Production and investigation of the bioperformance of oligosporogenic mutants of Bacillus thuringiensis strain BLB I

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PRIMA: Thematic Area 1

Water management (RIA) Research and innovation action

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SAFWA

Aims to release to the market an innovative solution combining a new competetive biopesticide to cultural trainings aiming to reduce land and water pollution through new agricultural practices

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I. INTRODUCTION

- **The control of insect pests has been based mainly on the use of chemical pesticides**

Highly persistent in the environment

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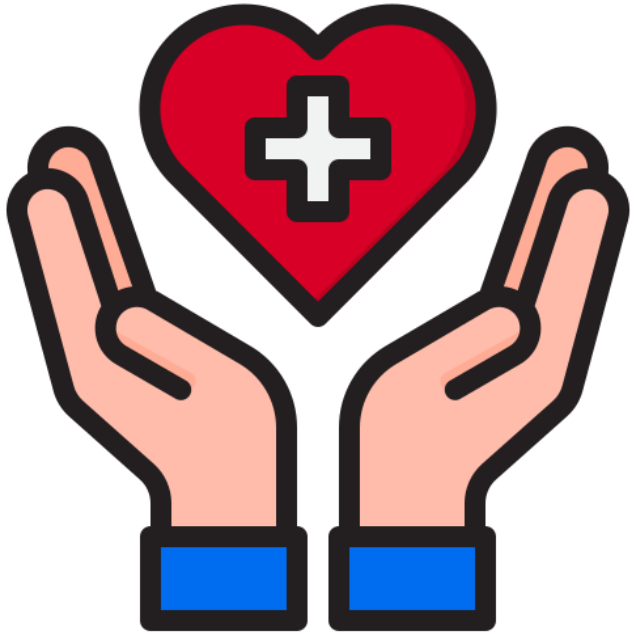
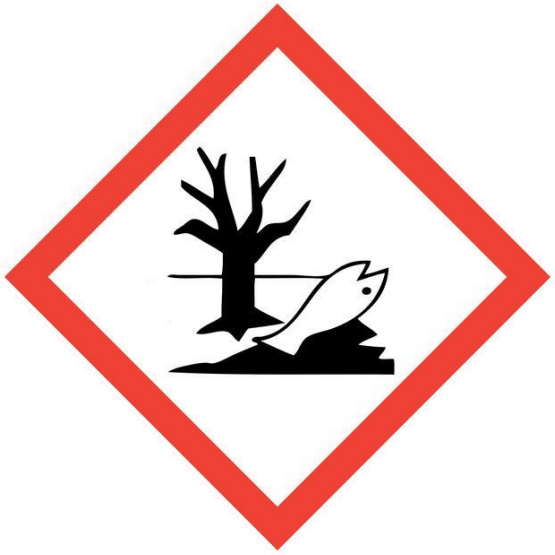
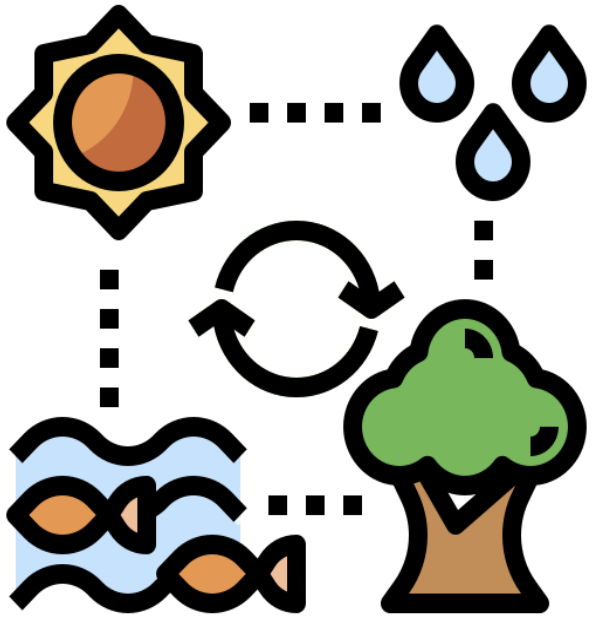
1. INTRODUCTION



Human being health



Non-target organisms



The ecosystem

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I. INTRODUCTION

2007

A necessity to reduce the use of these products

Natural formulations known as
Biopesticides

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1. INTRODUCTION

Biopesticides ?

