

Brassica napus endophytes have the potential to protect the crop against the blackleg disease causal agent, the fungus *Leptosphaeria maculans*

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INTRODUCTION

The World population is going to achieve 8.000 million people imminently¹.

➔ Finding a sustainable and efficient way to feed the growing population is one of the major challenges of this generation.

Important environmental problems are being caused by chemical fertilizers and biocides, for what new approaches are needed (Figure 1):

➔ Plant growth promoting (PGP) bacteria & Biological control agents (BCA)²

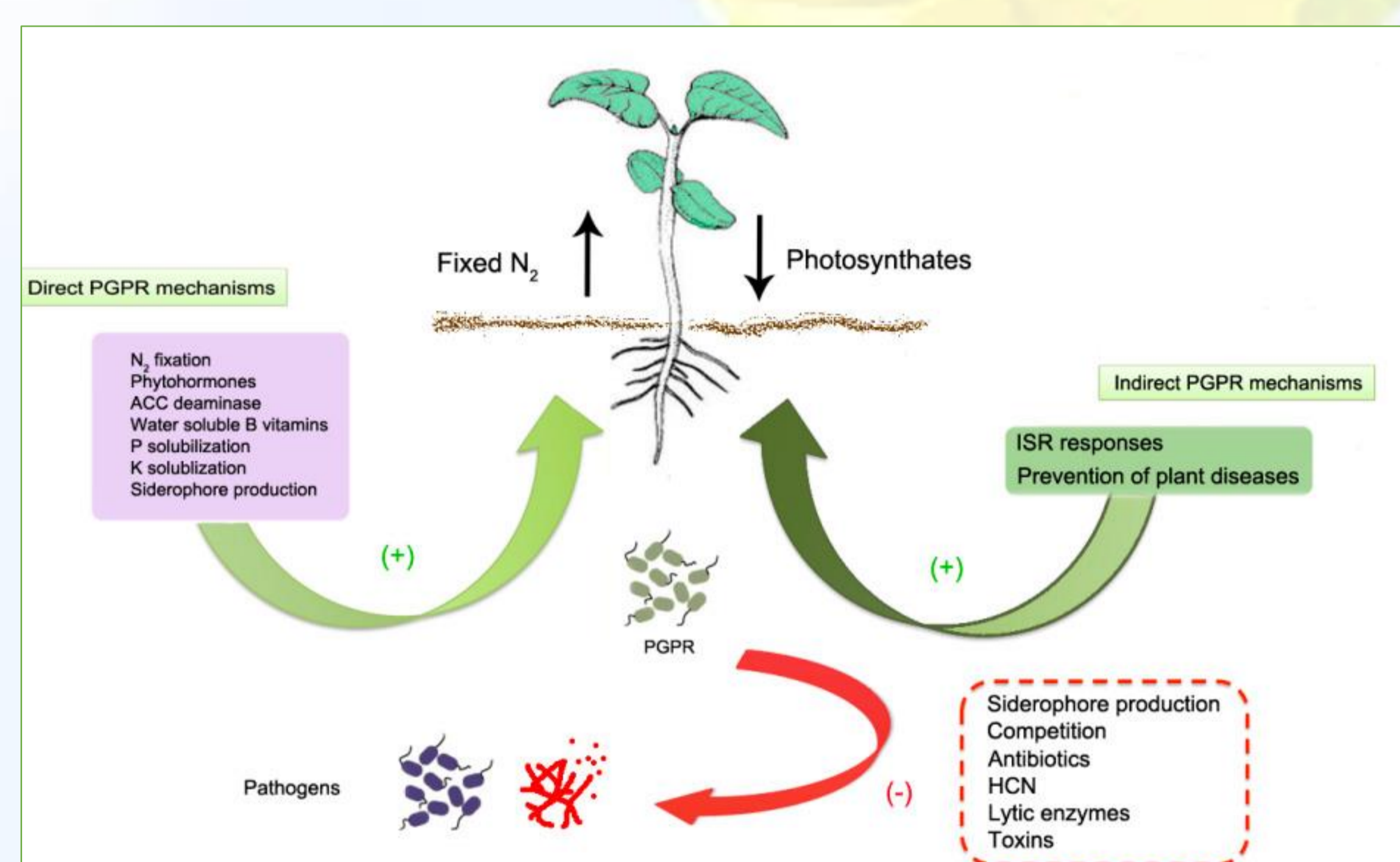


Figure 1. Mechanisms of Plant Growth Promoting bacteria².

