UNIVERSIDAD DE LA FRONTERA

Facultad de Ingeniería y Ciencias

Doctorado en Ciencias de Recursos Naturales



WHEAT CULTIVAR INFLUENCE OVER NITROGEN-FIXING BACTERIAL COMMUNITIES IN AN ANDISOL AND A NOVEL PGPB TRACKING METHOD FOR *AZOSPIRILLUM SPP*. BASED ON CRISPR LOCI-TARGETED PCR

DOCTORAL THESIS IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE DOCTOR OF SCIENCES IN NATURAL RESOURCES

JOAQUÍN IGNACIO RILLING TENORIO

TEMUCO-CHILE

2020

"Wheat cultivar influence over Nitrogen-fixing bacterial communities in an Andisol and a novel PGPB tracking method for *Azospirillum spp.* based on CRISPR loci-targeted PCR"

Esta tesis doctoral fue realizada bajo la supervisión y dirección del Dr. Milko Alberto Jorquera Tapia del Departamento de Ciencias Químicas y Recursos Naturales, Facultad de Ingeniería y Ciencias, Universidad de La Frontera y ha sido aprobada por los miembros de la comisión examinadora.

Dr. Dr. Andres Quiroz DIRECTOR DEL PROGRAMA DE DOCTORADO EN CIENCIAS DE RECURSOS NATURALES

.

Dr. Milko Jorquera Tapia Profesor Guia de Tesis

Dr. Francisco Chavez

Dr. Mauricio Schoebitz

Dra. Monica Rubilar DIRECTOR ACADÉMICO DE POSTGRADO UNIVERSIDAD DE LA FRONTERA

Dra. Graciela Palma

Dra. Maria de la Luz Mora

Thesis summary

"The use of plant growth-promoting bacteria (PGPB) as environmental-friendly fertilizers has been explored since the 1990s. Despite the advantages that this technology could offer, the tracking of such inoculants has been unsuccessful due to several caveats provided by soil nature (competition with native microorganisms, adaptation, compatibility with host plants, among others). Thus, the search for potential tracking methods to assure inoculants colonization are demanded. In this thesis, we firstly explored the local bacterial communities from the rhizosphere and endosphere of four winter wheat cultivars, to assess the environment our selected PGPB would colonize. Then, a CRISPR-specific loci targeted PCR assay, aiming to track *Azospirillum sp.* B510 colonization in wheat plants rhizosphere and endosphere in sterile and unsterile substrates was designed. As results, all wheat cultivars shown similar composition and structure in terms of microbial communities. In parallel, the tracking of the B510 strain was successful in rhizosphere of wheat plants in sterile and unsterile substrates, although its tracking in the root endosphere was not feasible. This assay however, was proposed to be tuned in order to apply the technique to other plants, inoculants, and matrixes in the future."