- ❖ Derived from natural sources
- ❖ Non toxic to non target organisms
- ❖ Targeting specific pests

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I. INTRODUCTION

Biopesticides derived from *Bacillus thuringiensis* account for 90% of the global market for microbial biopesticides by 2021

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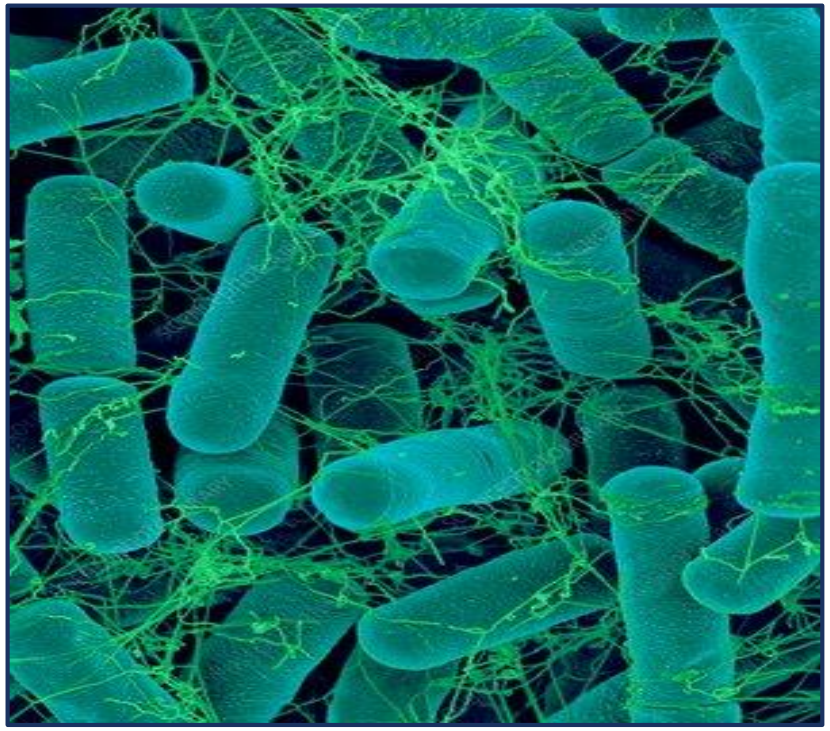
I. INTRODUCTION

Bacillus thuringiensis



- ✓ **Gram-positive, spore-forming**
- ✓ **Rapid and host-specific action**
- ✓ **No side effects on non target organisms**

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1. INTRODUCTION

Cry toxins produced by *Bacillus thuringiensis*

Delta -endotoxins

- More than 800 genes
- Produced during the sporulation phase
- 20-30% of dry weight of cells
- Synthesized as inactive protoxins of 70 kDa or 130 kDa.

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1. INTRODUCTION

Bacillus thuringiensis-based formulations offer several advantages

Accumulation of a high proportion of spores in the environment

Imbalance in the bacterial population

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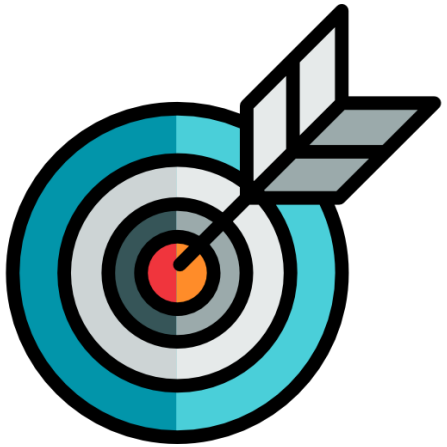
2. OBJECTIVES

Eco-friendly biopesticide



- ✓ **Isolate new oligosporogenic/asporogenic and delta-endotoxins hyperproducing *Bacillus thuringiensis* BLB1 mutants by classical mutagenesis**
- ✓ **Screening for new protease-overproducing mutants**
- ✓ **Bioassays of oligosporogenic mutants against *Ephestia kuehniella* larvae**

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3. RESULTS

- Classical mutagenesis of the BLB1 strain
- Determination of the delta-endotoxin production rate of oligosporogenic mutants
- Study of the protease production by oligosporogenic mutants
- Evaluate the toxicity of oligosporogenic mutants against *Ephestia kuehinella* larvae

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Optimization of BLB1 strain classical mutagenesis

Taguchi table L12

X1: Nitrous acid concentration (transition, microdeletion)

X2: Nitrous acid exposure time

X3: Concentration of acridine orange (indel, frameshift)

X4: Acridine orange exposure time

X5: Exposure time to UV light (point mutations, frameshift)

Factors	level 1	level 2
X1	30mg/ml	70mg/ml
X2	30min	60min
X3	15µg	35µg/ml
X4	15min	60min
X5	2min	45min

Experience	X1	X2	X3	X4	X5
1	1	1	1	1	1
2	1	1	1	1	1
3	1	1	2	2	2
4	1	2	1	2	2
5	1	2	2	1	2
6	1	2	2	2	1
7	2	1	2	2	1
8	2	1	2	1	2
9	2	1	1	2	2
10	2	2	2	1	1
11	2	2	1	2	1
12	2	2	1	1	2

