Biology and Fertility of Soils (2020) 56:439–442
https://doi.org/10.1007/s00374-020-01466-9

INTRODUCTION

Application of beneficial microorganisms and their effects on soil, plants, and the environment: the scientific legacy of Professor Yoav Bashan

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Published online: 8 May 2020

Yoav Bashan was born in Haifa, Israel, in 1951, and died in Auburn, Alabama, in 2018. He started his scientific career in the 1980s, initially in the Hebrew University and in the Weizmann Institute (Israel), through 1990 when he moved to La Paz (Mexico) where he was actively working until his death. During his career, Professor Bashan was involved in many aspects of plant-bacteria interactions, microbial-assisted ecological restoration of disturbed arid environments, environmental microbiology of the hot deserts, and the development of bacterial inoculants for agriculture and environmental purposes. His key contributions of a nearly 40-year scientific career will be summarized in this Introduction.

Contribution to scientific terms and concepts

Since its introduction by Kloepper et al. (1980), the term plant growth-promoting rhizobacteria (PGPR) had been used to refer to beneficial bacteria associated with roots. However, in 1998, Professor Bashan proposed two new terms for general scientific use that would seem to encompass all the plant beneficial bacteria: "biocontrol plant growth-promoting bacteria" (Biocontrol-PGPB) and "plant growth-promoting bacteria" (PGPB) (Bashan and Holguin 1998). Since then, the term PGPB is considered synonymous with PGPR, although it is a more inclusive one, as it comprises also endophytic bacteria, phyllosphere bacteria, and bacteria associated with microalgae.

Most of Yoav Bashan's research was focused on the PGPB *Azospirillum* spp. After years studying the mode of action of the bacteria, he proposed two hypotheses: in 1990, he published the Additive Hypothesis, stating that the effect of *Azospirillum* on plants cannot be reduced to one specific mechanism, but to several mechanisms operating either at the same time or sequentially (Bashan and Levanony 1990). In 2010, he presented the updated Multiple Mechanisms Hypothesis, based on the assumption that there is no single mechanism involved in promoting plant growth with *Azospirillum*, but rather a combination of a few or many mechanisms in each specific case of inoculation. The mechanisms may vary with plant species, *Azospirillum* strain, and environmental conditions prevailing during the interaction (Bashan and de-Bashan 2010).

In 1986, Professor Bashan developed an alginate-based encapsulated inoculants of *Azospirillum brasilense* (Bashan 1986); that was the start point of the use of synthetic inoculants as an alternative to the delivery of PGPB in the field. Years after, he overhauled the initial inoculant and proved that by reducing the size of the inoculant there was a possibility to develop a highly effective powder-like formulation (Bashan et al. 2002).

Work on plant-microorganisms interaction of arid ecosystems

Prof. Bashan contributed significatively to the knowledge of the interactions between microorganisms and plants in the

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desert. His studies focused on the Sonoran Desert (Mexico), where agriculture is limited, but environmental problems such as desertification, loss of soil fertility, and lack of water are pervasive. Years of fundamental studies on the microbiology of the desert and the effect of PGPB and mycorrhizae on the establishment and growth of native plants, including the climax plant giant cardon cactus, resulted in long-term field experiments of restoration, and a set of manuscripts documenting the success (Bashan et al. 2009a, 2009b, 2012; Lopez-Lozano et al. 2016; Moreno et al. 2017).

On a more ecological approach, he studied the phenomenon of rock weathering by microbial-assisted cacti, showing that both endophytic and rhizospheric bacteria associated with these desert plants are mainly responsible for the solubilization of minerals from desert rocks, allowing the plants to establish in the absence of soil (Puente et al. 2004a, 2004b, 2009; Lopez et al. 2009, 2011, 2012).

Additionally, from his work on arid mangroves, he established two fundamental parameters for their conservation and restoration: (1) promoting functional hydraulic conditions that allow the free passage of tides within the ecosystem (Bashan et al. 2013a) and (2) maintaining the interactions between mangrove species and their associated microorganisms, mainly plant growth-promoting microorganisms (PGPB) including nitrogen fixers and phosphate solubilizers, without which trees cannot develop (Holguin et al. 1992; Puente et al. 1999; Rojas et al. 2001; Toledo et al. 2001; Vovides et al. 2011).

