Legume and microbial genetic resources in the face of climate change: innovation for a sustainable agriculture of the future

Antonio M. DE RON*; A. Paula RODIÑO; J. Dorinda POSE

Biology of Agrosystems (BAS). Misión Biológica de Galicia (MBG), Spanish National Research Council (CSIC). Pontevedra, Spain.

* amderon@mbg.csic.es

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Abstract

The excessive use of nitrogen fertilizers in intensive agriculture, aimed at meeting global food demand, generates serious environmental impacts, such as the contamination of surface and groundwater due to nitrate accumulation and increased emissions of nitrogen oxides (NOx), powerful greenhouse gases that contribute to global warming and climate change. In response to this problem, symbiotic nitrogen fixation is emerging as a sustainable alternative for reducing dependence on chemical fertilizers. In this context, the BAS group identified in 2020 the bacterium *Burkholderia alba* in common bean roots in the experimental fields at the MBG-CSIC (Pontevedra, Spain). This is a relevant finding given that this bacterium had only been previously identified in South Korean soils in 2018. Through inoculation trials on various legumes of interest for human food and animal feed, it was shown that *B. alba* is a promiscuous symbiotic bacterium, capable of associating with multiple legume species, a unique characteristic not described in other known symbiotic bacteria. This discovery opens new perspectives for its application as a bioinoculant in sustainable agriculture, as it could optimize biological nitrogen fixation and reduce the use of synthetic fertilizers, thus reducing greenhouse gas emissions and mitigating their impact on climate change. An innovative approach is to explore the potential of *B. alba* as a bioinoculant for legumes, evaluating its efficacy in promoting plant growth and nitrogen fixation. In addition, potential synergistic interactions with other symbiotic bacteria should be investigated to maximize their agronomic and environmental benefits.

Resumen

El uso excesivo de fertilizantes nitrogenados en la agricultura intensiva, destinada a satisfacer la demanda mundial de alimentos, genera graves impactos ambientales, como la contaminación de aguas superficiales y subterráneas por la acumulación de nitratos y el aumento de las emisiones de óxidos de nitrógeno (NOx), potentes gases de efecto invernadero que contribuyen al calentamiento global y al cambio climático. En respuesta a esta problemática, la fijación simbiótica de nitrógeno se perfila como una alternativa sostenible para reducir la dependencia de los fertilizantes químicos. En este contexto, el grupo BAS identificó en 2020 la presencia de la bacteria *Burkholderia alba* en raíces de judía común en parcelas experimentales en la MBG-CSIC (Pontevedra, Spain). Se trata de un hallazgo relevante dado que esta bacteria solo se había identificado previamente en suelos de Corea del Sur en 2018. Mediante ensayos preliminares de inoculación en diversas leguminosas de interés para alimentación humana y animal, se puso de manifiesto que *B. alba* podría ser una bacteria simbiótica promiscua, capaz de asociarse con múltiples especies de leguminosas, una característica única no descrita en otras bacterias simbióticas conocidas. Este descubrimiento abre nuevas perspectivas para su aplicación como bioinoculante en la agricultura sostenible, ya que podría optimizar la fijación biológica de nitrógeno y reducir el uso de fertilizantes sintéticos, reduciendo así las emisiones de gases de efecto invernadero y mitigando su impacto en el cambio climático. Un enfoque innovador sería explorar el potencial de *B. alba* como bioinoculante para leguminosas, evaluando su eficacia para promover el crecimiento vegetal y la fijación de nitrógeno. Además, se deberían investigar posibles interacciones sinérgicas con otras bacterias simbióticas para maximizar sus beneficios agronómicos y ambientales.

Keywords: agrobiotechnology, bioeconomy, environmental microbiology, plant genetic resources, sustainable agriculture.

Background

Climate change is a natural process that has recently been accelerated or aggravated by anthropogenic factors (Stern and Kaufmann 2014). It is a challenge for global agriculture, affecting productivity, biodiversity, and food security. Plant genetic resources for food and agriculture, as well as microbial resources, are strategic tools for a resilient, sustainable, and innovative agriculture of the future. The valorization and efficient use of legumes and their symbiosis with beneficial microorganisms is necessary within the framework of European and global sustainability strategies (De Ron 2015). Agricultural production, the basis of food security, uses nitrogen fertilizers that emit highly potent greenhouse gases that produce global warming and climate change.