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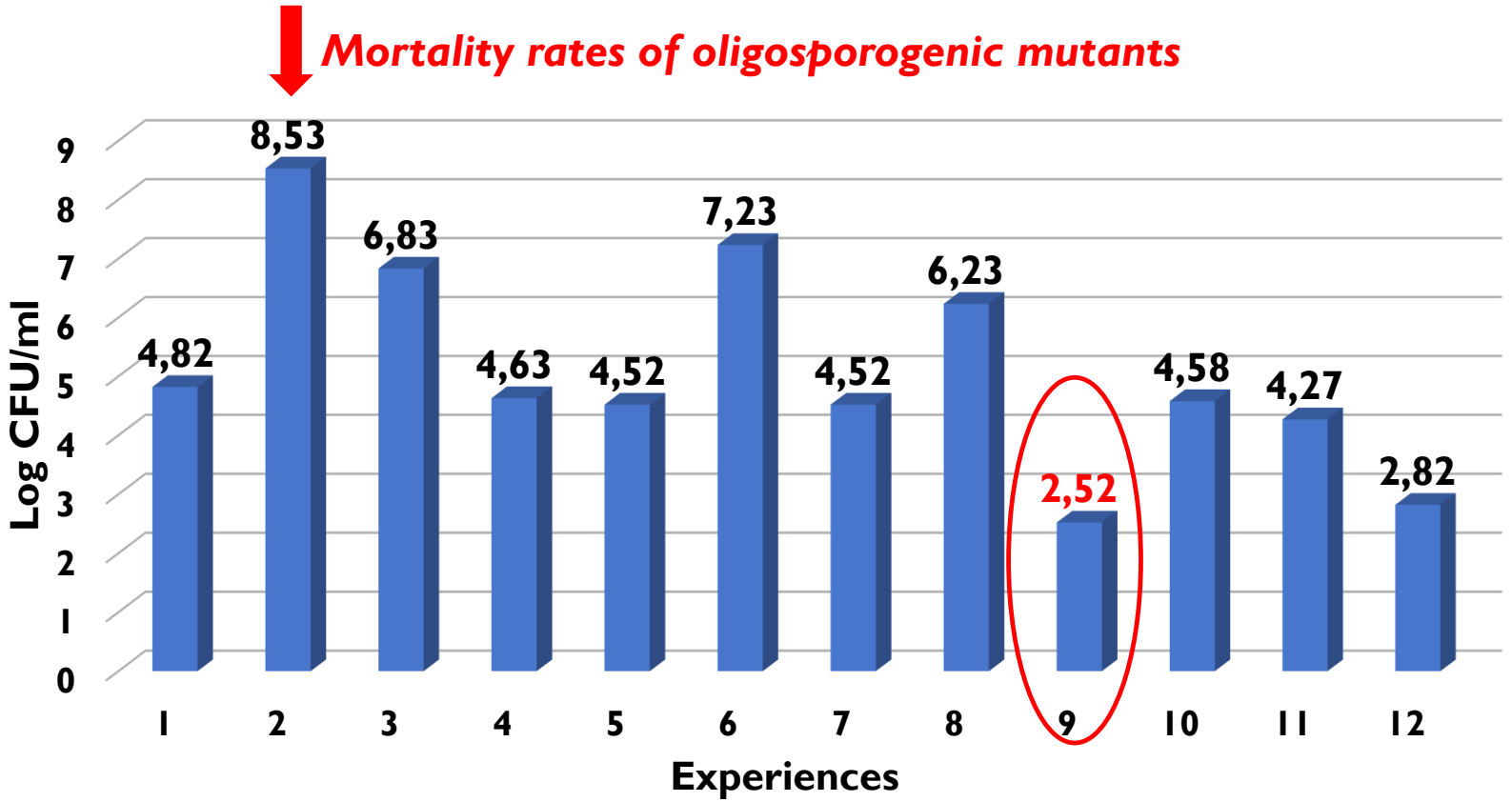
Optimization of BLB1 strain classical mutagenesis