Editorial policies

Professor Bashan gave a fundamental contribution to the policy that guides *Biology and Fertility of Soils*, not only for his competent review of the assigned manuscripts but also for publishing three editorials. The first one demonstrated that it is a mistake to use tricalcium phosphate (TCP) as a sole source of P to isolate and characterize phosphate solubilizing bacteria (PSB) (Bashan et al. 2013b). He also published an editorial about the need for the disclosure of the microbes and other constituents present in microbe-based or pesticide-based products used in research (Bashan et al. 2016) and an editorial about the errors that can be produced when using fresh weight instead of dry weight during studies aiming at determining the effect of PGPB on plant growth (Bashan et al. 2017).

Bashan Foundation and Bashan Institute of Science

Beyond his scientific career, Yoav Bashan worked to spread science mostly to developing countries, through the creation of the Bashan Foundation and the Bashan Institute of Science. The Bashan Foundation was created in 1999, as a private, nonprofit organization, with the sole mission to promote high-quality, scientific research by providing free access to research papers and research data supported by the Bashan Foundation via its website (www.bashanfoundation.com). In 2016, he created the Bashan Institute of Science (www.bashanis.org), located in Alabama (USA). The institute is a nonprofit scientific research organization with the mission of creating novel cutting edge scientific advances by continuously exploring the unknown, development of new scientific ideas and concepts for the benefit and advancement of mankind, and enhancing quality of life. Prof. Bashan established that the driving force behind its operation is the constant needs of society for better living, done through creating new knowledge and new, and continuously changing, scientific solutions and new technologies, mostly in biology.

Some research needs suggested by Professor Bashan: career and legacy to the scientific community

As stated in Pereg et al. (2016), for a successful plant-PGPB interaction, plants must harbor the bacteria attached to or inside their tissues. However, most studies do not include tracking or monitoring of the bacteria after their application. Since his early works, Prof. Bashan was searching for available, easy, and effective methods that will allow the clear visualization of attachment and colonization of the bacteria on plant roots of the plants after inoculation. According to Rilling et al. (2019), methods based on reporter genes, immunological reactions, and nucleic acids have been applied to track or monitor PGPB in seeds, soils, or plants after inoculation. Some of these approaches were implemented by Prof. Bashan. Thus, for the immunological detection of *Azospirillum brasilense*, enzyme-linked immunosorbent (ELISA) (Levanony et al. 1987) and immuno-gold labeling (Levanony et al. 1989) assays were developed; Lopez et al. (2011) followed the endophytic colonization of bacteria inoculated in small cactus by immunostaining; de-Bashan et al. (2010) traced *A. brasilense* Sp6 in the rhizosphere of *Atriplex lentiformis* by denaturing gradient gel electrophoresis (DGGE); Bacilio et al. (2004) followed root colonization of *Azospirillum lipoferum* tagged with the green fluorescent protein gene (*gfp*). Finally, Trejo et al. (2012) applied fluorescent in situ hybridization (FISH) to visualize colonization of *A. brasilense* in sorghum roots, and Posada et al. (2016) developed a FISH species-specific probe for the identification of *Bacillus pumilus* in banana roots. In summary, and as clearly presented in Rilling et al. (2019), albeit the fact that methods for monitoring colonization are difficult to implement and time consuming, finding the correct one is paramount for the assessment of a successful inoculation and interaction between the plant and the PGPB.

The special issue

As his main research topic, this special issue dedicated to Professor Yoav Bashan contains contributions related to the use of PGPB as inoculants. There are 9 original manuscripts dealing with different aspects of isolation and characterization of PGPBs; development of mixed inoculants; inoculation procedures; analysis of persistence and colonization of the inoculated bacteria; effect of the inoculation on growth of crops such as winter wheat, ancient wheat, and sugarcane; biofertilizers for biocontrol of pathogens; and changes in rhizosphere microbial communities as a result of inoculation.

Besides the research manuscripts, there are two reviews. One deals with bioweathering in arid lands mediated by plant-microbe interactions and proposes a conceptual model of rock weathering where microbial associates induce higher root exudation of organic acids in succulents. The other review discusses the evolution of the research on the agronomical use of *Azospirillum* and its uses for environmental purposes and biotechnological applications beyond the agricultural industry. Finally, a novel hypothesis is proposed to explain the plant growth promotion capability of these bacteria.

Lastly, there is an Editorial written mainly by Prof. Bashan, proposing that manuscripts submitted to *Biology and Fertility of Soils* must contain exact protocols of fermentation and identity of microorganisms that form advanced consortia within microbe-based products.

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