

INTRODUCTION

Plant are innate chemical factories which produce a variety of chemical diversity having applications in different spheres revolving around the existence of humans on the earth thereby providing food, medicine and shelter. Use of plant and plant products dates back many thousand of years to the time when vegetables were the only source of therapeutics. Shaman and healers tried teas and extracts which were derived from natural sources through trial and error and had some efficacy to treat human diseases. These experiences were recorded and subsequently minor improvements were done which were documented in Chinese, Ayurvedic, European and African systems of medicine. The modern medicine and pharmaceutical sciences are also closely associated with plants and their biological effects on human system as a therapy to many diseases (Saxena and Kumar 2002). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan 1999).

Plants still are in mainstay despite the orientation towards synthetic chemistry as they provide diverse chemical templates which are developed into pharmacophores for use as medicine. Today, 11% of the 252 drugs considered as basic and essential by World Health Organization were exclusively of plant origin (Rates 2001). Further, a study by Fabricant and Farnsworth (2001) has indicated that 80% of the plant-derived drugs are related to their original ethnopharmacological use.

The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care needs, and about 85% of traditional medicine involves the use of plant extracts (Nolan and Labbe 2005). Therefore, about 3.5 to 4 billion people in the world rely on plants as sources of drugs. Infectious bacterial diseases are a major burden responsible for morbidity and mortality after cardiovascular diseases as they account for 13.3 billion deaths, or approximately 25% of the total global deaths (WHO 2002). This is attributed to growing antimicrobial drug resistance which is becoming a global public threat. The golden era of antimicrobial drug discovery has faded as antimicrobial drugs do not pack the same punch due to reduced susceptibility of the infectious bugs towards the current drugs. Frequently misuse and overuse of antimicrobials in many developing countries, had led to emergence of resistant pathogens which have been responsible for morbidity, mortality and cost of health care (Sharma *et al*. 2005). Nature serves as a reservoir for both drugs and bugs. The increased residual concentration of these antimicrobial drugs in the environmental reservoirs is predominantly responsible for the emergence of superbugs or multidrug resistant microbes. Some important human pathogens that have recently been reported to have acquired antibiotic resistance are; *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* (WHO 2002). Clinicians and clinical microbiologist's world over are facing a difficult situation with increase in drug resistant human pathogens and the quantum of chemical diversity getting narrowed as all possible biological matrices are being continuously being screened totally governed by serendipity in finding a new chemical template.

Plants, especially roots, face a variety of stresses during nutrient and resource absorption and assimilation from the soil. The stressors could be adjoining roots and rhizospheric microflora. To survive and flourish they develop extraordinary array of defense molecules (phytoalexins and phytoanticipins) have been looked at with a perspective of crop protection abilities but their potential in terms of medical applications in particular as antimicrobial drugs to overcome antibiotic resistance has been overlooked.

Soil is acting as a reservoir of pathogenic as well as non- pathogenic microflora depending upon the location and input in the soil in terms of manure application and the

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quality of irrigation water being received. Rhizosphere is the dynamic zone around the root which is characterized by the presence of root exudates and high microbial diversity of beneficial microbes. The high titers of human pathogenic bacteria in the soil alters the dynamics of soil microbiology in the rhizosphere and demands exploration of the biochemical mechanisms being used to resist attack by pathogenic microbes on the symbiotic bacterial flora and the plant root systems. This could be the new facet which could be efficiently exploited for discovery of new antimicrobial drugs.