The fungus *Leptosphaeria maculans* is the causal agent of the most damaging disease of *Brassica napus* crops, a research found that for every unit of increase in disease severity, a 17.2 per cent loss in plant seed yield can be expected (Figure 2)³.

A sustainable way to control this disease is needed to prevent crop losses. To this purpose, a screening of bacterial canola endophytes with antifungal potential is proposed to find an efficient BCA.

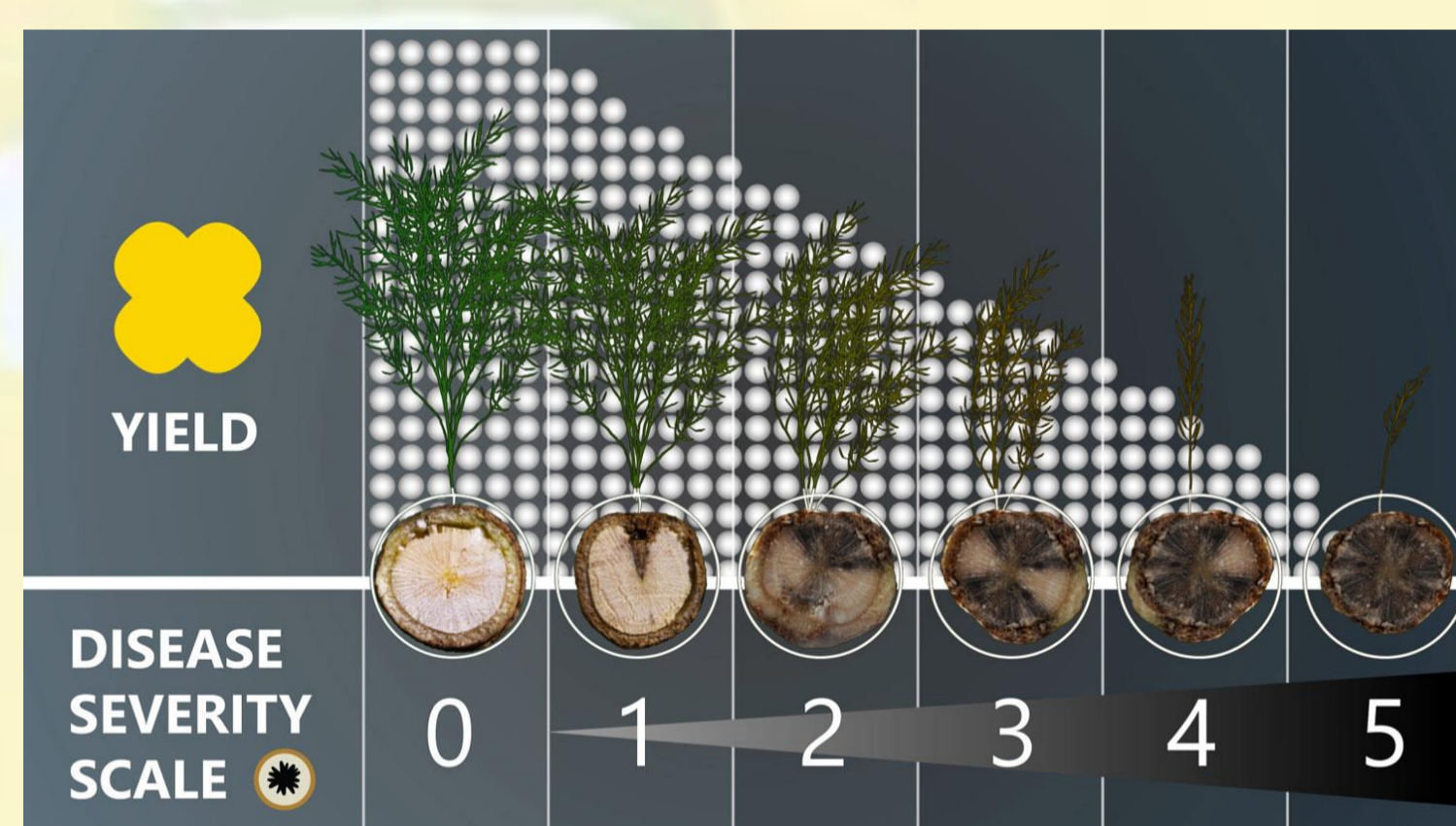


Figure 2. Disease severity and yield lost.