Mortality levels proving the effectiveness of mutagenesis

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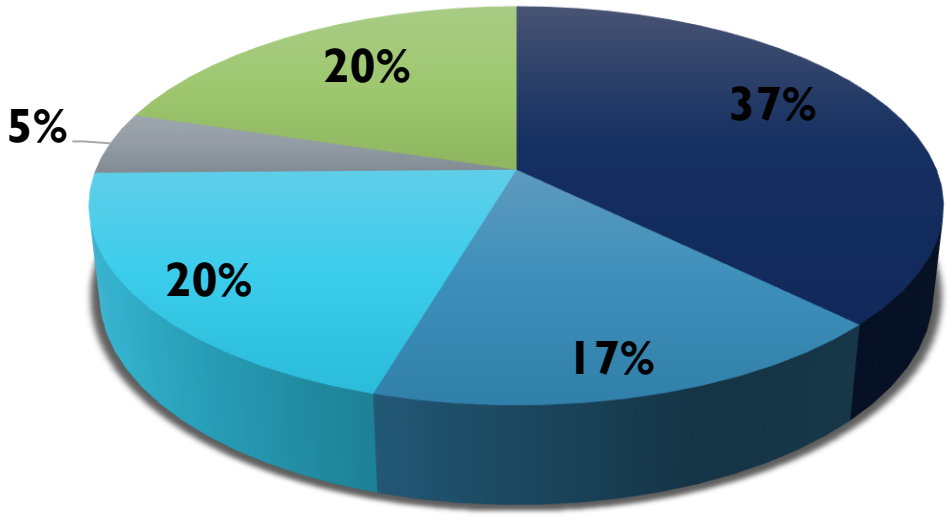
Optimization of BLB1 strain classical mutagenesis: determination of the contribution of factors



a new cycle of classical mutagenesis using optimal conditions:

- **X1: 30mg**
- **X2: 30min**
- **X3: 35µg/ml**
- **X4: 15min**
- **X5: 2min**

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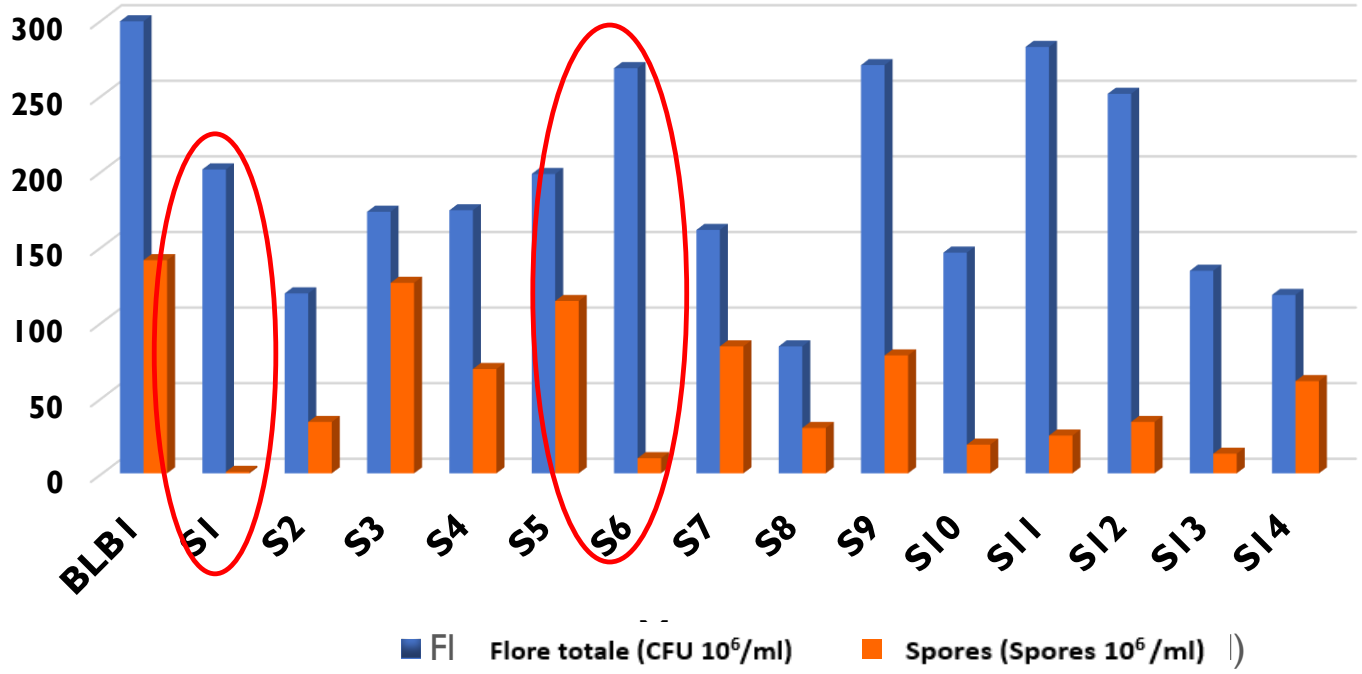
Optimizing classical mutagenesis of the BLB1 strain: Cell and spore counts