The BAS group identified in 2020 the bacterium *Burkholderia alba* in common bean roots in the experimental fields at the MBG-CSIC, that had only been previously identified in South Korean soils in 2018 (Lee et al. 2018). Legumes mitigate these effects through their ability to associate symbiotically with nitrogen-fixing bacteria, such as *Burkholderia alba*, which could associate with different legumes and limit the use of nitrogen fertilizers and their gas emissions. The efficient and commercial development of biofertilizers/ biostimulants for legumes is limited by the specificity of symbiotic bacteria with different crops. Initial data indicate that *Burkholderia alba* could be a promiscuous bacterium capable of associating with different legumes, something that has not been described for any other symbiotic bacteria. Therefore, it is a strong candidate bacterium suitable for the production of legume biofertilizers/ biostimulants.

The legumes

Legumes are a group of plants essential for human and animal nutrition, known for their high nutritional value due to the protein content of the grains and their ability to enrich the soil. They include species such as beans, lentils, chickpeas, broad beans, peas, and soybeans, among others. These plants belong to the Fabaceae (or Leguminosae) family that is characterized by producing pods containing edible seeds (Clemente and De Ron 2016).

Legumes are a key source of plant-based protein, especially in diets where meat and/or fisch is scarce or uncommon. For example, soybeans contain all the essential amino acids, while others, such as lentils and beans, are rich in iron, fiber, B vitamins (such as folic acid), and minerals like magnesium and zinc. Furthermore, their low glycemic index makes them ideal for people with diabetes. Despite their benefits, their consumption has declined in some regions due to a preference for processed foods. The Food and Agriculture Organization of the United Nations (FAO) is promoting their reintroduction to combat malnutrition and improve agricultural sustainability. Innovations such as legume flours and healthy snacks seek to revitalize their demand.

In conclusion, legumes are a pillar of global food security, combining nutrition, accessibility, and low environmental impact. Including them in the daily diet is a step toward a healthier life and a more sustainable planet.

Legumes are the main source of plant protein for human food and and animal feed. These include:

- Human food: common bean (*Phaseolus vulgaris*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), cowpea (*Vigna* spp.), pea (*Pisum sativum*), peanut (*Arachis hypogaea*), faba bean (*Vicia faba*).
- Animal feed: alfalfa or lucerne (*Medicago sativa*), clover (*Trifolium* spp.), peanut (*Arachis hypogaea*), soybean (*Glycine max*), pea (*Pisum sativum*).

Legumes play a crucial role in environmental sustainability thanks to their unique ability to fix atmospheric nitrogen into the soil through a symbiosis with bacteria such as *Rhizobium*. This process reduces the need for chemical fertilizers, decreasing nitrate pollution and greenhouse gas emissions. Furthermore, their cultivation improves soil fertility, preventing erosion and promoting crop rotation. They require less water than other protein crops, contributing to water resources conservation. Due to their adaptability, legumes can grow in marginal soils, helping to recover degraded lands. Their inclusion in agroecological systems promotes biodiversity and reduces dependence on external inputs, consolidating them as a pillar of sustainable agriculture.

In 1987, the MBG-CSIC (Pontevedra) started a program for the collection, conservation, and evaluation of legume germplasm. Initially focused on common beans, the program was later expanded to include peas, cowpeas, and lupins. Part of the collection was collected by the MBG-CSIC, and a significant part was received from the Plant Genetic Resources Centre (CRF-INIA-CSIC) and other national and international institutions (De Ron et al. 2022).

The common bean

The common bean is a crop native to the American continent, from the area between northern Mexico and northeastern Argentina, according to archaeological, botanical, historical, and linguistic data. Archaeological evidence, mainly seeds, were found in the Andes (Peru, Chile, Ecuador, and Argentina) and Mesoamerica (Mexico, Central America, and the southeastern United States), dating back to 10,000–8,000 BC in the central-southern Andes and to 6,000 BC in different locations in Mesoamerica. According to currently known data, the bean was domesticated by humanity in the two aforementioned areas, and the so-called genetic pools of this crop originated there. For this reason, it is usual referring to the "Andean" and "Mesoamerican" varieties of beans, referring to the genetic origin of the varieties currently cultivated worldwide (Santalla et al 2001).

The first varieties of common bean arrived in the Iberian Peninsula from Central America around 1506, due to the trade that existed at that time with the islands of Central America, and from South America after 1532. It is very likely that the bean was introduced to Spain by merchants who brought the seeds with them as a curiosity, due to their varied shapes and colors. Columbus called the small beans he identified on his voyages to America "white beans". These beans corresponded to Mesoamerican-type bean varieties reminiscent of the seeds of another legume, the broad of faba bean (*Vicia faba* L.), cultivated at the time in many parts of Spain. Farmers began to grow the new legume seeds, abandoning or reducing the cultivation of broad beans in many places. In this process of crop substitution, the common bean inherited the names "haba", "faba" or "habichuela" common in many parts of Spain.

