

Could the efflux pumps be responsible of the reduced sensitivity to tebuconazole, fengycin and surfactin in *V. inaequalis* ?

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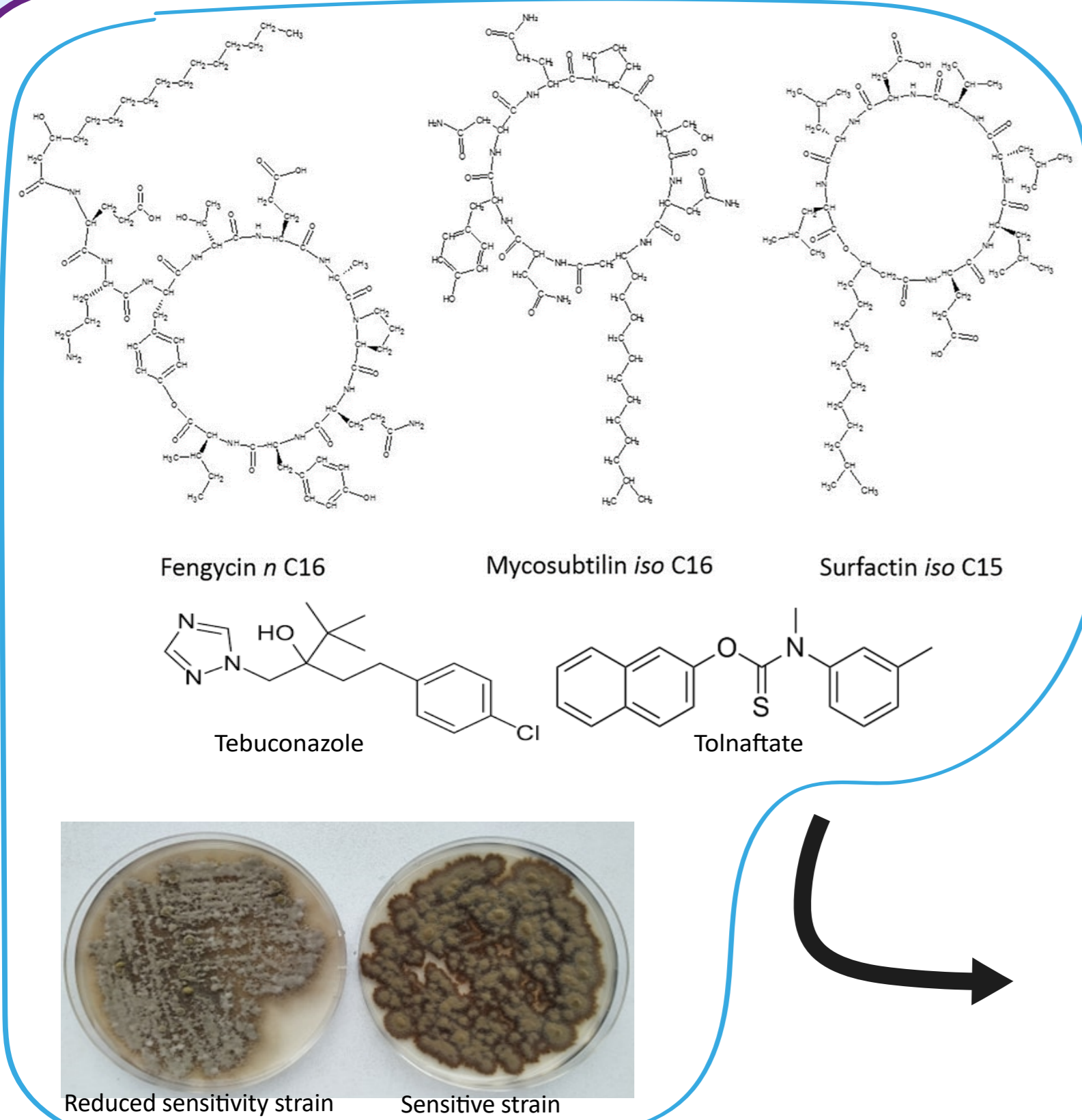
Introduction

Apple scab caused by *Venturia inaequalis* leads to important economic losses. In France, to fight this plant pathogen many fungicidal treatments are required (treatment frequency indicator was 22.2 for fungicide^(a)). However, the use of these fungicides causes soil and water pollution but also cases of fungicides resistance of *V. inaequalis*. Several mechanisms may explain fungicide resistance. **Overexpression of efflux pumps** is one of them.