14 oligospogenic mutants

Number of spores and cells produced by oligosporogenic mutants



S1 and s6 the more reduced spore rates

Reduced spore numbers do not affect cell viability

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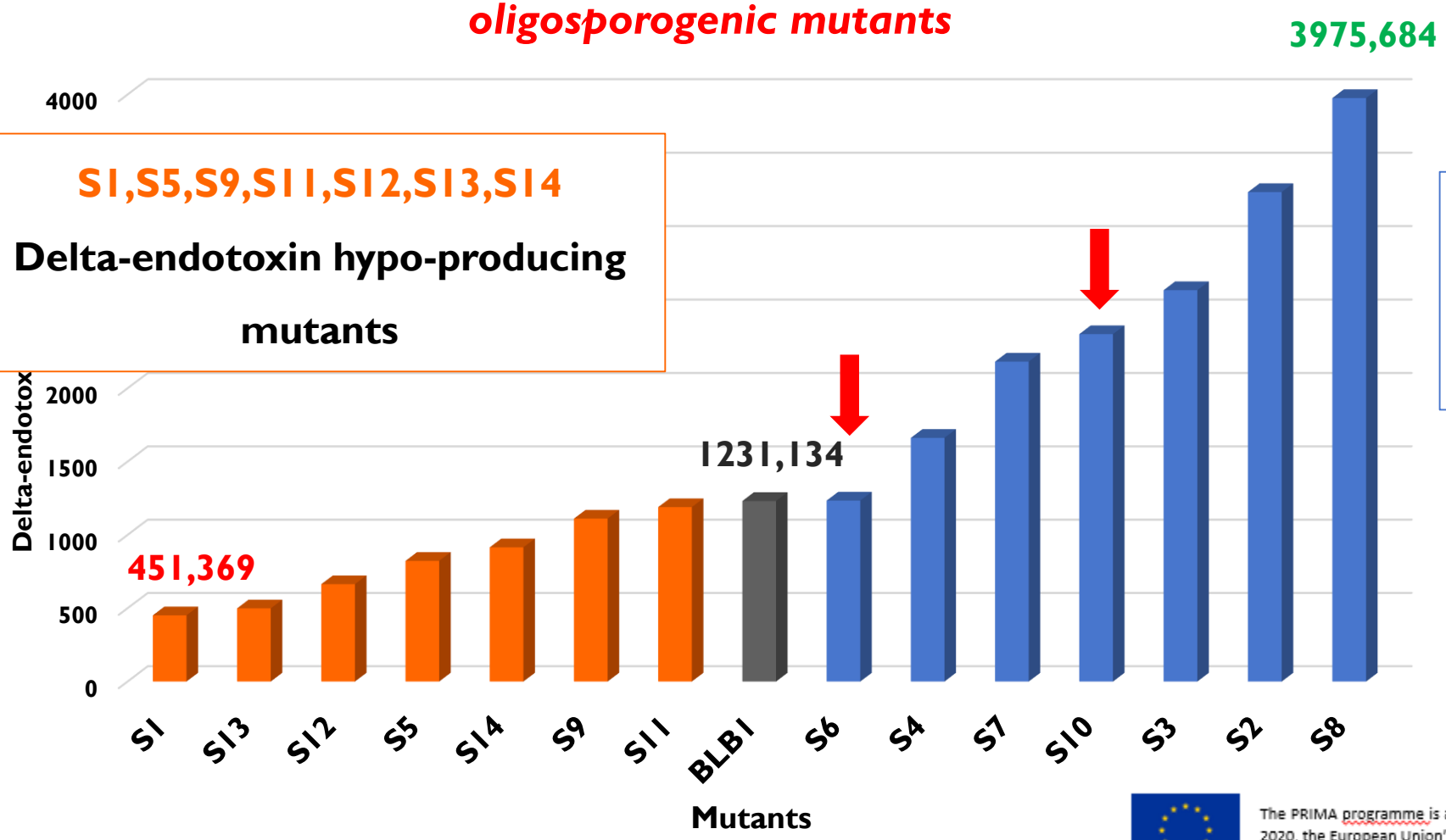
Determination of delta-endotoxin production