Furthermore, before Columbus explorations, grain legumes of Afro-Asian origin were cultivated in the Iberian Peninsula. These legumes arrived with the Arab expansion and have left their mark on Andalusian agriculture. Among these legumes, the "alubia" (in Arabic, "lubiya" a name derived from the Greek "lobós" or "lóbia") stands out. This is the species *Vigna unguiculata*, a legume whose cultivation is very limited today, although it is still appreciated in some areas of Spain, and especially in Portugal. In Spain, it is known as "caupí" (a name derived from the English "cow pea") and as "carilla" (black-eyed bean), while in Portugal it is commonly called "feijão frade". It is logical to assume that, like the broad bean, the alubia was replaced by the common bean in the Spanish countryside, with the newly arrived bean inheriting a new name, "alubia" currently used in many parts of Spain.

The common bean is the most important grain legume for direct human consumption on a global scale. Current bean germplasm collections show a wide range of phenotypes, although in many countries local varieties are being replaced by elite cultivars, concentrating agricultural production on an increasingly small number of cultivars with the consequent genetic erosion or loss of biodiversity. This crop has spread across all continents in recent centuries, giving rise to a complex genetic structure outside its areas of origin and domestication (Mesoamerica and South America).

Several evidences indicate that European germplasm contains greater than expected additional diversity, especially in Southern Europe, and particularly in the Iberian Peninsula, where the common bean was introduced at the beginning of the 16th century and has been documented as a secondary center of domestication of the species (Santalla et al. 2002, De Ron et al. 2016). The integration of omics data into bean germplasm documentation databases and its combination with genetic, phenotypic, and agroecological data is opening a new era for the valorization and efficient use of common bean genetic resources as the main grain legume for human consumption in Europe and globally.

Currently, the BAS group bean germplasm collection at the MBG-CSIC includes 800 accessions of *Phaseolus vulgaris* (common bean) and 42 of *P. coccineus* (scarlet bean). This plant material has been studied from a genetic, agronomic, quality, and biotic and abiotic stress tolerance perspective, primarily for use in plant breeding. This collection also serves as the basis for studies on the evolution of the species *P. vulgaris* (figure 1).

Figure 1. Partial view of the BAS group beans collection at the MBG-CSIC.

In recent years, dry bean production in Spain has been in sharp decline, primarily due to irregular yields and the low commercial quality of the beans. Therefore, the competitiveness of Spanish beans in the national and international markets requires improved quality, with greater added market value. Consumers currently demand higher quality food, as well as information about nature, production methods, and specific characteristics of legumes. These demands have led to the production of quality beans under Protected Geographical Indications and Protected Designations of Origin in different production areas of Spain, which is providing significant support for the production and consumption of native bean varieties in these areas.

Symbiosis with rhizobia and symbiotic nitrogen fixation (SNF)

Meeting the food needs of a growing population has led to the adoption of inappropriate agricultural practices, forcing agriculture to occupy more marginal lands where nutrient availability can be compromised by the physical and chemical conditions of the soil. Improper use of chemical fertilizers, which have an indirect cost related to the consumption of nonrenewable energy required for ammonia synthesis.

Environmental implications, including the contamination of surface and groundwater by excess nitrates and increased production of the greenhouse gas nitrogen oxide, lead to a loss of soil fertility. To improve common bean production, it is crucial to expand the identification of new, competitive, and efficient legume microsymbionts. In this context, more than 20 species of rhizobia, including alpha- and beta-proteobacteria, have been described as symbionts in the bean crop.

Symbiotic nitrogen fixation (SNF) (Vance 1997) is important for agricultural sustainability and represents an ecological alternative to the excessive application of nitrogen fertilizers, particularly in Europe, and an economic alternative to the limited access to nitrogen fertilizers in developing countries. The BAS group has conducted several studies on the bean-rhizobia relationship using different bean genotypes and rhizobia strains in different soil types. Gaining sufficient knowledge about the characteristics of rhizobia populations in Spanish soils will be valuable for developing strategies to improve SNF and thus increase bean yield at low production costs and with a limited environmental impact.

Recent research on legumes and their symbiotic rhizobia began at the MBG-CSIC in 1990 by the Legume group, currently the Biology of Agrosystems group (BAS). However, research on legumes and their interactions with rhizobia started in the 1930s. Indeed, a 1943 letter from José M. Rosell, Director of the Spanish Institute of Root Bacteriology (Barcelona, Spain), to the Director of the MBG-CSIC, Cruz Gallástegui, revealed research on legumes such as lupins and their associated rhizobia. Additionally, during those years, some field trials of lupin and alfalfa varieties were conducted at the MBG-CSIC (De Ron et al. 2019).