RHIZOSPHERE AND ROOT BIOCHEMISTRY

Plant root are remarkable factories as they produce an array of bioactive compounds in the process of adjusting to the biotic as well as abiotic stresses in the rhizosphere. Some prominent sources from where the soil receives the human pathogenic microflora include hospital effluents, effluents from sewage treatment plants, water from poultry farms (Chitnis *et al*. 2000, 2004; Saxena 2007). Irrigation of fields and plantations with gray water and application of untreated or fresh manure or sewage sludge does not have total positive influence as is perceived since it adds heavy metal content, excess of labile organic matter and pathogenic microorganisms which can bioaccumulate within the crop or the plant (Mena *et al*. 2003). A number of microorganisms have been a cause of disease on plant leaves and roots (Walker *et al*. 2004; Jha *et al*. 2005). *Pseudomonas aeruginosa* colonizes in lungs and also establishes as a biofilm on roots of *Arabidopsis thaliana* and sweet basil (Walker *et al*. 2004). Internalization of the *E. coli* 0157: H7 have been reported in lettuce plant as well as in tissues of healthy cucumbers (Solomon *et al*. 2002; Johannessen *et al*. 2005). The interactions of these pathogenic microbes with rhizosphere flora are in a dynamic state involving biochemical signaling amongst the interacting microbial communities. If there is a negative interaction wherein the rhizosphere flora succeeds then it shall lead to inhibition of the pathogenic microbes thereby preventing their infection. Use of non-pathogenic *Streptomyces* sp. to curb the growth of pathogenic *Streptomyces scabies*. *Pseudomonas flourescens* has also been implicated to control *Erwinia carotovora* subsp. *atroseptica*, a pathogen of potato by production of antimicrobial compound 2,4-diacetylphloroglucinol (Cronin *et al*. 1997). These metabolites could be harvested in *in vitro* systems for their possible use as antimicrobials against drug-resistant pathogenic bacteria and fungi. Plants have already been selected as model systems for understanding the process of pathogenesis of plant as well as animal pathogens. Clinical isolates of Pseudomonas species have been found to incite soft rot like symptoms in variety of plants like tomatoes, onion and lettuce (Kominos *et al*. 1972). Arabidopsis ecotypes have exhibited severe soft rot symptoms on infection by a human pathogen UCBB_PA14. Some other multihost *Pseudomonads* like PAK, PAO-1 have also been identified (Rahmne *et al*. 1997). *Enterococcus faecalis* has also been found to colonize or incite disease in *Arabidopsis thaliana* (Jha *et al*. 2005). Clinical isolates of *Salmonella* from Centre for Disease Control (CDC), USA have been found to get into tomato roots and exist as endophytes, thus enhancing the chances of food borne diseases (Guo *et al*. 2002). A remarkable example of plant roots exhibiting a defense response to the challenge by pathogenic bacteria is by elicitation of rosmarinic acid, a multifunctional caffeine ester by sweet basil (*Ocimum basilicum*). Bacteria have been found to possess a common strategy to attack plant as well animals. Staphylococcal model of pathogenesis of *A. thaliana* leaves and roots have indicated reminiscent symptoms of bacterial pathogenesis by the plant pathogenic counterpart which included the colonization of leaves and the trichomes. However one study indicated that Arabidopsis mutants hyperaccumulating salicylic acid (precursor of analgesic aspirin) did not allow colonization of the *Staphylococcus aureus* indicating the difference chemotypes elaborated by plants could be important in designing new drugs for exploitation as antimicrobials (Shah 2003; Prithviraj *et al*. 2005). *Pseudomonas aeruginosa* has been found to form biofilm in case of cystic fibrosis in humans as well as on plants. Screening root exudates by plant to resist biofilm formation in plant could also be exploited for using them along with antimicrobial drugs after discovering the potential of humus mucus secretion as potential biofilm formation inhibitors (Singh *et al*. 2002). Mutualistic interactions between the plant roots and microbes can be exploited wherein the microbes produce better congeners than the original chemical templates i.e., they could serve as elicitors of the previously existing compounds as well as production of new phytoalexins/ phytoanticipins. Tropane alkaloids are found in plant roots like the thiarubrine and terthienyl which are found in *Calendula officinalis* roots are classical nematicides. Other important root compounds having pharmacological activities include Ginkgolides from maidenhair trees (*Ginkgo biloba*) which are used in heart ailments, dementia and senility, forskolin from Indian herb *Coleus forskolii* for used of treatment of Asthma and finally Camptothecin from Chinese medicinal herb *Camptotheca acuminata* which is the latest anticancer drug of plant origin (Flores and Curtis 1992; Flores *et al*. 1996). Plant roots have also been investigated for their antibacterial and medicinal properties. A variety of them belong to the classes of saponins and alkaloids (Hassan *et al*. 2003). Root culture of different species has been carried out to profile secondary metabolites which have potential health benefits (Flores and Curtis 1992). Roots can be modulated to enhance it biosynthetic capacities for production of secondary metabolites by exposing them to a variety of stressors which could be natural or synthetic in nature under *in vitro* conditions (Verpoorte *et al*. 1998; Pasqua *et al*. 2005) or precursor in experiments for biotransformation or through the expression foreign genes i.e., transgenics. Hairy root cultures have been a prominent method indicating the biosynthetic capabilities. About 200 species of dicot plants have been developed into hairy root cultures for harnessing the bioactive compounds produced by the roots. Enhancing biosynthetic capabilities of roots can be achieved by growing microbes with hairy roots as a co-culture or challenging them with plant and animal pathogenic microbes. It has been previously reported that co culture with fungi like the vesicular arbuscular mycorrhizae has led to the accumulation of different secondary metabolites in roots of barley (*Horedeum vulgare*) (Peipp 1997). Thus metabolites produced in a plant as a result of stress are in demand in the food, agriculture as well as pharmaceutical industry (Ramchandra and Ravishankar 2002). Plant sesquiterpeniods have been found to possess significant antimicrobial activity. They have also been documented to be induced in response to infection by a living cell (Stoessel *et al*. 1976). Potato hairy root cultures produce a variety of phytoalexins viz. rishitin, lubimin, phytuberin and phytuberol when the elicitor treatment of a fungus (*Rhizoctonia bataticola*) with cyclodextrin was used as an elicitor treatment for screening antimicrobial compounds and thus serves as model system for production of antimicrobial agents (Komaraiah *et al*. 2003). *Beta vulgaris* (sugar beet) was also found to produce viscosinamide when infected by the fungus *Pythium ultimatum* (Thrane *et al*. 2000).

