

## Biosafety of Novel Bioinoculants

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### Abstract

With rapid advances in microbial discovery and inoculant technology, the realm of microbial inoculants has seen a significant shift from the conventional range of microbes viz., *Rhizobium*, *Azospirillum*, *Azotobacter*, etc. to include a wide range of bacterial and fungal genera that colonize the rhizosphere and promote plant growth in a myriad fashion. Such microbial strains with beneficial traits have been included under the umbrella terminologies of Plant Growth Promoting Rhizobacteria (PGPR) and Plant Growth Promoting Rhizofungi (PGRF). While this is definitely a welcome step, it brings along with it a host of pertinent questions, of which the biosafety of the microbial strains used for inoculant formulation is of paramount importance. This rationalization becomes imperative in the present scenario, where the etiology and pathogenesis of several hitherto unknown or lesser known bacterial species are being deciphered, and opportunistic pathogenic properties are being attributed to several commonly occurring environmental microbes. Another issue that needs to be factored in this paradigm is the possible horizontal gene transfer between naturally occurring microbes and the introduced inoculant strains. This assumes significance since horizontal gene transfer amongst organisms plays a larger role in the context of environmental protection and evolving antibiotic resistance. Hence a judicious analysis of the benefits and risks associated with novel microbial inoculants need to be addressed, before its eventual usage. Therefore it is imperative for microbiologist's agronomists and plant protection scientists to be aware about the latest trends in biosafety, in order to make informed decisions in their day to day work. Hence this article will primarily focus on the need for ensuring the biosafety of the newer bioinoculants, and the relevant regulatory frameworks that are in place internationally.

**Keywords:** Bioinoculants; Biosafety; Plant Growth Promoting Rhizobacteria, Plant Growth Promoting Rhizofungi (PGRF); Cartagena protocol; World Health Organization (WHO)

### What is Biosafety?

The term biosafety can be broadly described as the measures that need to be taken up for the prevention of large-scale loss of biological integrity, with a primary focus on both ecology and human health. It can also be described as the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release in the environment [1]. It is to be noted here that though biosafety primarily focuses on human health, the well-being of the ecosystem and its biological integrity are also of paramount importance. Though biosafety principles and practice have been in place in different countries worldwide, they were internationally rationalized and structured by the Cartagena Protocol on Biosafety to the Convention on Biological Diversity [2], as a supplement to the Convention on Biological Diversity. This protocol seeks to protect biological diversity from the potential risks posed by genetically modified organisms produced by modern biotechnology. The main focus of this protocol is the potential risks arising from the utilization of Living Modified Organisms (LMOs). The Cartagena Protocol on Biosafety makes clear that products from new technologies must be based on the precautionary principle and thereby allows developing nations to balance public health and economic benefits. This "precautionary principle" when applied for risk assessment and containment of potentially harmful organisms, can serve as an excellent guideline for national level risk profiling and hazard alleviation. Individual nations are at liberty to

legislate on the restrictions that are to be put in place for ensuring the wellbeing of the population and environment.

### Risk Classification of Microbes

Based on the risk profile of individual microbes, each country classifies the microbial agents in that country by risk groups based on pathogenicity of the organism, modes of transmission and host range of the organism. Country wise classification of risk groups may be influenced by existing levels of immunity, density / movement of host population presence of appropriate vectors and standards of environmental hygiene. A guiding principle for this exercise is the classification of infectious agents into risk groups by the World Health Organization (WHO), which proposed a four tier classification of infectious organisms [3], as described below.

- **WHO Risk Group 1** (no or low individual and community risk): A microorganism that is unlikely to cause human disease or animal disease
- **WHO Risk Group 2** (moderate individual risk, low community risk): A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited
- **WHO Risk Group 3** (high individual risk, low community risk): A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available

- **WHO Risk Group 4** (high individual and community risk): A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Though this system of classification has been recommended by the WHO, primarily for laboratory practice, most countries tend to follow a four tier system for classification of infectious agents. Individual nations are at liberty to assign individual microbes to appropriate risk groups and legislate a suitable legal frame work to alleviate the risks caused by infectious agents. Hence it may be observed that the same microbe may be assigned to different risk groups by different nations depending on the prevailing conditions.

