Recent Trends in Biotechnology

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RECENT TRENDS IN BIOTECHNOLOGY

Editors

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SCIENTIFIC PUBLISHERS (INDIA)

P.O. BOX 91 JODHPUR

Published by:
Pawan Kumar
Scientific Publishers (India)
5-A, New Pali Road, P.O. Box 91
Jodhpur – 342 001
Tel.: +91-291-2433323
Fax.: +91-291-2512580
E-mail: info@scinetificpub.com
www.scientificpub.com

ISBN: 81-7233-369-2

Lasertype set: Rajesh Ojha
Printed in India

PREFACE

National Conference on *Recent Trends in Biotechnology* was organized by the Department of Biotechnology, Amravati University, Amravati during February 3-5, 2000. The untiring efforts of Late Dr. B.B. Kadu, the organizer of the conference made it a grand success by enriching the knowledge of several participants. Dr. Kadu was hopeful of bioindustrial development in the country and had earnest desire for creation of a strong base for fundamental and fruitful research in the field of Biotechnology. Due to his sudden death he could not translate his ideas into actions. His idea of publication of the proceedings remained incomplete. The faculty members of Biotechnology Department took this mission of publication of the proceedings to fulfil the desire of the departed great Biotechnologist and to disseminate the knowledge of the current aspects of biotechnology for the benefit of Biotechnologists, Botanists, Zoologists, Pharmacologists and people loving the new and emerging discipline.

The proceedings include recent achievements in biotechnology, transgenics, bioprocessing, mushroom technology, plant tissue culture, biofertilizers and molecular biology.

We are thankful to all the contributors for their cooperation and also for submission of the manuscript. The help rendered by the researchers of Biotechnology department during the editing work is thankfully acknowledged. We thank Dr. (Mrs.) Kalpana Kadu for floating idea of publication of proceedings.

We are grateful to Professor Sudhir Patil, Hon'ble Vice Chancellor of Amravati university, Amravati for constant encouragement. We also express our sincere gratitude to Professor G.V. Patil, Hon'ble ex-Vice Chancellor for showing keen interest and for encouragement in bringing out the proceedings.

Lastly, we would like to thank M/S Scientific Publisher (India), Jodhpur for publication of the proceedings.

AMRAVATI **Editors**
May 15, 2004

FOREWORD

The Department of Biotechnology of Amravati University had organized a National Conference on ***Recent Trends in Biotechnology*** in the year 2000. I must acknowledge the sincere and dedicated efforts of Dr. B.B. Kadu, who was instrumental in organizing this National Conference. It was ultimately sad demise of Dr. Kadu, which has resulted in a heavy loss to the academics in the University. However, the committed students and admirers of Dr. Kadu have made all efforts to fulfil his cherished dream and the National Conference came to a reality.

The proceedings of this National Conference are now being printed by the Scientific Publishers (India), Jodhpur, Rajasthan. This definitely is a matter of credit and praise for the academic community of Amravati University in general and teachers of the Biotechnology Department in particular.

I am sure that these proceedings shall be of great help to the faculty members, students, academicians and researchers to upgrade their knowledge and enlarge the vistas of wisdom. I congratulate the faculty members and the participants of the conference for bringing out such an academically sound volume on biotechnology.

Dr. Sudhir Patil
Vice-Chancellor,
Amravati University
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Recent Trends in Biotechnology
Rai, Chikhale, Wadegaonkar, Thakare & Ramteke (eds.)
Scientific Publishers (India), Jodhpur (2004)

1

ECOLOGICALLY COMPETENT *RHIZOBACTERIA* FOR PLANT GROWTH PROMOTION AND DISEASE MANAGEMENT

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INTRODUCTION

Every year, severe global economic losses to agricultural crops are encountered due to plant diseases caused by more than sixty pathogens. Of various strategies of disease management, dominantly used chemical based strategies has resulted in serious imbalances in the agro-eco-systems. This had led to find alternative control approaches i.e., Integrated Pest Management (IPM) or Integrated Plant Disease Management (IPDM) for crop disease management. This shift towards non-chemical strategies is likely to correct the imbalance in public approach. Biological control of insect pests using microorganisms to suppress phytopathogens is a successful alternative approach to the use of synthetic agrochemicals.