IN VITRO ANTIBIOSIS ASSAYS

49 *B. napus* bacterial endophytes were grown in Petri plates with PDA medium for a week. Then, plugs of the *L. maculans* fungus were placed in the plate and incubated for another week.

Within the isolates, 13 endophytes with high *in vitro* antifungal potential were found, *P. brassicacearum* CDVBN10 and *P. orientalis* CDVBN20 strains showed the strongest antifungal activity (Figure 3).

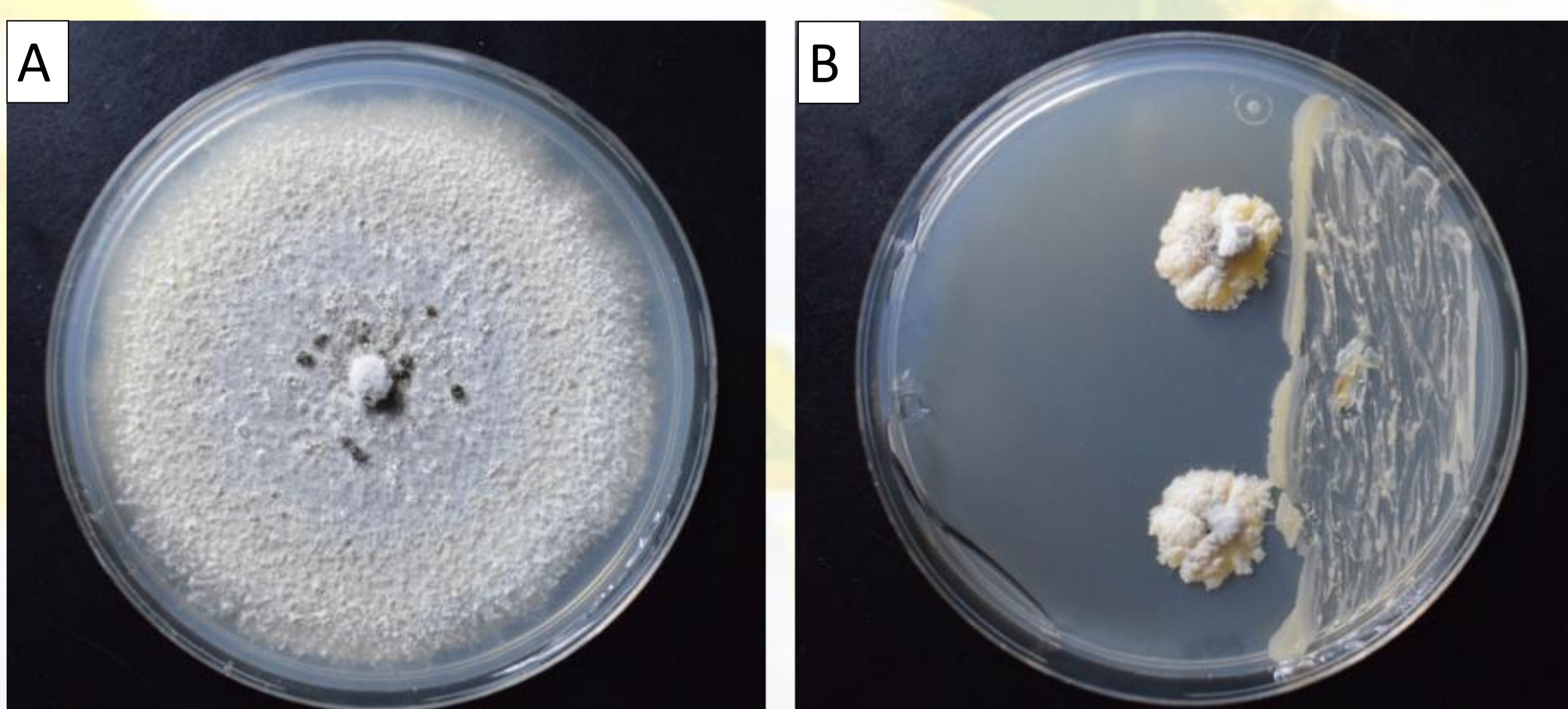


Figure 3. Antibiosis assays against the fungus *L. maculans*. (A) Control plate with fungal plugs and no bacteria, (B) *P. brassicacearum* CDVBN10 bacteria streak and *L. maculans* plugs.