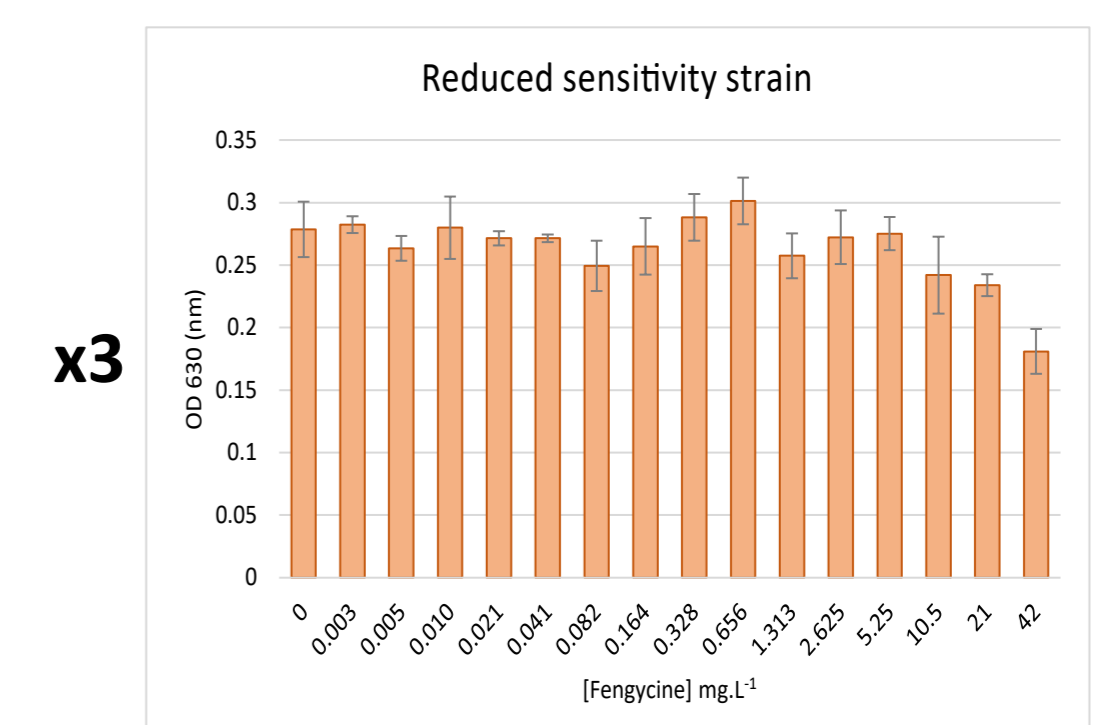
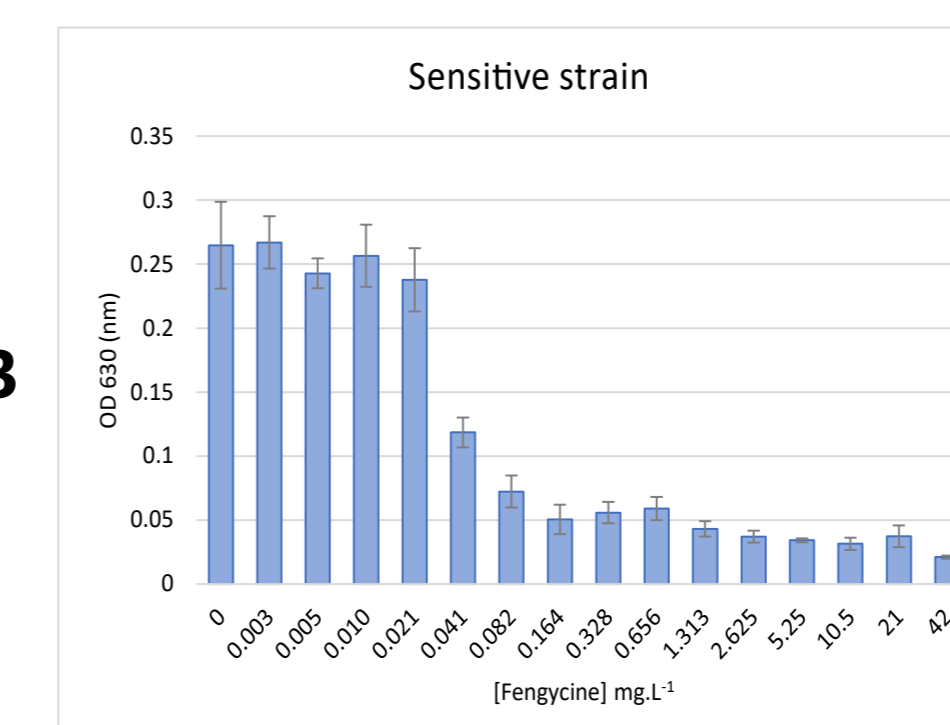
Lipopeptides from *Bacillus* sp. are environmentally friendly and potential alternatives as **biocontrol agent**. In this study, three lipopeptides were tested: fengycin (F), mycosubtilin (M) and surfactin (S). The three lipopeptides are tested against two strains of *V. inaequalis*: one strain sensitive to tebuconazole and one strain with reduced sensitivity to tebuconazole. Tebuconazole (Tb) is the reference active substance. The tolnaftate is used as an indicator of efflux pump overexpression^(b-c).