During the last 50 years, the control of most of the plant diseases has fully relied on the use of disease resistant cultivars, improvement in cultural methods and the application of agrochemicals. Although, this has resulted in increased production of agricultural commodities, it has also led to the development of pesticide resistance in pathogens. The increasing public awareness about these problems has stimulated interest in the research on the use of biological control methods for controlling plant diseases[1].

Past decade has witnessed increasing interest in the role of *Rhizobacteria*; which can variously have positive, negative or neutral effects on plant growth[1]. Rhizosphere is inhabited by diverse group of microbes. Some of these bacteria not only benefit from the nutrients excreted by the plant roots but also beneficially influence the plant in direct or indirect way resulting in a stimulation of its growth[2]. The term PGPR or ``yield-increasing bacteria" (YIB)[3] has been used since 1974 in a broad sense and includes *Rhizobacteria* that promote plant growth through disease suppression and/or other processes[3-5]. The *Rhizobacteria* most commonly referred to as plant growth promoting *Rhizobacteria* (PGPR) are those with a major function in the inhibition of plant pathogens[6-7]. These PGPR can be classified according to their beneficial effects. For instance, nitrogen fixers, hormone producers and biocontrol agents[2].

Rhizosphere as a site for plant microbe interactions

Roots, as they grow through the soil, ooze organic substances in their wake, thus, providing a veritable feast for the plant's microbial friends and foes. Rhizosphere is under the direct influence of the roots of higher plants and therefore considered as intense ecological habitat for microorganisms. It is associated with a distinct, diverse community of metabolically active soil microbiota that carry out biochemical transformations. Among these, the production of biologically active metabolites, particularly the plant growth regulators (PGRs) by *Rhizobacteria* is of prime most importance[8].

1. PLANT GROWTH PROMOTING *RHIZOBACTERIA* (PGPR)

Rhizobacteria have the potential to increase plant growth directly by releasing phytohormones, fixing nitrogen in the rhizosphere, solubilising insoluble forms of nutrients such as phosphate, promoting mycorrhizal function[9] and regulating ethylene production in such root[6]. Besides this, some *Rhizobacteria* have the capacity to suppress major plant pathogens[6-7].

In addition to the well-known *Azatobacter* and *Azospirillum* sp., a number of other bacteria may be considered as PGPR, including various species of *Pseudomonas, Acetobacter, Alcaligenes, Klebsiella, Enterobacter, Xanthomonas*, and *Bacillus* sp.[10,11,6,8]. Nehl *et al.*[1] reported that seed inoculation with *Rhizobium leguminosarum* significantly increased the root growth of *Canola* and *Lettuce* and concluded that this bacterium should be considered as a PGPR.

Important attributes of PGPRs

Almost all PGPRs are capable of producing a variety of primary and secondary metabolites, which in turn serve as plant growth regulators (PGRs). Among the proposed mechanisms of action of PGPR, production of PGRs in the rhizosphere is most vital[12]. Schroth *et al.*[13]. recognized the importance of PGRs produced by *Rhizobacteria* and the susceptibility of plant roots to bacterial colonization. Production of PGRs is widespread among many Rhizobacteria viz., *Azotobacter, Azospirillum, Rhizobium* sp. and *Mycorrhizae*. It has been proposed that PGRs released as secondary metabolites by these inoculants may contribute to plant growth promoting effects, but only a few studies have directly demonstrated this cause-and-effect relationship.

Different PGRs excreted by Rhizobacteria include —

(a) Indole acetic acid (IAA)

Schroth *et al.*[13] reported that *P. syringae* was capable of producing copious amounts of IAA, leading to increased shoot-root ratio in sugar beet when applied as seed inoculant. Similarly, Duberkowsky *et al.*[14] observed that IAA produced by *Pseudomonas fluorescence* strain had a stimulatory effect on the root development of black currant softwood cuttings. Gutierrez-Maflero *et al.*[15] detected auxin-like compound in culture filtrate of *Bacillus pumilus* and *B. licheniformis*, which was capable of increasing aerial length, aerial surface, number of leaves and total nitrogen content. This effect was higher in bacterial metabolite treated non-nodulated plants than in IAA treated plants. Production of phytohormone is a mechanism of action for the enhanced plant growth response[16].