The symbiotic bacterium *Burkholderia alba*

The genus *Burkholderia* comprises more than 100 species distributed in diverse ecological niches, including soils, plants, insects, industrial and hospital environments, and human clinical samples. Among them, some strains have relevant applications in agriculture, industry, bioremediation of recalcitrant xenobiotics, biocontrol, and plant growth promotion.

The nitrogen-fixing capacity of *Burkholderia* was initially described in *B. vietnamiensis* and subsequently confirmed in more than 15 species that efficiently associate with legumes, particularly in the genus *Mimosa* (Sheu et al. 2015). Furthermore, *Burkholderia* strains have been isolated from common bean nodules in Morocco, Mexico, and Brazil. In 2020, in field trials at the MBG-CSIC, it was observed that the growth of bean plants inoculated with the control strain *Rhizobium etli* CFN42 was lower than that of control plants that were neither inoculated nor treated with nitrogen fertilizer

(Osmoform NXT), and the MBG-CSIC BAS group identified *Burkholderia alba* in root nodules in these bean plants. This finding is noteworthy, as *B. alba* had previously been isolated only from soils in the Republic of Korea in 2018 (Lee et al. 2018), suggesting its potential as a relevant legume symbiont.

This opens up interesting possibilities regarding the possible use of this new symbiont, *B. alba*, including the association with other symbionts, as a bioinoculant and biostimulant for nitrogen fertilization of beans in sustainable agriculture that avoids the emission of greenhouse gases to contribute to the mitigation of climate change.

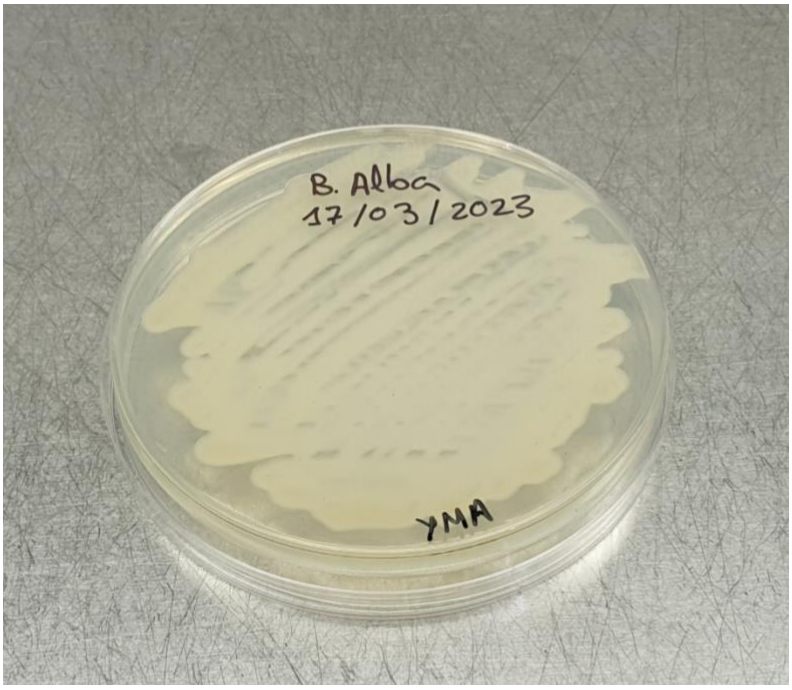

Figure 1. Petri dish with culture of the bacterium *Burkholderia alba*.

It should be a relevant innovation to use *B. alba* as a bioinoculant/bioestimulant for fertilizing beans and other legumes, with the goal of improving symbiotic nitrogen fixation (SNF) and its activity as a plant growth-promoting rhizobacteria (PGPR), including association with other microsymbionts.

Microbial consortia: biofertilizers and biostimulants

Microbial consortia, composed of bacteria (e.g. *Rhizobium*, *Azospirillum*) and fungi (e.g. mycorrhizae) (Rodiño et al. 2025), act as biofertilizers by fixing nitrogen, solubilizing phosphates and producing phytohormones, improving nutrient availability. As biostimulants , they promote plant growth through the synthesis of indoleacetic acids (IAA) and siderophores, increasing tolerance to abiotic stress. Their application in sustainable agriculture reduce the use of chemical fertilizers by up to 30%, according to studies in corn and wheat crops. The synergistic combination of selected strains maximizes their effectiveness, as demonstrated by the *Pseudomonas* - *Bacillus consortium* in rice, increasing yields by 25%.

For all these reasons, the use of microbial consortia in a scientific and sustainable agriculture of the future is one of the main innovative actions and contributions to climate change mitigation.

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