An in-silico platform for screening and testing bactericides targeting *Candidatus Liberibacter asiaticus* for Huanglongbing (HLB) management

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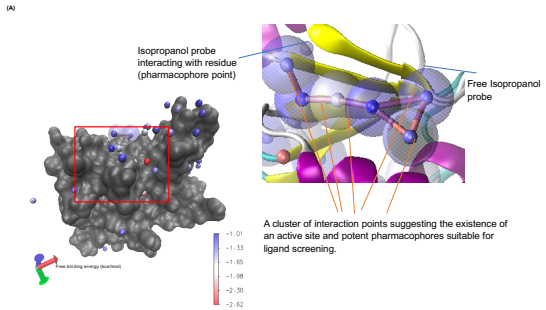
Background

- Only a few studies have utilized biochemical target-based ligand screening targeting critical enzymes for the pathogen, ***Candidatus Liberibacter asiaticus (CLas)*** that causes Huanglongbing disease (HLB) on citrus trees.
- Conserved proteins such periplasmic metal binding protein (PMBP), enoyl-acyl carrier protein reductase I (FabL), beta-Hydroxyacyl-acyl carrier protein dehydratase (FabZ), von Willebrand factor type A domain-containing protein (vWAP) and Serine/tyrosine phosphatase (STP) are potential candidates for CLas inhibition since they control critical metabolic pathways of the bacteria.
- The Enhanced Ligand Exploration and Interaction Recognition Algorithm (ELIXIR-A), was used screening of pharmacophores leading to the identification of inhibitors.
- In this work, serine/tyrosine phosphatase (STP) an enzyme known to regulate CLas and Candidatus Liberibacter solanacearum (CLsol) metabolic activity was selected for finding CLas inhibitors.

Method

- Inhibitor screening comprised of several steps including the development of the STP homology model, identification of active sites and pharmacophores, inhibitor screening, and *in silico* confirmation before experimental verification. Simulation platforms including ELIXIR-A, Autodock Vina, Visual Molecular Dynamics (VMD), Drugui, Nanoscale Molecular Dynamics (NAMD) were used for *in silico* analyses.
- The antimicrobial efficacy was verified in vitro using CLsol.



A) Hotspot distribution when NAMD simulations were performed with select probes on the CLsol STP enzyme. The red-spot depicts a binding hotspot with highest affinity. The area demarcated by the red box depicts pharmacophore distribution at the primary site.
B) B) Multiple pharmacophores were clustered around the CLsol STP enzyme implying potential binding site(s).

A new in-silico strategy to screen and identify inhibitors for causal agent of Huanglongbing (HLB), 'Candidatus Liberibacter asiaticus'

Ligand: Small Molecule Library including 13,190,317 molecules.
Receptor: homology molecular model of serine/tyrosine phosphatase using the plant pathogen whole genome sequences.

Virtual high-throughput screening: ~10 molecules were selected using ELIXIR-A platform

2 molecules were moved to Ligand & Receptor Interaction study

In-vitro study

Lead drug

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Results

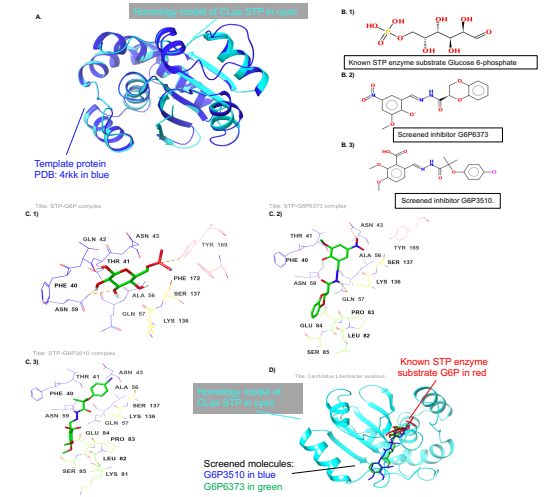
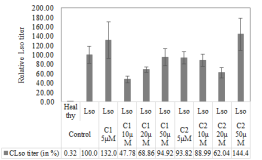


Figure A) The homology model of CLas STP (cyan) overlaid with the template (PDB ID: 4RKK in blue). B) structural information of the screened compounds; 1) Known STP enzyme substrate G6P; 2) Screened inhibitor G6P6373; 3) Screened inhibitor G6P3510.
C) The interactions of STP with G6P, G6P6373 and G6P3510, respectively.
D) G6P (red) occupying the active site and an isolated ligand (blue) occupying the same site as G6P on CLas STP (cyan).

Compound name	Glucose 6-phosphate	G6P3510	G6P6373
IUPAC Name	[(2~{R})-1-[(1,4~{S},5~{R},6~{S})-3,4,5,6-tetrahydroxycyclohexyl]-2-oxidanyl-propyl] dihydrogen phosphate	4-[(~{S})-[(2~{R},3~{R})-3-(4-chlorophenoxy)-2-methyl-propanoyl]hydrazinylidene]methyl]-2,3-dimethoxybenzoic acid	2-[[(3~{R})-2,3-dihydro-1,4-benzodioxine-3-carbonyl]hydrazinylidene]methyl]-6-methoxy-4-nitrophenolate
Octanol-water partition coefficient logP	-3.31	3.90	-0.87
molecular weight	260.13	420.85	372.31
number of hydrogen bond acceptors	9	8	10
number of hydrogen bond donors	6	2	1
Number of "Rule of five" violations	1	0	0
Number of Rotatable Bonds	7	8	5
Enzyme inhibitor score	1.23	-0.19	-0.54
Average docking affinity on CLasI STP, kcal/mol ± std (n=10)	-4.74 ± 0.196	-5.38 ± 0.379	-5.41± 0.438
Average docking affinity on CLas STP, kcal/mol ± std (n=10)	-4.38 ± 0.379	-5.30 ± 0.283	-5.27± 0.320



In-vitro dose-response assay of screened molecules on CLsol.

Discussion

- The screened compounds had higher binding affinities than the original substrate Glucose 6-phosphate on serine/tyrosine phosphatase (STP) enzyme of CLas and CLsol.
- The in-vitro experiments indicated that two screened compounds can inhibit the growth of the CLsol cultures.

Future work

- Test the two screened compounds in-vitro on pathogen CLas.
- Extend the screening work to other essential enzymes.

Acknowledgements

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