(b) Gibberellins (GAs)

The discovery of GAs as a class of natural hormones represent a classic example of the microbe-plant interaction. Bottini *et al.*[17] found that GA and Iso-GA excreted by *Azospirillum lipoferum* are involved in growth promotion of seedling root of *Graminae*. Reinfection of sterilized seeds with epiphytic microorganisms also leads to an increase in endogenous hormones (GAs) and increased growth rate.

(c) Cytokinin

Microbially produced cytokinins may play an important role in stimulating plant growth and development. Reddy *et al.*,[18] and Young *et al.*,[19] screened various Rhizobacteria for their biocontrol activity and reported that bacterial strain with biocontrol activity induced root elongation on cucumber and tomato suggesting a role for PGRs in plant growth. Hoflich[20] also observed cytokinins stimulated growth and yield increase in winter wheat, winter rape, oil radish, mustard and peas in pot and field experiments in response to inoculation with *Pseudomonas fluorescence* (isolated from rhizosphere of winter wheat).

(d) Ethylene

Ethylene produced by soil microorganisms contributes to the carbon pool of soil. Carbon is biologically active within an extremely low concentration range, and thus can increase plant growth and crop productivity. Recently, Glick and co-workers[5] in their studies of growth promotion by *Pseudomonas putida* GR 12-2, postulated that binding of *P. putida* GR 12-2 to the seed coat lower the level of 1-aminocyclopropane-1-carboxylic acid (ACC) from germinating seeds and thereby result in root elongation[6, 21-22].

2. BIOLOGICAL AGENTS

Suppressive soils contain Rhizobacteria that are able to control plant disease caused by fungi or bacteria. The mechanism responsible for this biocontrol activity includes competition for nutrients and niche, induced systemic resistance (ISR) and the production of antifungal metabolites (AFMs)[2]. Most of the identified *Pseudomonas* biocontrol strains produce AFMs, of which phenazine, pyrrolnitrin, 2, 4-diacetylpholoroglucinol (DAPG), pyoluterin, viscosinamide[23] and tensin[24] are the most frequently detected classes. Viscosinamide prevents the infection of sugar beet by *Pythium ultimum*[25].

(a) Rhizobacteria for biocontrol of plant diseases

A beneficial organism used to control a pathogen is referred to as Biological Control Agent (BCA)[26] or often, as an antagonist. Biological control of pathogens is species specific. Free-living beneficial Rhizobacteria have been extensively reviewed as beneficial crop inoculants, for improving plant growth[27-29]. Most approaches for biocontrol of plant diseases have used single biocontrol agent with inconsistent performance, because single organism is not likely to be active in all soil environments in which they are applied or against all plant pathogens. Control of a wide spectrum of pathogens by applied antagonists can largely be fulfilled by three main approaches : (i) modifying the genetics of biocontrol agent to be operable against more than one pathogen, (ii) developing strain mixtures with superior biocontrol activity and (iii) altering the environment to favor the biological control agent[30].

Many PGPR are known to promote the growth of several annual crops[31-33] by increased uptake of nitrogen[34], iron through siderophores and phosphorus, synthesis of IAA[5.35.21-22]. In addition, PGPR effectively lowers plant ethylene levels[22-23]. In this lies the importance of rhizobacteria as potential candidate for biocontrol and plant diseases management. Dube[36] has reviewed the information available on disease suppression by fluorescent pseudomonads involving antibiosis and competition.

(b) Characteristics of an ideal bacterial biocontrol agent

Over the last 20 years, several investigations have been initiated to find biological control agents for the suppression and control of plant diseases caused by various fungi, bacteria and viruses. Despite the numerous reports of successful experiments, there has been limited commercial success because of inconsistent field performance. Schroth *et al.*[13] have pointed out that using biocontrol strategies, plant diseases will continue to be erratic until there is better understanding of various factors which, influence the establishment and interaction of diverse communities in rhizosphere. To be an ideal antagonist the biological control agent should have (1) genetic stability, (2) high consistent efficacy, (3) ability to survive under adverse environmental conditions, (4) effectiveness against a wide range of pathogens on a variety of fruits and vegetables, (5) amenability for growth on an inexpensive medium in fermentor, (6) stability of the end products during storage, (7) non-production of secondary metabolites that might be toxic to humans, (8) strain resistance to standard fungicides and (9) compatibility with other chemical and physical treatments of the commodity such as heating and waxing.