Since siderophores have been related to the inhibition of other microbes, its production was evaluated on modified M9-CAS-agar medium plates⁵. Orange halos around the colonies were appreciated for *P. brassicacearum* CDVBN10 and *P. orientalis* CDVBN20, which indicates the biosynthesis of these molecules.

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IN SILICO ANALYSES OF ANTIBIOSIS POTENTIAL

Genome sequences of *P. brassicacearum* CDVBN10 and *P. orientalis* CDVBN20 strains were annotated on the antiSMASH 5.0 platform⁵. As can be seen in Figure 4, 12 and 15 different BGCs were respectively predicted.

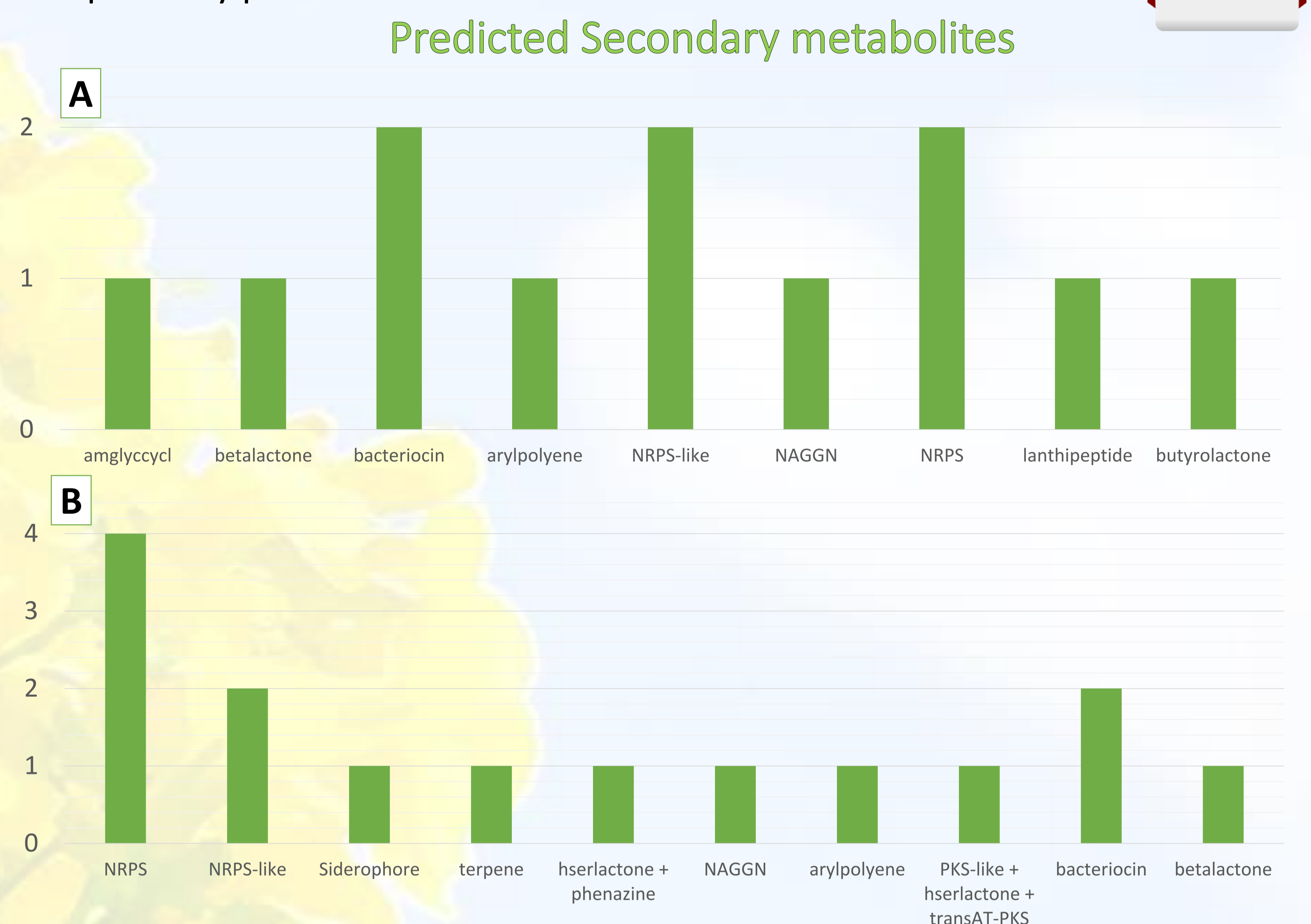


Figure 4. Distribution of the different BGC predicted by antiSMASH database for (A) *P. brassicacearum* CDVBN10 and (B) *P. orientalis* CDVBN20 genomes sequences.

