WEEK OF MICROBIAL TECHNOLOGIES

ABSTRACTS BOOK

7-11 NOVEMBER, 2022
LJUBLJANA, SLOVENIA

Jožef Stefan Institute

SURF BIO

Surfbio project has received funding under the European Union's Horizon 2020 research & Innovation programme under grant agreement N° 952379

BOOK OF ABSTRACTS

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Organising committee: Jožef Stefan Institute, ICCRAM University of Burgos, AXIA Innovation, Wageningen University and Research, Ghent University, Helmholtz-Zentrum Dresden-Rossendorf.

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PREFACE

The Week of Microbial Technologies –MicroTechWeek– was a five-day summit full of project meetings and open public events, such as an industrial workshop, a poster session and a hands-on training, aimed at gaining knowledge on the applications of surface and colloid biology in different industrial sectors. It was jointly organised by the European projects SURFBIO and GREENER.

The main goal of this event was networking between EU projects and stakeholders, sharing applications of surface and colloid biology and planning new initiatives based on microbiology technologies.

This book gathers the contents generated in the SURFBIO industrial workshop and in the poster session. Professionals from international companies and organisations contributed knowledge from different perspectives, creating very fruitful roundtables for the project partners.

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ABSTRACTS INDUSTRIAL WORKSHOP

PSEUDOMONAS SPP. IN BIOCONTROL OF CROWN GALL DISEASE: NEW APPROACHES

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INTRODUCTION

Crown gall is an economically important and widespread plant disease caused by tumorigenic bacteria that are commonly affiliated with the genera Agrobacterium, Allorhizobium and Rhizobium. Novel and an atypical group of tumorigenic agrobacteria belonging to the genus Rhizobium ("tumorigenes" clade) was identified as a causative agent of crown/cane gall on blackberry, rhododendron and blueberry in Serbia and Germany (Kuzmanović et al., 2018 and 2019). Efficient measures to control crown gall disease were not reported till nowadays, so assessment and application of alternative biological control measures would contribute to sustainable agricultural production and environmental protection. The aims of the study were 1) identification of candidate bacterial strains that could be employed for biological control 2) to analyse phytobiome of the treated and non-treated crops and 3) to perform a whole-genome sequencing of a few most promising biocontrol strains.

STATE OF THE ART

Antimicrobial activity of ten biocontrol candidates from rhododendron and 27 additional antagonistic strains were tested in vitro against the tumor-inducing strain Rhizobium sp. rho-6.2. The six most efficient Pseudomonas and Bacillus strains were tested in vivo, using co-inoculation and preventive inoculation strategies in controlled greenhouse conditions on tomato plants as a model system in four replicas and randomized. Tumors from the most effective treatments were sampled, and then total DNA was isolated and subjected to the next-generation sequencing (NGS). Direct analysis of bacterial communities using Illumina MiSeq sequencing of 16S rRNA gene amplicon libraries was performed to assess the microbial ecological effect, with complete bioinformatic and computational biology analysis conducted. Also, a whole-genome sequencing of a few most promising antagonistic strains was performed.

RESULTS

Among six antagonistic strains, the most efficient in co-inoculation strategy against pathogenic Rhizobium sp. rho-6.2 were two Pseudomonas strains (R-6.10 and R-11.20), which reduced a tumor size 92.86%. The same Pseudomonas strains were less effective in preventive treatments (15.38 and 30.77%). Although Bacillus strains exhibited high in vitro antimicrobial activity, their in vivo activity was in preventive treatment only 15.38%, whilst in co-inoculation strategy was detected as moderate (42.86%). Bacillus and Pseudomonas strains applied together increased biocontrol activity with 38.6% of tumor's reduction. In analyzed treatments, was detected the dominant presence of Proteobacteria followed by a moderate presence of Actinobacteriota and Firmicutes. On the genus level, the most abundant, both in negative control and treatments, were representatives of Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium group (18,53% - 71,81%) followed by Pseudomonas spp. (2,76%- 36,46%). According to alpha diversity indexes

on the genus level, the highest values were detected in the negative control, pre-treatment with Pseudomonas sp. R-6.10, co-inoculation with Pseudomonas spp. R-6.10 and R-11.20 individually. Analysis of beta diversity by the DPCoA matrix exhibited that the co-inoculation and positive control groups were well separated, whilst preventive treatment overlapped both the co-inoculation and positive control samples. Differential abundance analysis on a genus level revealed a statistically higher presence of Stenotrophomonas and Asanoa in preventive treatments and Dyadobacter and Pandoraea spp. in their positive control. In the co-inoculation strategy, Pseudolabrys and Asanoa were prevalent in treatments and Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium was detected as prevalent in positive control. Whole-genome sequencing and preliminary comparative genomics analyses revealed that the best biocontrol candidates, Pseudomonas strains R-6.10 and R-11.20 represent two new species, most closely related to P. graminis and P. fildesensis, respectively.

DISCUSSION

The Pseudomonas species exhibited the most prominent activity in vivo. Pseudomonas genus is rich in species with the potential for biocontrol of wide spectra of pathogens. Their activity is based on the production of variety of antimicrobial compounds (Dimkic et al., 2022). Also, silencing quorum sensing or quorum quenching is one of their biocontrol strategies by attenuating the virulence of the pathogen (Zhang et al., 2021). Metabarcoding analysis showed differences between treatments, mainly on the level of less presented genera. Best candidates for biocontrol of crown gall, Pseudomonas spp. R-6.10 and R-11.20 originating from the crown gall tumor, confirms the previously established hypothesis that plants are the best sources of biocontrol agents (Janisiewicz et al., 2013).

CONCLUSIONS

The selected Pseudomonas strains could be further tested as an alternative strategy for the biocontrol of crown gall disease and the potential involvement of the quorum quenching mechanism will be determined. Crown gall tumors have shown to be a great source of antagonistic isolates Pseudomonas sp. R-6.10 and Pseudomonas sp. R-11.20 identified according to WGS as the two new species that further needs to be described.

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