(c) Source and mode of action of biocontrol agents

Rhizosphere, has been the primary source of plant growth promoting strains which, can act as biocontrol agents, such as *Trichoderma, Gliocladium, Serratia, Streptomyces, Agrobacterium, Bacillus* sp. and *Fluorescent pseudomonads*. Understanding the mechanism of action is important, because, it gives much idea in, determining the maintenance, enhancement and implementation of BCAs. BCAs interact with phytopathogens directly or indirectly by following mechanisms[26].

(i) Competition for nutrients and space

BCAs compete with the phytopathogens for available resources and thereby restrict them to colonize plant roots. Competition for micronutrients frequently occurs in soil, e.g., competition for Fe is one of the modes of action by which fluorescent pseudomonads limit the growth of pathogenic fungi and reduce disease incidence and severity. Under conditions of Fe stress, these bacteria produce siderophores, (Pyoverdin/ pseudobactin), which show higher affinity for Fe than fungal siderophores. Number of research papers and review articles have correlated bacterial antagonism with siderophores[34,37]. Other micronutrients (Cu, Mn, Zn etc.) also play important role in soil suppressiveness[38] for controlling plant diseases caused by soil borne pathogens.

(ii) Antibiotic and bacteriocin mediated antagonism

Antagonism is a common phenomenon responsible for biocontrol of many pathogens. It results from production of one or more secondary metabolites by a microorganisms toxic for another. A variety of different secondary metabolites such as, bacteriocins, enzymes and volatile compounds have been reported to be involved in suppression of different pathogens[39]. These antimicrobial compounds act by inducing fungistasis, inhibition of spore germination, lysis of fungal mycelia or by exerting a fungicidal effect[39]. *P. fluorescence* produces a potent antifungal antibiotic, phenazine which has been used to control take-all disease of wheat caused by *Gaeumannomyces graminis*. Pyoluterin and Pyrrolnitrin are other important metabolites of fluorescent pseudomonads[39].

Agrobacterium radiobacter, a first commercially applied biocontrol agent to control crown gall in dicotes has been reported to produce agrocin 84 a type of bacteriocin, which specifically inhibits *A. tumifaciens*[39].

(iii) Predation and parasitism

Predation and parasitism occurs when the BCAs feed directly on or within the pathogen, i.e. the biocontrol agent may be a predator or a parasite of the pathogen. Mycoparasites, such as *Coniothyrium minitans* and *Sporidesmium sclerotivorum* have been tested as biocontrol agents, and some of them are efficient in controlling diseases caused by *Sclerotinia* sp. and other Sclerotia forming fungi[40].

(iv) Induced systemic resistance (ISR)

First evidence that PGPR induces defense associated changes in plant physiology directed against spatially distant pathogens was first reported in 1991[41]. The *Fluorescent pseudomonads* have been reported to induce systemic resistance in the plants[42], which provides protection against a broad spectrum of phytopathogens[43]. ISR pathway is induced when the plant is challenged by pathogenic organisms. Bacterial determinants that are claimed to produce ISRs when present in nanogram amounts this include siderophores, the *O*-antigen of lipopolysaccharide and salicylic acid[43]. Induced disease resistance is enhanced by the simultaneous activation of ISR and systemic acquired resistance (SAR) pathways[43-48].

(v) Siderophore bearing microbes as potential biocontrol agents

Siderophores produced by *Pseudomonas* sp. and similar other rhizobacteria like *Alcaligenes, Enterobacter, Bacillus* etc. have been implicated in the biological control of several diseases, like damping off of cotton caused by *Pythium ultimum*, root rot of wheat caused by *Pythium* sp., potato seed piece decay caused by *Erwinia carotovora*, vascular wilts caused by *Fusarium oxysporum*, and stem rot of pea nut caused by *Rhizoctonia solani* and *Sclerotium rolfsii*[39]. Various workers have reported the antagonistic action of *Rhizobium, Azotobacter* and *Azospirillum* on phytopathogens[49-50]. Saikia and Bezbaruah[51] reported that hydroxamate type of siderophore producing *A. chrococcum* RRLJ 203 was capable of inhibiting *F. oxysporum, F. udum, F. solani, F. moniliforme, Ustulina zonata* and *Fomes lamnensis*.

