



Quest for a next generation lipopeptide bio-control agent from the fish commensal bacterium '*Bacillus tequilensis* PP1'

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Background

Nearly all the vegetation in our planet earth is susceptible to invading pathogens, which significantly affect the crop and its yield. The chemical bio-control agents are the wide spread tool to get rid of plant pathogens. However, the excessive use of such chemical bio-control agents has the potential to wipe-out entire humanity. In this situation, advanced research in the development of eco-friendly bio-control agents is an absolute necessity

Objectives of the study: Isolation and purification of lipopeptide antibiotics and evaluating its biological activities such as antifungal and biosurfactant property.

Materials and methods

- Collection and authentication of fish
- Screening for antibiotic producing bacteria
- Precipitation (CaCl₂, 1%)
- Dialysis (2000 NMWCO)
- Size exclusion chromatography (Sephadex G-25)
- Detection of peptide (Ninhydrin test, LCMS, UV-visible spectrophotometry)
- Antifungal Study (Disc - Diffusion)
- Evaluating the surfactant property



Fig 1
Chelon parsia

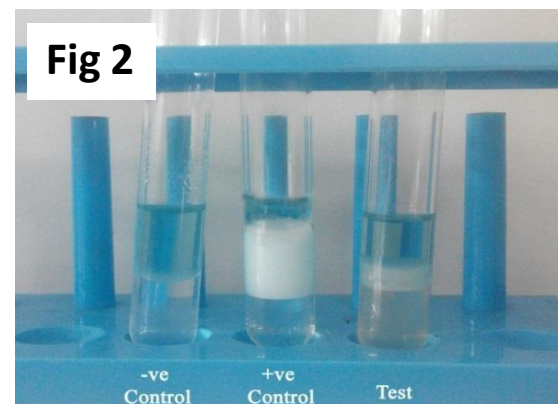


Fig 2

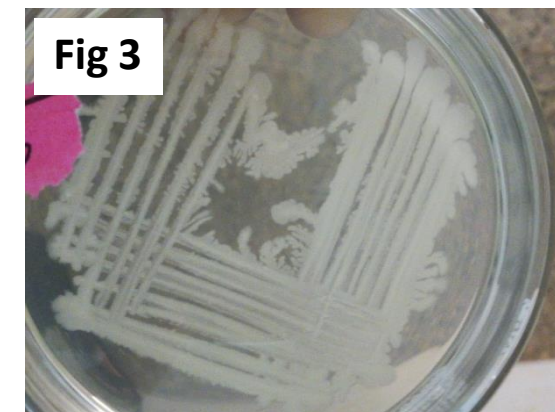


Fig 3

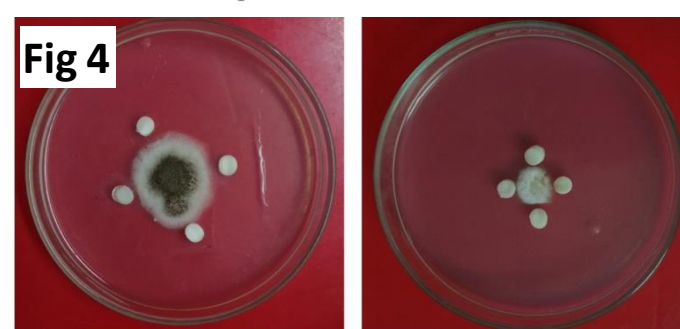
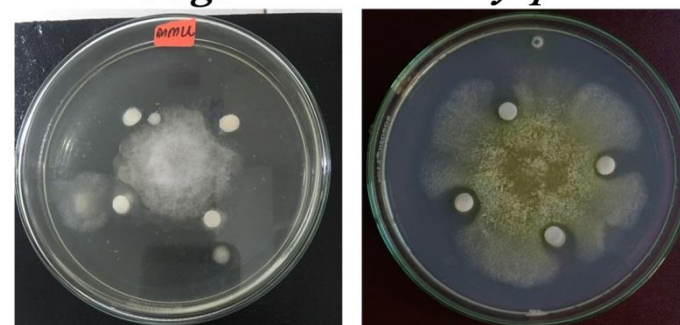


