

Genomics of the plant growth promoting bacterium FZB42 - towards a novel generation of biofertilizers

Xiao Hua Chen¹, Helmut Junge², Yueqiu He³, Qi Wang⁴, Xuewen Gao⁵ and Rainer Borriss¹

¹ Bakteriengenetik, Institut für Biologie, Humboldt Universität, Berlin, Germany, ²ABiTEP GmbH Berlin, Germany, ³Yunnan Agricultural University, Kunming, China, ⁴Chinese Agricultural University, Beijing China, ⁵Nanjing Agricultural University, Nanjing China

The explosion of the world population and the demand of high quality of the life required a higher productivity of agriculture leading to the enhanced use of chemical fertilizers and pesticides. Now, environmental pollution becomes a big problem for our planet. To solve this problem, alternative routes for solving recent and future nutrition problems are a pressing need. One of the most promising approaches is partial or complete substitution of harmful agrochemicals as chemopesticides and chemofertilizers by environmental microorganisms, which are supporting plant growth and acting against a large spectrum of plant pathogens including bacteria, fungi and nematodes (1). *Bacillus* based bioformulations have important advantages compared to other microbial biocontrol and biofertilizer agents:

1. *Bacillus* endospores with long term viability guarantee constant product quality over a long period of time
2. Living spores can be applied directly to seeds or plant roots. Spores are able to germinate and multiply in this environment and will produce the active substances during colonizing plant surfaces or cells.
3. Although the underlying mechanisms are not completely understood, during last years, much progress has been obtained in analyzing the molecular basis responsible for the beneficial effect on plant growth exerted by those bacteria.

The plant root bacterium *Bacillus amyloliquefaciens* strain FZB42 has been isolated by ABiTEP GmbH (2), a German biotechnology company specialized on screening and production of plant growth promoting bacteria. FZB42 is characterized by its beneficial effect on plant growth and, in addition, by its ability to act as biocontrol agent suppressing competing phytopathogenic organisms living in the vicinity of plant roots. Numerous laboratory and field trials performed in Europe, the US and Asia including China confirmed the beneficial effect exerted by FZB42 and its utility for a productive agriculture with simultaneous reduction of harmful chemical fertilizers and pesticides (<http://www2.hu->

berlin.de/chinapgp/).

To comprehensively understand the molecular mechanism underlying plant growth promotion, we have sequenced the whole genome of FZB42. FZB42 is the first plant growth promoting Gram-positive bacterium which has been completely sequenced (3). Its relatively small genome of 3,916 kb containing 3,693 protein-coding sequences is characterized by the absence of extended phage insertions which are typical for the closely related *B. subtilis* 168 genome but contains on the other hand several DNA islands in whose genes unique in FZB42 were found clustered. They are defined by local deviations in the tetranucleotide usage patterns (OUV) from the signature of the whole FZB42 genome. Island 7 is located between 1,164 and 1,193 kb corresponding to a region occupied by prophage 4 in *B. subtilis* 168. Several of the genes clustered in this 28,745 bp DNA island exhibit striking similarity to genes involved in extracellular arabinogalactan hydrolysis, galactose uptake by a sugar specific PTS IIABC, and catabolism (Leloir pathway) in enterococci, lactobacilli, and *Erwinia carotovora*. It can be assumed that acquisition of this molecular toolbox consisting of several elements derived from other soil and plant associated bacteria unrelated to *Bacilli* enhanced the ability of FZB42 to utilize additional plant-derived polysaccharides of its rhizosphere environment. A possible advantage in competing with other plant associated organisms.

The FZB42 genome harbors an unappreciated potential to produce secondary metabolites, many of them with antibiotic activity. In addition to five gene clusters known from *B. subtilis* to be involved in non-ribosomal synthesis of secondary metabolites, four giant gene clusters directing synthesis of two antimicrobial peptides, bacillomycin D and a hypothetical dipeptide, and two polyketides, difficidin and macrolactin, were identified (4-6). In total, FZB42 utilizes more than 8.5% of its whole genomic capacity to synthesize antimicrobial substances. The impressive genetic capacity to produce antagonistic acting secondary metabolites not only enables FZB42 to cope successfully with competing organisms within its natural environment, but also protect the plants from the pathogenic bacteria and fungi.

Phytohormone-like acting compounds previously have been suggested to be involved in the phytostimulatory action exerted by FZB42. Analyses by HPLC and GC MS with culture filtrates of FZB42 confirmed the presence of the plant growth hormone indole-3-acetic acid (IAA, 7). A tryptophan-dependent pathway of IAA synthesis in FZB42 was proposed using the whole genome sequence information.

In summary, our results demonstrate that *Bacillus amyloliquefaciens* FZB42 uses versatile ways to promote plant growth and to suppress its competing microflora, enabling the

organism to act as biofertilizer and as biocontrol agent as well. Based on the information available from the whole genome sequence we are now ready to develop new strategies to better exploit the positive effects exerted by FZB42 and related bacteria for a productive agriculture. One example is combining of secondary metabolites present in culture broth with living spores, able to germinate on plant surfaces. At present, field trials performed in different parts of the People Republic of China are underway in order to examine more carefully the beneficial effect on Chinese crops exerted by FZB42. These experiments are performed in frame of a Chinese German collaboration project¹ with the aim to extend application and improve quality of beneficial bacteria used in Chinese agriculture. The German partners involved in this program are Humboldt University Berlin and the AbiTep GmbH Berlin. The main Chinese partners are Yunnan Agricultural University, Kunming, Chinese Agricultural University, Beijing, Agricultural University Nanjing, and the FengHe Co. Ltd. Kunming, Yunnan province (detailed information is available under <http://www2.hu-berlin.de/chinapgp/>). In near future, we plan to produce and to introduce into the Chinese market high quality level bioproducts, which should enhance significantly plant growth, by a joint venture enterprise formed by German and Chinese companies. Stepwise substitution of classical agrochemicals by bioformulations prepared from beneficial *Bacillus* strains will lead to a better practice of sustainable land use and avoiding further risks of water pollution. The present program performed in collaboration by Chinese and German scientists will hopefully speed up this process.

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¹ A step directed to sustainable agriculture by use of bacterial formulations which support plant growth and suppress plant diseases caused by phytopathogenic microorganisms. Leading German institution is Humboldt University, Institut für Biologie, Bakteriengenetik. Contact: Prof. Rainer Borriss rainer.borriss@rz.hu-berlin.de (<http://www.chinapgp.net>)