Concentrations of delta-endotoxins produced by oligosporogenic mutants



S1,S5,S9,S11,S12,S13,S14

Delta-endotoxin hypo-producing mutants

S2, S3, S4, S6, S7, S8, S10

Delta-endotoxin hyper-producing mutants

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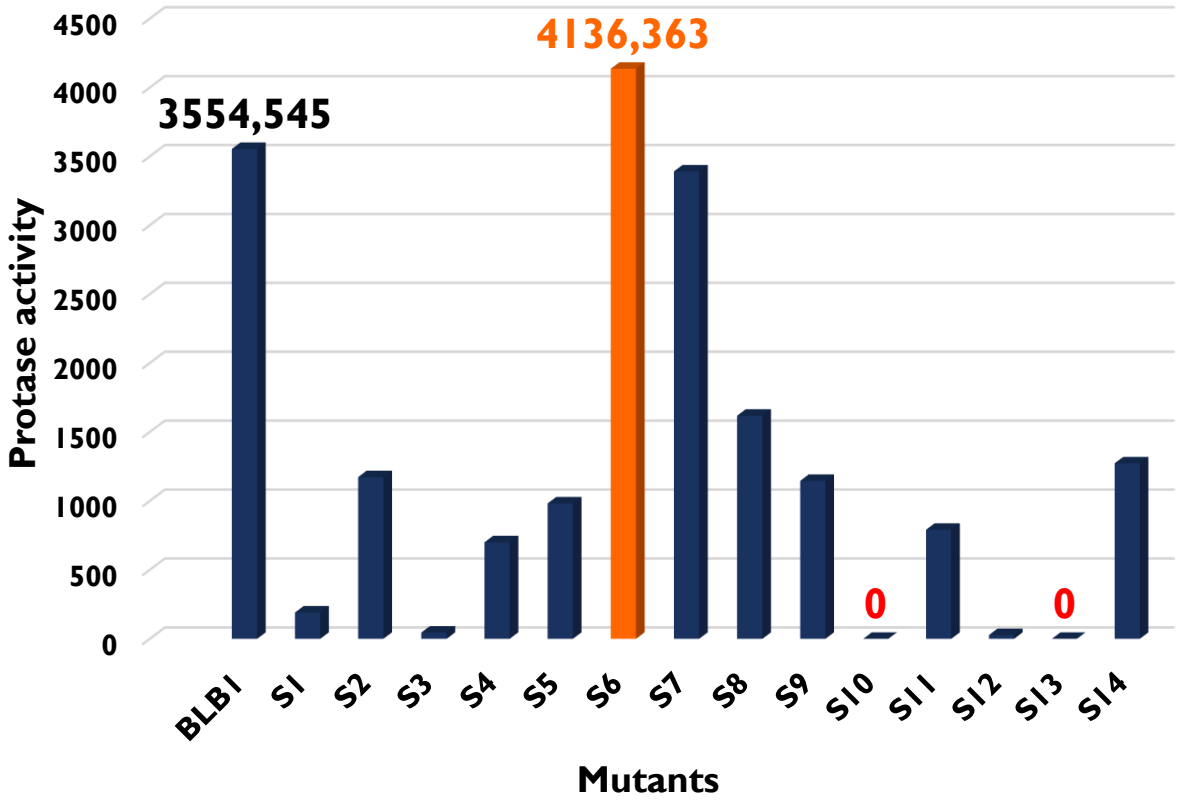
Determination of protease production

Protease activity of oligosporogenic mutants



14 oligosporogenic mutants

- **S6:** overproducer of protease: 4136,363 UI
- **S10 et S13:** total loss of enzymatic activity: 0UI