## Biosafety versus Utility

It is quite a common knowledge that the soil and the interior tissues of plants are treasure troves of microbes and harbor quite a number of potential bioinoculant strains. If one were to survey both these environments, it's quite common to come across strains that have been assigned the opportunistic pathogenic status. But in reality many of these strains may not harbor pathogenic determinants, though the possibility of they acquiring the pathogenic arsenal via horizontal gene transfer cannot be ruled out. In the recent past, number of studies have reported the beneficial traits of microbial strains with possible human health considerations and there is ever increasing body of knowledge on the possible agricultural benefits of such microbes. Table 1, presents a non-exhaustive list of such microbial strains.

Organism	Source of Isolation/ Crop on which effect was recorded	Effect	Reference
<i>Enterobacter aerogenes</i>	Apples	Reported to control <i>P. cactorum</i> infection of apples in nursery soils	[4]
<i>Serratia plymuthica</i>	Cotton	Controls <i>Rhizoctonia solani</i>	[5]
<i>Enterobacter cloacae</i>	Cucumbers	Increased the yield of cucumbers	[6]
<i>Enterobacter ludwigii</i>	Lolium perenne L.	First report on the PGPR features of <i>E. ludwigii</i>	[7]
<i>Enterobacter</i> sp.	Coconut rhizosphere	Production of phytohormones, siderophores and antibiotics	[8]
<i>Enterobacter</i> sp.	Broccoli	Promoted growth of <i>Brassica oleracea</i> (broccoli)	[9]
<i>Serratia plymuthica</i>	Peppers	Biocontrol agent for management of <i>Phytophthora capsici</i>	[10]
<i>Acinetobacter calcoaceticus</i>	Wheat rhizosphere	Improved plant growth in pot and field studies. Has the ability to solubilise P, produce IAA and siderophores	[11]
<i>Enterobacter asburiae</i>	Mustard	Possesses the inherent ability to produce growth regulators in the presence of fungicides	[12]
<i>Enterobacter arachidis</i> sp. nov	Groundnut	A novel methylophilic nitrogen-fixing bacterial strain	[13]
<i>Escherichia coli</i>	Maize	Significantly enhanced plant growth and nutrient uptake	[14]
<i>Stenotrophomonas maltophilia</i>	Hazelnut seedlings	Increased plant growth promotion	[15]
<i>Bacillus cereus</i>	Arabidopsis	Induced resistance against a broad spectrum of pathogens including <i>Pseudomonas syringae</i> pv. tomato DC3000	[16]
<i>Enterobacter cancerogenus</i>	Pigeon Pea	Growth promotion observed in pigeon pea	[17]
<i>Enterobacter</i> sp.	Maize	Enhanced nitrogen accumulation and significantly, improved growth of maize seedlings over controls	[18]
<i>Enterobacter radicincitans</i>	Wheat	Biological nitrogen-fixing endophytic bacterium with growth-promoting effects on a variety of crops	[19]
<i>Pseudomonas aeruginosa</i>	Cowpea	Solubilizes phosphate, produces IAA, siderophore, HCN and ammonia	[20]
<i>Aspergillus flavus</i> , <i>Aspergillus fumigatus</i>	Rhizosphere of sugarcane, groundnut and paddy fields	P solubilization and IAA production	[21]
<i>Pseudomonas aeruginosa</i> stain NJ-15	Soil isolate	Antagonistic to a wide range of plant pathogenic fungi	[22]

<i>Pseudomonas aeruginosa</i> strain Zapa	Tomato	Positive for root colonization, indole acetic acid, salicylic acid, siderophore production and inhibited the growth of wide range of plant pathogenic microorganisms	[23]
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**Table 1:** A non-exhaustive compilation of the reported instances of plant growth promotion by microbial strains with possible human health concerns

Though it would be unfair to brand any of the above cited environmental microbial strains as pathogenic, solely based on their taxonomic assignment, until and otherwise proven by the principles laid out in the Kochs postulates [24], or by the detection of potentially pathogenic factors like toxins, under *invitro* conditions, the above information is meant to give the reader an insight on the crossroads between agricultural utility and biosafety of novel microbial inoculant strains.