Product	Origin	Structure ^(b-d-e)	Properties ^(b-d-e)
Fengycin	From <i>Bacillus</i> sp.	Partially cyclic + β -OH fatty acids	• Fungitoxic activity (filamentous fungi) • Biosurfactant
Mycosubtilin		Cyclic heptapeptide + β -NH ₂ fatty acids	• Antifungal (yeast and fungi) • Biosurfactant
Surfactin		Cyclic heptapeptide + β -OH fatty acids	• Antiviral, antitumour and anticoagulant activities • Stimulation of plant defense • Biosurfactant
Tolnaftate	Chemical active substance	Thiocarbamate	• Antifungal agent in medicine • Indicator of multi drug resistance strain
Tebuconazole		Azole	Antifungal agent in agriculture

Methods

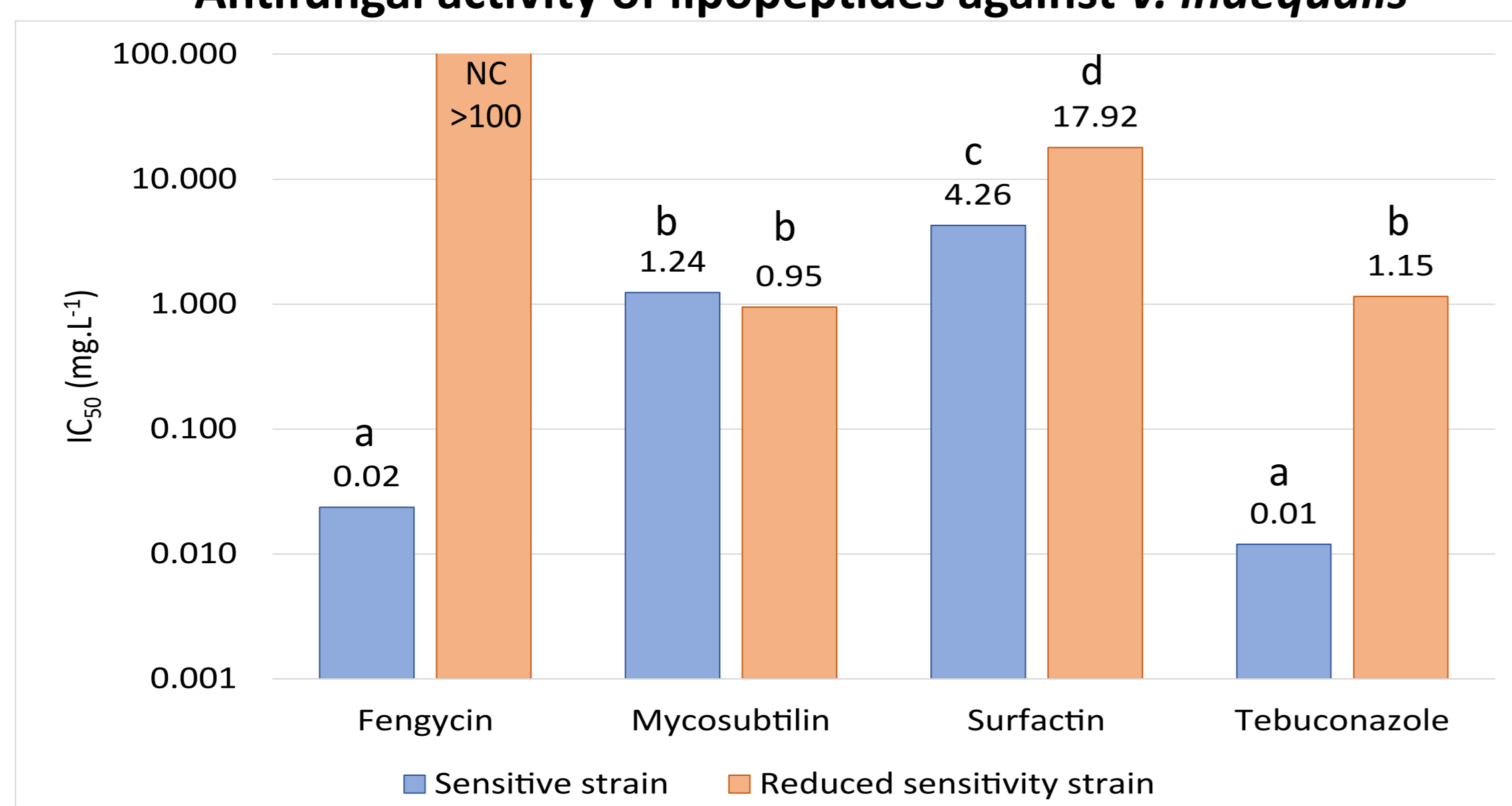


- The antifungal activity of the three lipopeptides (F, M and S) and two fungicidal active substances (Tn and Tb) is evaluated in a microplate test. The products are solubilized in 100% DMSO (60mg.L⁻¹ for lipopeptides and 40mg.L⁻¹ for active substances). Then, a range of 16 concentrations in glucose peptone is performed.
- A spore suspension calibrated at 1.10⁵ sp/mL is made after 14 days of incubation in the dark and 6 days under UV. After inoculation, the plates are incubated for 6 days under agitation and in the dark.
- After obtaining the OD of 3 independent experiments, an IC₅₀ value is obtained by non-linear regression with a 95% confidence interval. An ANOVA was performed to determine the differences in activity between the molecules.



