Letter to the Editor

An integrated pipeline for the pest management of Bactrocera oleae

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Bactocera oleae (Diptera: Tephritidae) is the most destructive pest of olive orchards worldwide. It is growing its larvae in the mesocarp of olive fruits, thus causing extensive crop damage and significant reduction of olive oil production (Tzanakakis 2003). *Bactocera oleae* is responsible for the destruction of 5% of the total olive oil production (30% in the Mediterranean countries), with direct impact on a financial level that exceeds 800 million dollars per year. During the last 5 years, 95 % of the world olive oil has been produced in Spain, Italy and Greece, where *Bactocera oleae* is endemic (Kounatidis *et al.* 2009). Controlling its population is a priority for producers. Until recently, the main measures against olive flies is to lure them using pheromone traps. However, the excessive use of insectides renders the flies resistant to them. In this direction biological control came up with the use of parasitoids and shortly after the sterile insect technique was introduced. As effective as they are, those techniques are hazardous to both wildlife and the environment.

Olive fly has been reportedly associated with many different bacterial species and one of the most important is *Erwinia dacicola*. While most fruit flies' larvae can't develop on unripe fruit, olive fly larvae can metabolize very efficiently nutrients from green olives. The main inhibitory substance of unripe olives is oleuropein – a phenolic glycoside whose levels are very high in the unripe olive fruit (Savio *et al.* 2011). It has been examined that asymbiotic larvae can't develop on unripe olives, while wild type larvae can normally develop. *Erwinia dacicola* is a co-evolved symbiont to the olive fly that renders it a non-cultivable bacterium. *Erwinia dacicola* can be found intracellularly in the larval midgut caeca cells while in the adult fly, it resides extracellularly in foregut cells (Estes *et al.* 2012). This transition, common in many insect symbionts, is essential for their survival during the insect metamorphosis. The extracellular localization in the adult fly is also important for the transmission of *Erwinia dacicola* vertically to the next generation by staining the eggs as they pass through the ovipositor. *Bactrocera oleae* larvae depends on the biochemical machinery of the candida endosymbiotic *Erwinia dacicola* to process and metabolize nutrients from unripe olives (Pavlidi *et al.* 2017). This could lead to a massive decrease on olive fly's population during the first two or three generations of the year, until olive fruit is fully developed, and eventually the full annual population.

Therefore, there is great need to lower the concentration of *Erwinia dacicola* on adult flies. In this direction a three-dimensional homology-based model was designed according to Vlachakis *et al.* (2014). The model was stereochemically and energetically evaluated and was superposed to its template. It was confirmed that the model retained the fold of its template, but more importantly, it shared similar physicochemical and kinetic profile with the X-ray structure it derived from. The main hinderance in *in silico* drug design and high throughput virtual screening is the toxicity and non-specific binding of potential inhibitors. This issue in the real world

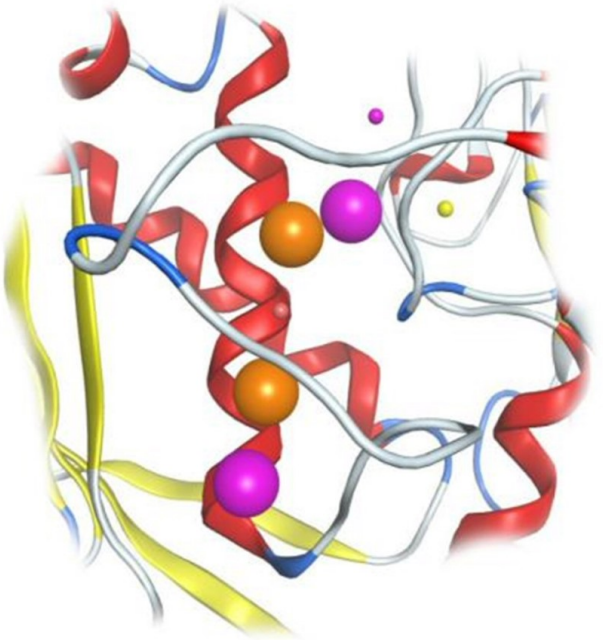

Figure 1. The consensus pharmacophore model for *Erwinia dacicola*. The pharmacophore spheres represent and characterize molecules on schematic 3D level by identifying the essential properties of molecular recognition for a low molecular weight inhibitor compound to optimally interact with the fly's endosymbiotic bacterium.

eventually escalates and results in resistance to the designed agents. Therefore, there is dire need for novel approaches and new strategies to be deployed. In this direction, a pharmacophore was designed for the identification of the possible common binding interactions in a series of potential targets of *Erwinia dacicola*. The latter pharmacophore models can be overlaid and reduced to their shared features so that common interactions are retained. Such a consensus pharmacophore can be considered as the largest common denominator shared by a set of active molecules. This novel pipeline is expected to act both in a parallel and serial mode. Blocking multiple targets accumulatively will be more destructive for the survival of the target organism (i.e. *Erwinia dacicola*) and in case the bacterium establishes some form of full or partial immunity in one of our pharmacological targets the chances are that the rest targets will remain valid. The commonly reduced pharmacophoric features includes two heavy aromatic regions and two hydroxy-like groups. The rest of the pharmacophore features showing in smaller spacefill spheres are unique for the given site and protein, so they are being ignored on the basis that they don't satisfy all three targets in this study. Electrostatic surfaces were drawn to be used as a filtering criterion for the screening process. It was found that all 3 sites shared a mainly positively charged binding site. Consequently, and based on both the electrostatic surface study and the pharmacophore modelling the ideal compounds should be quite rigid, contain at least two aromatic rings and some -OH or -COOH groups.

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