### Biosafety - Risk Profiling and Evaluation

Since microbes are known to be omnipresent and omnipotent, it's not uncommon to find a microbial strain with superior bioefficacy from the most unusual of places, and likewise the description of taxonomically novel strains has seen a quantum shift with the improvements in nucleotide sequencing techniques. A common situation that most soil microbiologists find themselves often, is the presence of excellent plant growth promoting strains belonging to the genera *Stenotrophomonas*, *Acinetobacter*, *Enterobacter*, etc. from the soil environment. Though all strains of the above mentioned may not be pathogenic, the incidence of opportunistic infection by members of these genera is on the rise. Apart from these, several novel species with beneficial traits tend to emerge on the radar of an enterprising soil microbiologist. Though such strains are widely prevalent in the environment, they tend to exist in equilibrium in nature which ensures a natural biosafety net. But when such stains are selected and their population levels are raised to threshold levels to attain the desired effects of inoculation, it may raise public health and ecological concerns. In such a scenario, it's imperative to define a paradigm for utilization of unconventional strains for bioinoculant production.

A crucial step in this direction is the accurate determination of the taxonomy of the organism, by the polyphasic approach. This could include a combination of phenotypic, chemotaxonomic, and genotypic methods. This crucial step can be followed by the safety evaluation framework provided by the American Biological Safety Association (ABSA). The preliminary risk assessment process can be made based on the risk group level of the microorganisms as referenced in the classification database for infectious agents ([http:// www.absa.org/riskgroups/](http://www.absa.org/riskgroups/)). In order to further assess the biosafety of a novel microbial agent, its toxicity can be determined by acute toxicity tests (usually carried out on small laboratory animals), and its environmental effects on select animal species like fishes, earthworms, pollinators, etc. The acute toxicity procedures followed in most nations include oral toxicity/pathogenicity, pulmonary toxicity/pathogenicity, intravenous toxicity/pathogenicity, dermal toxicity/pathogenicity, eye irritation/infectivity, and reports of hypersensitivity incidents [25]. Thus it can be observed that a combination of acute toxicity tests and environmental evaluation can give a fair estimate of the biohazard potential of a novel microbe. But this system of evaluation that is followed worldwide does not factor the effects of chronic exposure and the possibility of horizontal gene transfer with environmental strains.

### Biosafety - the Indian and Global Scenario

India has been a pioneer in enacting biosafety legislations, with special reference to microbial inoculants. Some of the important legislations that have been enacted are discussed here in brief. The Rules for Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells (1989) under the Environmental Protection Act (1986) is a pioneering legislation. This Act defines the term microorganisms as "Microorganisms shall include all the bacteria, viruses, fungi, mycoplasma, cell lines, algae, protozoans and nematodes indicated in the schedule and those that have not been presently known to exist in the country or not have been discovered so far". An essential feature of this act is the enlistment of human and animal pathogens viz., fungal agents, parasitic agents, viral, rickettsial and chlamydial agents and special category agents, into risk groups II and III based on their risk profiles. Another interesting feature of this legislation is the creation of a separate section called as "Plant Pests" to accommodate potential plant pathogens. This act stipulates that no person shall import, export, transport, manufacture, process, use or sell any hazardous microorganisms or genetically engineered organisms /substances or cells except with the approval of the Genetic Engineering Approval Committee, which functions under the Ministry of Environment and Forests, Government of India. But with advances in microbial discovery and pathogenesis, this risk classification needs to be periodically updated in order to uphold the highest levels of biosafety while simultaneously harnessing the benefits of novel microbes for the benefit of mankind.

A second legislation of relevance in India is the Insecticide Act (1968), which has an exhaustive list of microbial biological control agents published under Section 3 of the Insecticide Act, and is amended from time to time by gazette notifications. All microorganisms notified under this Act, these require toxicology testing and mandatory registration with the Central Insecticide Board and Registration Committee, under the Ministry of Agriculture, Government of India, before commercial usage. Apart from these the Recombinant DNA Safety Guidelines (1990) notified by the Department of Biotechnology, Ministry of Science and Technology, Govt. of India, defines the biosafety measures that need to be put in place for the research activities, large scale use and also the environmental impact during field applications of genetically altered material products. These guidelines have assigned microbes based on their modes of transmission, host range of the agent, availability of effective preventive treatments or curative medicines, capability to cause diseases to humans/animals/plants, epidemic causing strains in India. While bacteria and fungi are accommodated into risk groups II and III, viruses have been accommodated into risk groups II, III and IV. By enacting pioneering legislations, India has placed an effective biosafety net and has regulated the use of potentially hazardous microbes, both from the animal and plant pathogenic point of view.