Results

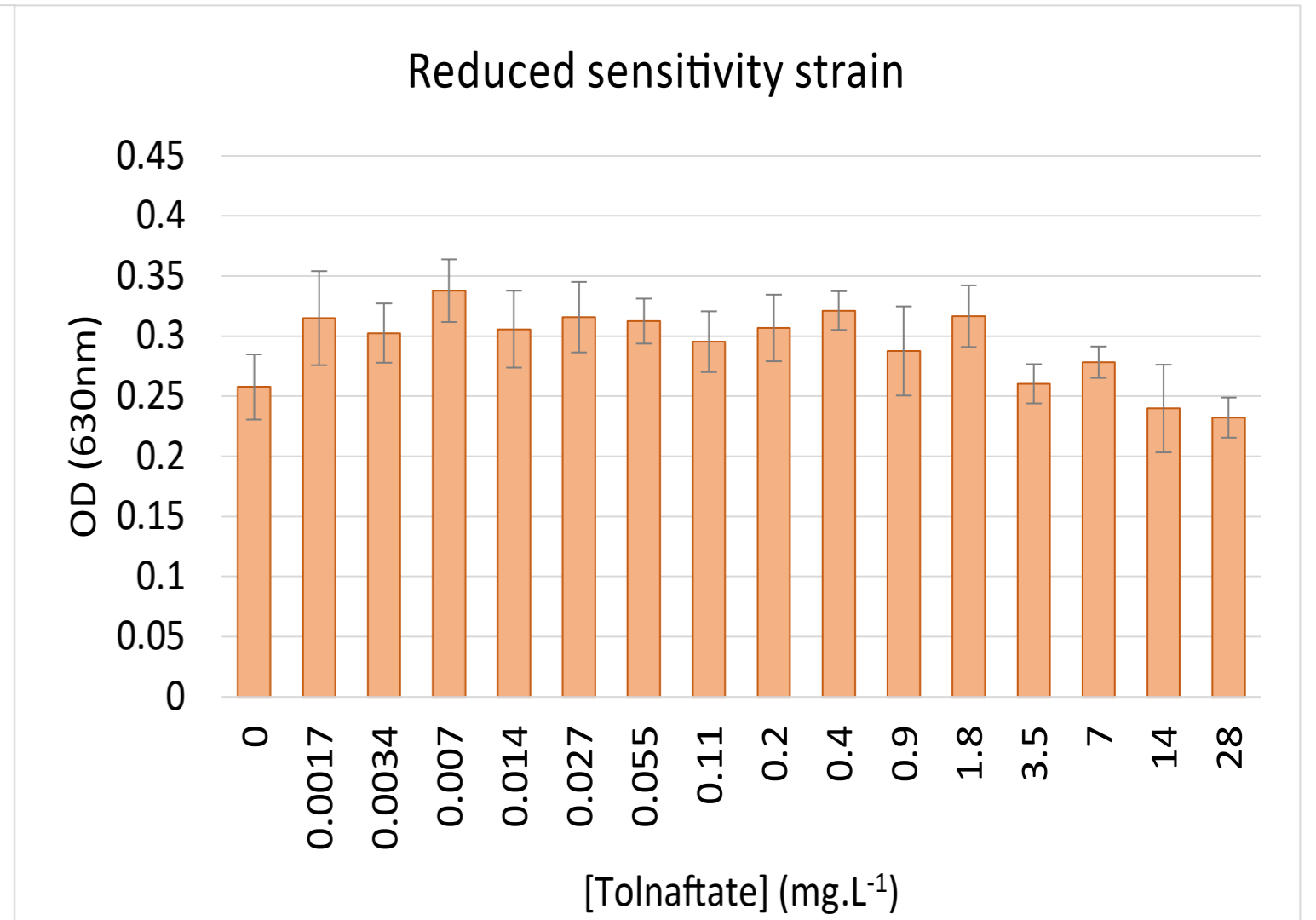
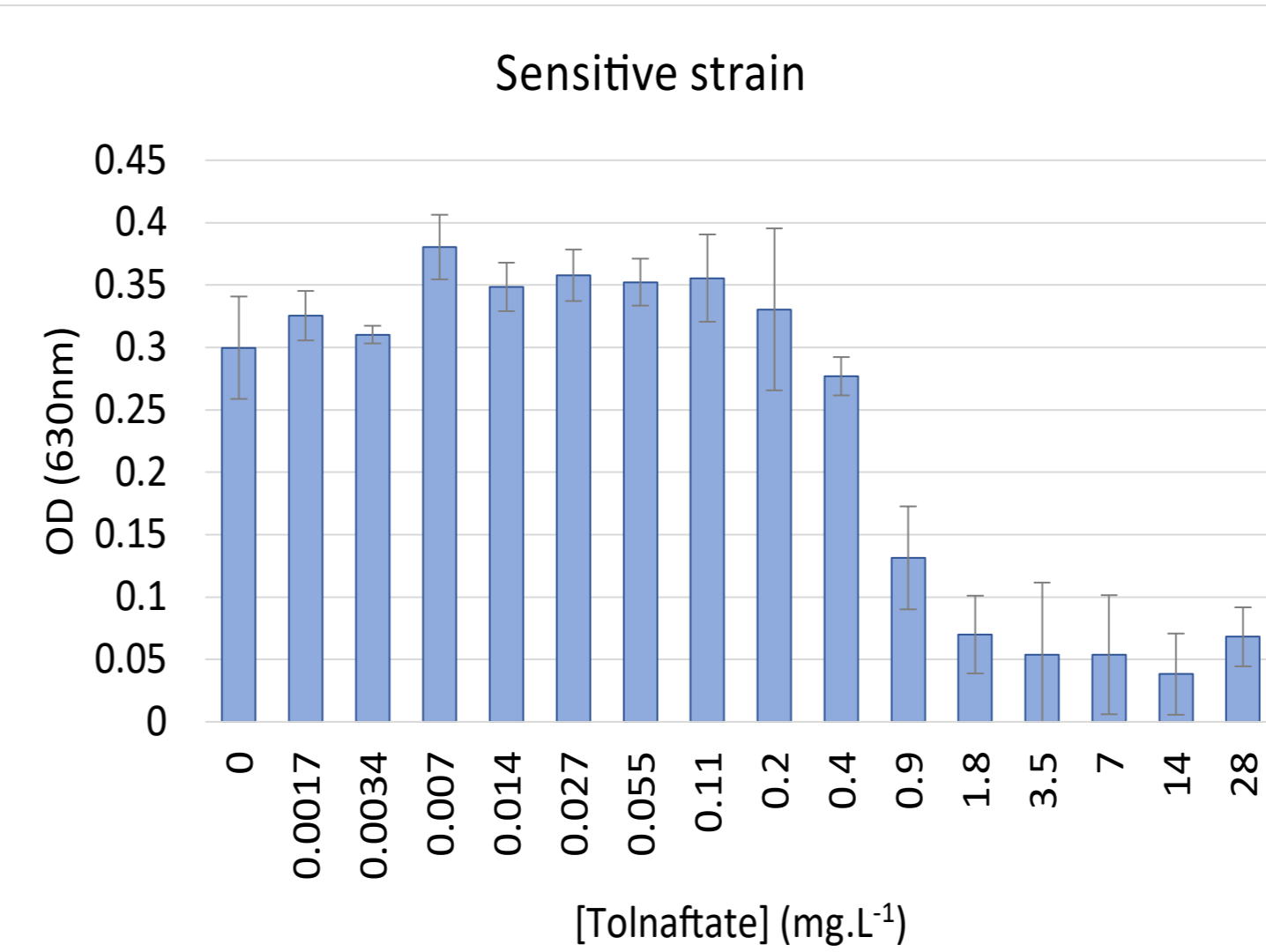
Antifungal activity of lipopeptides against *V. inaequalis*



- Fengycin has a higher activity level than mycosubtilin and surfactin in the tebuconazole sensitive strain.
- Fengycin has a similar IC₅₀ to tebuconazole in the sensitive strain.
- Mycosubtilin has an antifungal effect on the both strains.
- The strain with reduced sensitivity to tebuconazole has reduced sensitivity to fengycin and surfactin.

Tolnaftate activity against *V. inaequalis*

Tolnaftate is an indicator fungicide for multidrug resistance linked to the overexpression of efflux pumps.



- Antifungal activity of tolnaftate on the sensitive strain : IC₅₀ = 0.40 mg.L⁻¹ [0.39-0.59]
- No antifungal activity on the reduced sensitivity strain.

Conclusions

- Fengycin is the lipopeptide with the best activity, similar to tebuconazole on the sensitive strain.
- Mycosubtilin is the only lipopeptide with antifungal activity against both strains.
- The reduced sensitivity strain is resistant to tolnaftate -> indication of the role of efflux pumps

Perspectives

- Characterize tebuconazole resistance by other mechanisms :
 - CYP51 gene mutation
 - Overexpression of the CYP51 gene
- Investigation of increased efflux in the presence of pump inhibitor and azole/lipopeptides