Among the predicted BGCs, only in one cluster all the genes showed similarity to a previously described. This BGC belongs to *P. orientalis* CDVBN20 strain genome sequence and shows similarity to Pseudomonic acid A BGC from *Pseudomonas fluorescens* NCIMB 10586. This is an antibiotic substance that have demonstrated antifungal activity⁶ (Figure 5).



Figure 5. All genes from *P. orientalis* CDVBN20 predicted BGC shows similarity to *Pseudomonas fluorescens* NCIMB 10586 BGC

The other BGCs showed low or no similarity to any other known cluster in the antiSMASH database, for what we estimate that they might represent novel compounds, of which **some may have antimicrobial activity** (Figure 6)

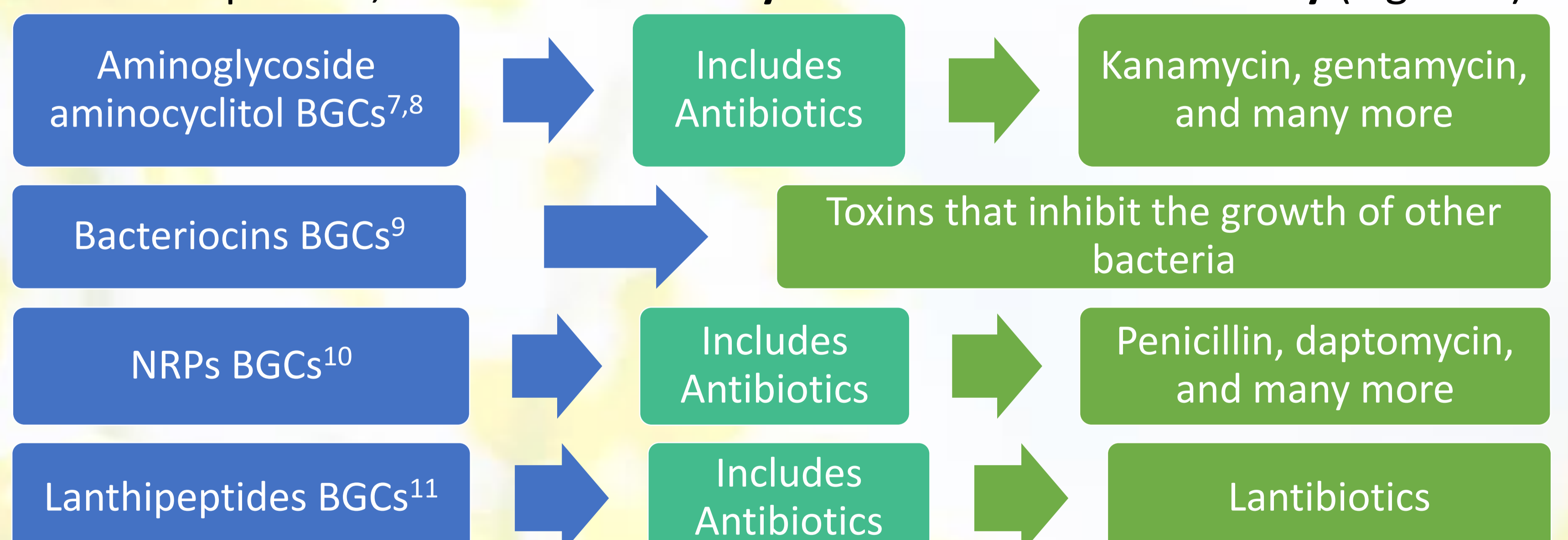


Figure 6. Predicted cluster types for *P. brassicacearum* CDVBN10 and *P. orientalis* CDVBN20 genomes sequences and the potential antimicrobial activity

Terpene compounds are generally considered plant metabolites, but its role in bacteria needs to be unveiled¹².

It was predicted a siderophore BGC for *P. orientalis* CDVBN20 genome.

Besides that, homoserine lactones clusters were predicted, they've been described as interkingdom communication molecules¹³ and phenazine molecules may also elicit induced systemic resistance (ISR) and interfere in tissue colonization, as described¹⁴.

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