## Biosafety - Future Requirements

Though most nations tend to follow an animal model based testing for the biosafety evaluation of microbial inoculants, it needs supplementation by an equally rigorous environmental evaluation especially from the point of horizontal gene transfer in order to assess the potential of novel inoculant strains to acquire/donate genes. This is more pertinent for bacterial species with relatives that pose health and biosafety concerns. For novel genera/species to be used as microbial inoculants, the supplementation of acute exposure studies (conducted on small animal models), with chronic exposure studies, in order to determine the long term effects of chronic exposure would be pave the way for their unhindered usage for the benefit of mankind. Another issue that rarely catches the attention of biosafety regulators is the widespread use of a whole gamut of nondescript organic manures and formulations that harbor numerous beneficial and potentially harmful microbes alike. Though bringing them under a biosafety framework would be a Herculean task, some biosafety guidelines need to be evolved in order to reduce the risk perception caused by a possible magnification of potentially harmful microbes contained within these nondescript formulations.

From the academic point of view, the issue of reporting the mere isolation of a particular microbial species, from clinical specimens without establishing the cause – effect relationship between the isolated microbe and disease/symptom incidence needs to be rationalized. This is a pertinent issue, since the mere report of an environmental microbe from the clinical environment does not necessarily render it pathogenic, but tends to send alarm bells across the scientific communities both clinical and non-clinical alike and creates a stigma around the reported microbial species. While a high degree of caution is to be exercised, in the selection of microbial inoculant strains, it also has to be ensured that potentially beneficial strains are not left behind due to the lack of rational evaluation procedures.

## Conclusion

In microbial inoculant technology, it is imperative that the fruits of modern science reach the target clientele in order to enhance food production, simultaneously a critical balance has to be maintained while viewing benefits derived from these technologies viz a viz the probable risks posed by the novel technologies and microbial strains used for inoculant formulation. In such a scenario the biosafety of novel microbial strains, is of paramount importance and suitable regulatory frameworks have to be put in place in order to ensure that benefits and risks associated with such novel strains are properly balanced and do not pose a challenge to the health of higher forms of life and the environment. Such a regulatory process has to be dynamic and has to keep pace with the rapid progress in scientific discoveries, in order to ensure the larger objective of enhancing food production with reduced chemical input usage.

## Disclaimer

The views expressed herein are those of the authors only. It may not necessarily be the views of the institution/organization the authors are associated with.