Research has been primarily oriented in enhancing the biosynthetic capacities of the hairy roots or the *in vitro* roots by providing a combination of biotic as well as abiotic stresses.

Phytophthora cinnamoni was used as an elicitor in *Ocimum basilicum* hairy root culture where it enhanced the production of rosmarinic acid by 2.67 times of the untreated hairy root (Bais *et al*. 2002). Elicitation also increases the potential antimicrobial activity of the plant root secondary metabolites. Approximately 49% of the 966 species subjected to elicitation studies indicate accumulation of the bioactive compounds having antimicrobial activity. It actually doubled the number of species which were documented to possess antimicrobial activity (Poulev *et al*. 2003). Thus roots are metabolic factories synthesizing an array of known metabolites in bulk without actually destroying the original plants.

Plants used as food as well as medicine encounter multidrug resistant pathogenic bacteria in their ecological niche and evolve themselves to resist the stress of invasion by producing a vast diversity of new chemical templates. These new defense compounds so produced within the plants as a response to invasion would have the potential to kill them or to restrict their reproduction with the plant as a result they may also exist as endophytes i.e., getting internalized without harming the plant systems and staying in a dormant stage. Internalization has been significantly being reported in *Listeria monocytogenes*, *Clostridium botulinum*, *Salmonella* spp. and *E. coli* O157:H7 (Jablasone *et al*. 2005; Aruscavage *et al*. 2006). Studies have largely been undertaken in evaluation of the crude plant, herb or spice extract as an antimicrobial under *in vitro* conditions (Shetty and Labbe 1998; De *et al*. 1999; Sagdic *et al*. 2003). Garlic extracts are also found to possess potential antimicrobial efficacy in particular towards *Helicobacter pyroli* (Sivam *et al*. 1997). These studies indicate that plants possess chemopreventive principles which can be used effectively to have a good health status (Lampe 2003).

Carrot slices when challenged with *Listeria monocytogenes* in a buffer under *in vitro* conditions had a significant killing effect indicating their anti-listerial activity (Nguyen and Lund 1992). Similarly effects of spices as antimicrobial preservatives in ready to eat foods have also been evaluated. *Syzgium aromaticum* (clove) oil has been found to be effective in controlling *Listera monocytogenes* in chicken frankfurters (Hao *et al*. 1998; Mytle *et al*. 2006). However there are hardly any studies wherein metabolite profiling of the same plant under normal and stress conditions has been compared to identify the induced metabolites responsible for their overt antimicrobial action. Hence it is immensely important to develop reliable *in vitro* and *in vivo* assay methodologies to have a phytochemical comparison to assess induction of stressed metabolite possessing antimicrobial activity. Further studies on *Pseudomonas aeruginosa* PA14 indicates that conservation in virulence mechanism exist to infect host of divergent origins i.e., animals and plants (Rahmne *et al*. 2000). This further substantiates the hypothesis that the metabolites synthesized by plants as a result of stressed induced by the invading bacteria would also be beneficial in resisting or killing it in the animal systems too as they would have a similar mechanism to overcome the virulent microbe (Baarlen *et al*. 2007). Consequently screening the stress metabolites due of microbial invasion of plants would have a massive potential in antimicrobial drug development.

FUTURE SCOPE

The roots are protected in the rhizosphere by the nature of their exudates which govern the interactions between the beneficial and harmful microflora and this leads to masking the true potential to biosynthesize potential phytoanticipins which could serve as lead structure for new antimicrobial drug development. Underground, modified stems also would serve as a novel biological matrix since their defense mechanism would be entirely different from roots in soil. *In vitro* challenge studies on the roots or hairy roots of different plants and underground stems to screen the stress metabolites or defence compounds in case the human pathogen can adopt as a plant pathogen opens up avenues in uncovering an array of biomolecules with a potential to be developed into new antimicrobial drugs.

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