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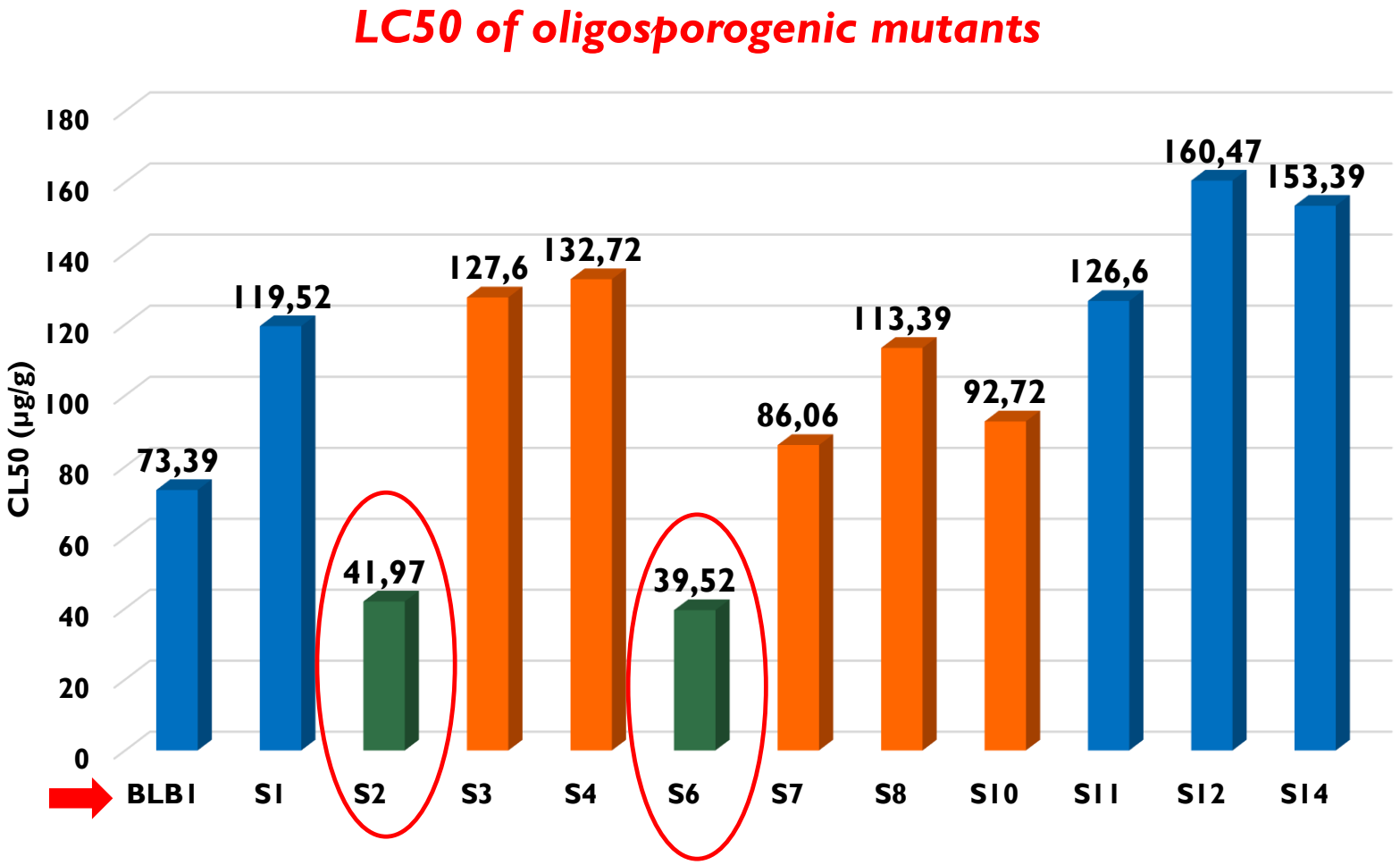
Bioassay of oligosporogenic mutants against *Ephestia kuehniella*



S2 and S6: Excellent candidate for pest control.

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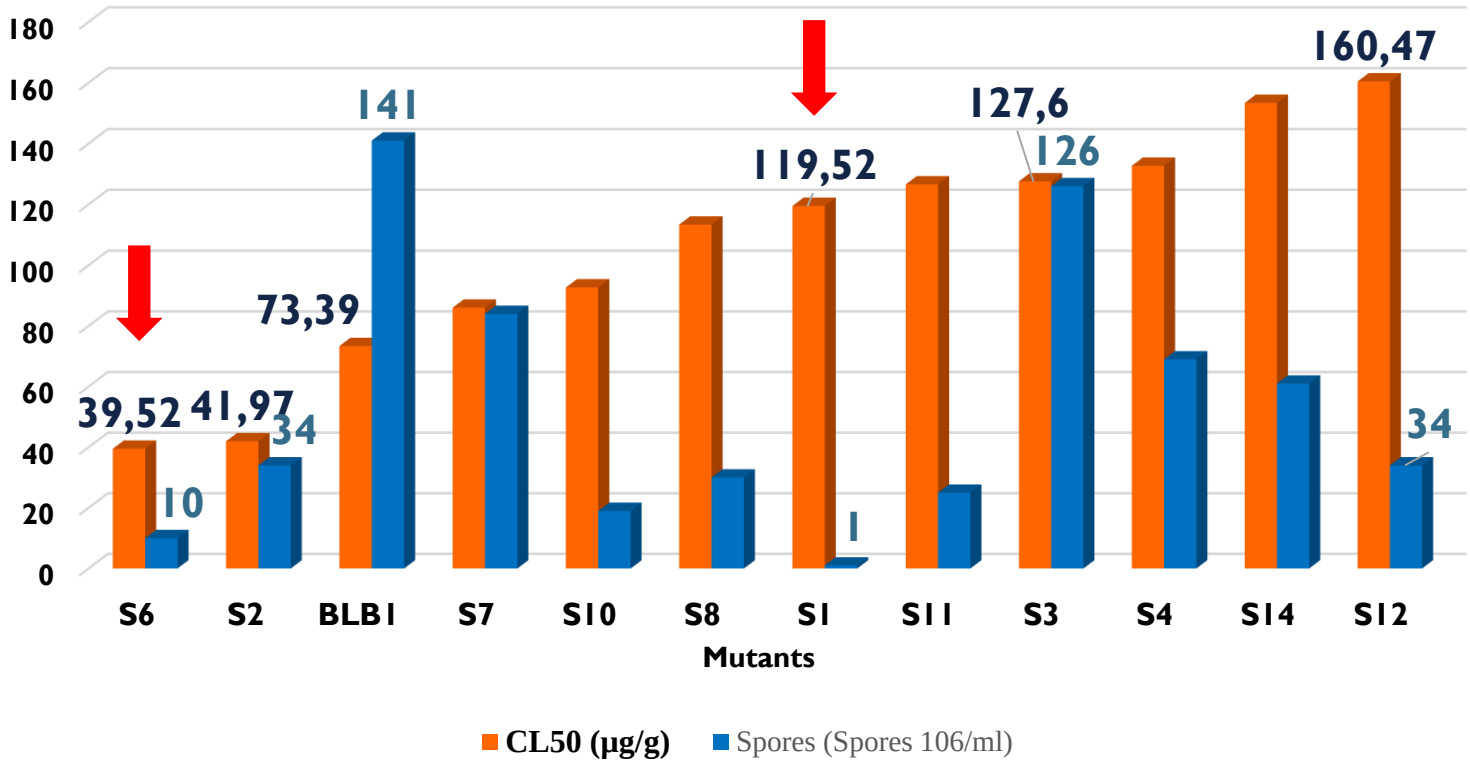
Bioassay of oligosporogenic mutants against *Ephestia kuehniella*