Fig 4
A.niger

F.oxysporum



M.racemosus

T.viride

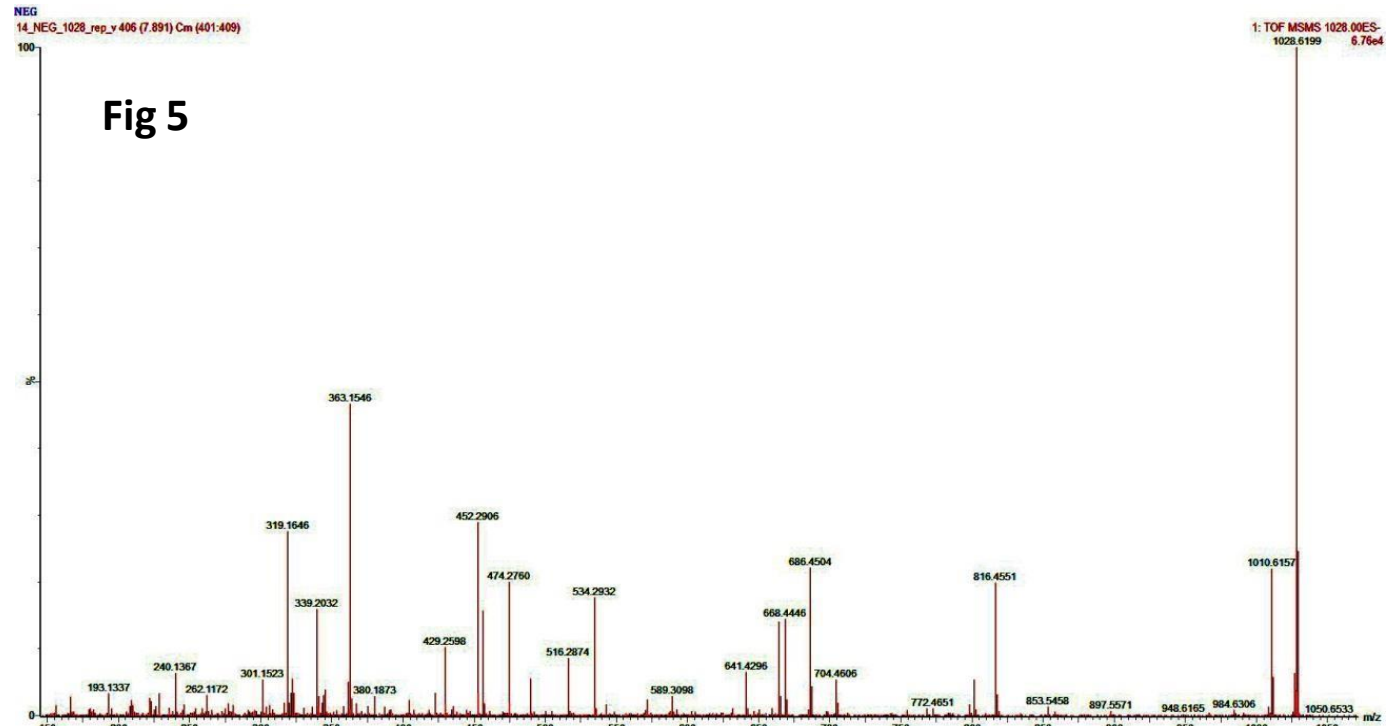


Fig 5

Table 1

Name of fungal strain	MIC (µg/ml)
<i>Aspergillus niger</i>	7
<i>Trichoderma viride</i>	6
<i>Mucor racemosus</i>	6
<i>Fusarium oxysporum</i>	12

Results

In the present study, A novel strain of *Bacillus tequilensis* PP1 (accession number MK648314) (Fig 3) isolated from the gills of an estuarine water fish *Chelon parsia* (Fig 1). The strain showed significant antagonistic property towards most of the phyto-pathogenic fungi. An unknown molecule with m/z 1028.61 was found to be the key molecule that contribute to antifungal activity. Primary structural study revealed that the molecule comes under lipopeptide class which contain a heptapeptide region and a fatty acid tail. However, the detailed structure of the molecule is under investigation. The isolated lipopeptide can inhibit the growth of phyto-pathogenic fungi such as *Aspergillus niger*, *Trichoderma viride*, *Mucor racemosus* and *Fusarium oxysporum* with minimum inhibitory concentration range (MIC) of 6 to 12 µg/ml. In addition to the antifungal activity, the lipopeptide was found to be a powerful surfactant with an emulsification index of 32.21 %. The antifungal activity against phyto-pathogens and the surfactant property of the proposed lipopeptide opens up its extended application in the development of eco-friendly biocontrol agent in agricultural field.

Conclusion

This study bring forward an antifungal lipopeptide (m/z 1028.61) derived from the fish-gill commensal bacterium *Bacillus tequilensis* PP1. The lipopeptide has the potency to develop it as a biocontrol agent for agricultural application. The proposal has been strengthened by its pleasing MIC values (6 to 12 µg/ml) against phytopathogenic fungi. In addition, the surfactant property of the molecule extends further possibilities. This is an ongoing research and the detailed structural identity of the lipopeptide is under investigation in our lab.

References

- Ongena M, Jacques P. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. (2008) *Trends Microbiol* 16(3):115-25.
- Penha RO, Vandenberghe LPS, Faulds C, Soccol VT, Soccol CR. *Bacillus* lipopeptides as powerful pest control agents for a more sustainable and healthy agriculture: recent studies and innovations(2020) . *Planta*. 251(3):70.

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