Siderophore producing microbes function as a biocontrol agent, by depriving the pathogen from iron nutrition thus resulting in increased yield of potato tuber[37]. Freitas and Pizzinato[52] also reported the inhibition of *Colletotrichum gossypi* by rhizobacteria resulting in plant growth promotion in cotton seedlings. Sindhu *et al.*[39] reviewed the role of rhizobacteria in inhibition of phytopathogens Table 1[39]. Johri *et al.*[53] have also reviewed the role of *Fluorescent pseudomonas* strains RBT 13 isolated from rhizoplane in tomato disease management. Strain was capable of producing siderophores, which exhibited antagonistic action against several bacterial and fungal plant pathogens. However, only few microbes in ecosystem have the ability to produce powerful siderophores, which can restrict the proliferation of phytopathogens in rhizosphere. Such strains become ecologically competent bicontrol agents provided that they exhibit strong root colonizing ability and synthesize important metabolites, which can support pesticidal activity of siderophore[28].

Table 1. Siderophore bearing rhizobacteria for controlling infestations in cash crops

Biocontrol organism used	Target pathogen/disease	Crop
Pseudomonas fluorescence	*Erwinia carotovara*	Potato
	Gaeumannomyces graminis	Wheat
	Take-all	Wheat
	Fusarium glycinia	Soybean
	Sarocladium oryzae	Rice
Pseudomonas putida	*Fusarium* sp.	Radish, Cucumber
	Wilt	Beans,
	Fusarium solani	Potato
	Erwinia carotovora	
Pseudomonas cepacia	*Fusarium oxysporum*	Onion
Bacillus subtilis	*Fusarium roseum*	Corn
Bacillus sp.	*Rhizoctonia, Pythium*	Wheat
	Root rot and Take-all	
Rhizobium sp.	*Macrophomina phaseolina*	Soybean
Bradyrhizobium sp.	*Fusarium solani*	Sunflower
	Rhizoctonia solani	Mungbean

Source : Sindhu *et al.*, 1997[39]

(d) Advantages and disadvantages of using biocontrol agents

With increasing interest in biological control of soil borne pathogens, several companies now have developed biocontrol agents as commercial products[54, 38].

The biological control agents have various advantages, such as —

1. BCAs are safer than the chemicals now in use.

2. They do not impose the problem of biomagnification, as they do not accumulate in food chain.
3. Their self-replication circumvents repeated application.
4. Target organisms seldom develop resistance towards BCA as happens with the use of chemicals.
5. Properly developed biocontrol agents are not harmful to ecosystem as they originate from nature.

The major disadvantages include —

1. Variability of field performance.
2. Establishment and viability of threshold population of biocontrol organisms on planting material or in soil may drop below the requisite level and a drop in viability below the requisite level may eliminate the possibility of biocontrol.
3. Many soil edaphic factors viz. temperatures, soil pH, clay content, interaction of biocontrol organism with other rhizospheric bacteria and with pathogens also affects the viability of biocontrol preparation.

3. DEVELOPMENT AND COMMERCIALIZATION OF BIOCONTROL AGENTS

In the recent past, huge amount of work has been carried out on the biocontrol of plant diseases, which has also led to the development of commercial bioproducts. Some of the commercially available bacterial biocontrol products are listed in Table 2.[55]. It has been observed that biocontrol products identified to date control relatively narrow spectrum of diseases on a particular host crop. Some of the decisions that determine whether a biocontrol product is commercialized are business decisions not based on science. Before approaching for commercial production, a company must asses many factors including demand for the product, potential market size and existing competing products (Formulation). Jacobsen has[56] suggested following criteria for the development of ideal biocontrol product.

(i) Biocontrol products must have a relatively wide spectrum of efficacy.

(ii) The efficacy of the bioproducts must be high, consistent and reliable.

(iii) Biocontrol products also must meet the acceptable standards for environmental and toxicological safety.

(iv) The bioproducts must have acceptable shelf-life without special storage requirements.

(v) Manufacture of the biocontrol products also must be cost effective and provide consistent product quality.