Relation between sporulation and toxicity



R=0.0480395 (Pearson correlation test)

No correlation between sporulation and toxicity

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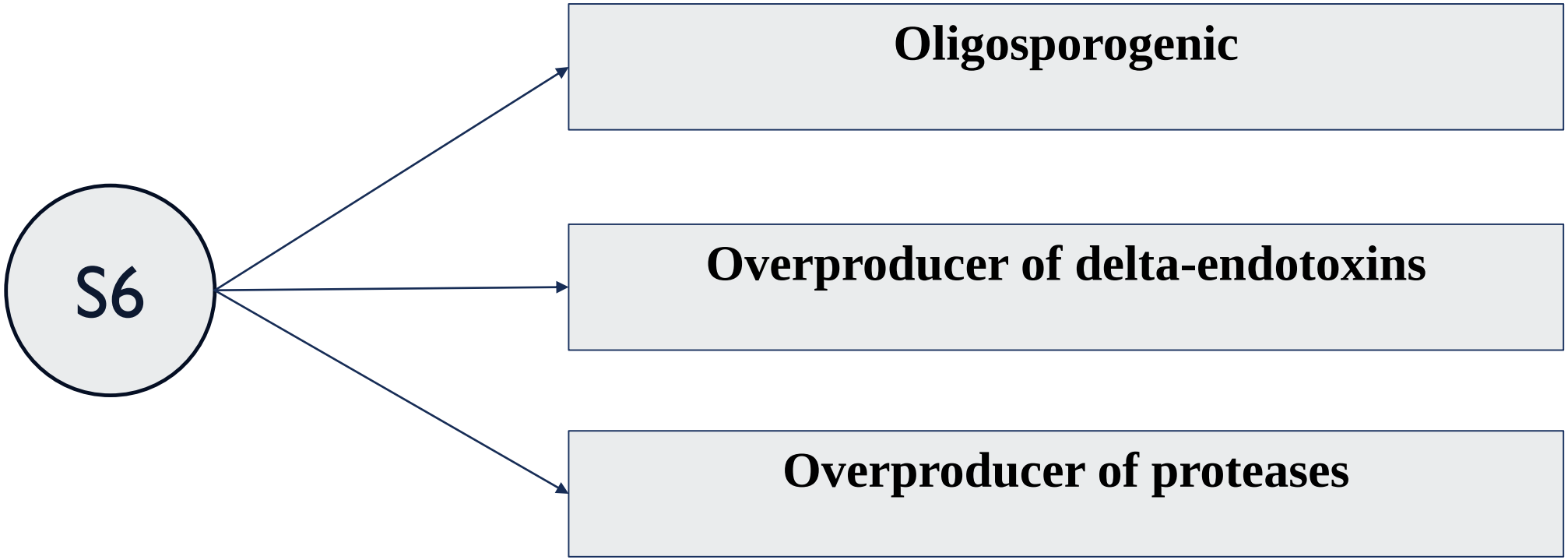
4. CONCLUSION

In this work, which makes part of the SAFWA project, we succeeded in obtaining :



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THANK YOU FOR YOUR ATTENTION

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