## References

1. Biosafety and the environment: An introduction to the Cartagena Protocol on Biosafety (2003) GE.03-01836/E, United Nations Environment Programme. (undated), 8.
2. Cartagena protocol on biosafety (2000) Secretariat on the Convention on biodiversity, Montreal.
3. Laboratory Biosafety Manual (2004) (3<sup>rd</sup>edn), World Health Organization, Geneva.
4. Brewster DT, Spiers A G, Hopcroft DH (1997) Biocontrol of *Phytophthora cactorum* in vitro with *Enterobacter aerogenes*. New Zeal J Crop Hort 25: 9-18.
5. Chernin L, Ismailov Z, Haran S, Chet I (1995) Chitinolytic *Enterobacter agglomerans* antagonistic to fungal plant pathogens. Appl Environ Microbiol 61: 1720-1726.
6. Georgieva O (2003) *Enterobacter cloacae* bacterium as a growth regulator in green house cucumbers (*Cucumis sativus* L.). Cucurbit Genetics Cooperative Report 26: 4-6.
7. Schoebitz M, Ribaudo C, Ciampiy L, Poncelet D (2007) Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from *Lolium perenne* L. rhizosphere. XVth International Workshop on Bioencapsulation, Vienna, Au. Sept 6-8, 1.
8. Gupta A, Murali G (2008) Siderophore production by plant growth promoting rhizobacteria. Indian J Agric Res 42: 153-156.
9. Zakria M, Ohsako A, Saeki Y, Yamamoto A, Akao S (2008) Colonization and growth promotion characteristics of *Enterobacter* sp. and *Herbaspirillum* sp. on Brassica oleracea. Soil Sci Plant Nutr 54: 507-516.
10. Shen SS, Piao FZ, Lee BW, Park C S (2007). Characterization of antibiotic substance produced by *Serratia plymuthica* A21-4 and the biological control activity against pepper *Phytophthora* blight. The Plant Pathology Journal. 23: 180-186.
11. Prashant S, Makarand R, Bhushan C, Sudhir C (2009) Siderophoregenic *Acinetobacter calcoaceticus* isolated from wheat rhizosphere with strong PGPR activity. Malays J Microbiol 5: 6-12.
12. Ahemad M, Khan MS (2010) Plant growth promoting activities of phosphate solubilizing *Enterobacter asburiae* as influenced by fungicides. Eur Asia J Bio Sci 4: 88-95.
13. Madhaiyan M1, Poonguzhali S, Lee JS, Saravanan VS, Lee KC, et al. (2010) *Enterobacter arachidis* sp. nov., a plant-growth-promoting diazotrophic bacterium isolated from rhizosphere soil of groundnut. Int J Syst Evol Microbiol 60: 1559-1564.
14. Nautiyal CS1, Rehman A, Chauhan PS (2010) Environmental *Escherichia coli* occur as natural plant growth-promoting soil bacterium. Arch Microbiol 192: 185-193.
15. Erturk Y, Cakmakci R, Duyar O, Turan M (2011) The effects of plant growth promotion rhizobacteria on vegetative growth and leaf nutrient contents of hazelnut seedlings (Turkish hazelnut cv, Tombul and Sivri). Int J Soil Sci 6: 188-198.
16. Niu DD, Liu HX, Jiang CH, Wang YP, Wang QY, Jin H L, Guo J H (2011) The Plant growth-promoting rhizobacterium *Bacillus cereus* AR156 induces systemic resistance in *Arabidopsis thaliana* by simultaneously activating salicylate- and jasmonate/ethylene-dependent signaling pathways. Molecular Plant Microbe Interact 24: 533-542.
17. Usha Rani M, Arundhathi, Reddy G (2011) *Bacillus cereus* and *Enterobacter cancerogenus* screened for their efficient plant growth promoting traits rhizobacteria (PGPR) and antagonistic traits among sixteen bacterial isolates from rhizospheric soils of pigeon pea. Afr J Microbiol Res 5: 2090-2094.
18. Ogbo F, Okonkwo J (2012) Some characteristics of a plant growth promoting *Enterobacter* sp. isolated from the roots of maize. Adv Microbiol 2: 368-374.
19. Witzel K1, Gwinn-Giglio M, Nadendla S, Shefchek K, Ruppel S (2012) Genome sequence of *Enterobacter radicincitans* DSM16656(T), a plant growth-promoting endophyte. J Bacteriol 194: 5469.
20. Bhakthavathalu S, Shivakumar S, Sullia SB (2013) Characterization of multiple plant growth promotion traits of *Pseudomonas aeruginosa* FP6,

- 
- a potential stress tolerant bio-control agent. Ann Biological Res 4: 214-223.
21. Priya S, Panneerselvam T, Sivakumar T (2013). Evaluation of Indole-3-Acetic Acid in phosphate solubilizing microbes isolated from rhizosphere soil. Int. J. Curr. Microbio App. Sci, 2(3), 29-36.
22. Bano N1, Musarrat J (2003) Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. Curr Microbiol 46: 324-328.
23. Hariprasad P1, Chandrashekar S, Singh SB, Niranjana SR (2013) Mechanisms of plant growth promotion and disease suppression by *Pseudomonas aeruginosa* strain 2apa. J Basic Microbiol .
24. Koch R (1884) Die Aetiologie der Tuberkulose, Mittheilungen aus dem Kaiserlichen Gesundheitsampte. 2: 1-88. In: TD Brock, editor. Milestones in Microbiology: 1556 to 1940. ASM Press. 116.
25. Young CC, Shen FT, Singh S (2012) Strategies for the exploitation and development of biofertilizer. In: Maheshwari DK (Ed.) Bacteria in Agrobiolgy: Plant Probiotics. Springer-Verlag Berlin Heidelberg. 127-139.