High-Throughput Screening assay development for pyocyanin biosynthesis inhibitors identification.

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Abstract

Pyocyanin is a phenazine pigment related to *P. aeruginosa* virulence, however no molecule that inhibits it bosynthesis has been identified so far. The assay described herein overcomes previous assay limitations and makes it possible to screen *in vitro* for pyocyanin biosynthesis inhibitors using HTS methods.

Introduction

Bacterial resistance is a complex phenomenon that has worldwide impact.[1] Although antibacterial drugs have increased the life expectancy around the world, molecules with bactericidal/bacteriostatic activity seem unable to overcome the bacterial resistance challenge.[2] An alternative approach is to modulate the virulence of pathogenic bacteria. Hence making it easier for the host immune system to fight the infection. Pyocyanin (PYO) is a *P. aeruginosa* virulence factor whose biosynthesis is carried out by 10 enzymes encoded at 3 operons (Figure 1).[3]

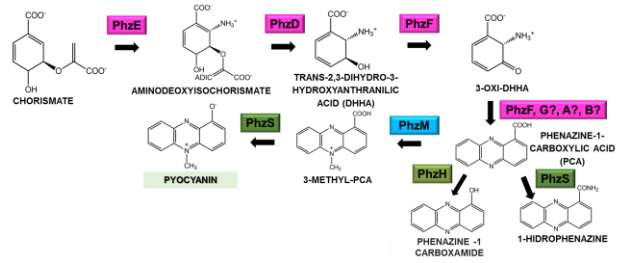

Figure 1. Pyocyanin biosynthetic pathway in *P. aeruginosa*. The operons responsible for each enzyme production are grouped according to color.

Despite the fact that five of them (PHZS, PHZD, PHZM, PHZG and PHZF) have already been kinetically and structurally characterized, no inhibitor has been reported for so far.

Results and discussion

Sensitive and low-cost conditions for screening are essential to reduce the number of false positives and avoid discarding promising molecules in the hit identification steps. Aiming to fulfill these requirements, thermal shift assays (TSA) were employed to investigate the effect of pH, organic solvents and additives on the stability of three enzymes of the pyocyanin biosynthesis pathway. Our data suggest, for example, that screening of phzS and phzD should be carried out at pH= 7.0 (ΔTm= 2.3 ºC, (ΔTm= 3.5 ºC), whereas phzM is more stable at pH= 6.0 (ΔTm= 3.1 ºC) (Figure 2). In addition, our results suggest that phzS screening can be carried out in the presence of 5%DMSO (ΔTm= 0.8 ºC) or EtOH (ΔTm= 1.9 ºC).

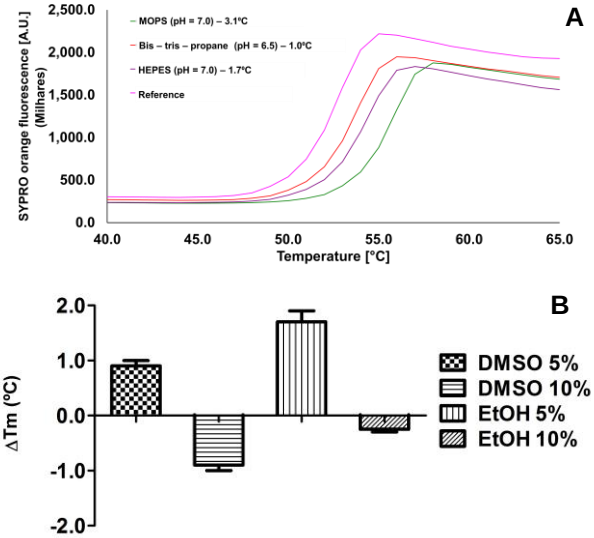

Figure 2. Thermal displacement of phzD due to pH variation (A) and phzS in the presence of organic solvents (B).

Conclusion

The TSA reported herein are useful to evaluate substances which absorb at 340 nM as well as other macromolecular targets of PYO biosynthesis pathway that have no spectrophotometric assay described in the literature. Therefore, this work lays the basis for the identification of pyocyanin biosynthesis inhibitors.

Aknowledgements

CNPq and FAPESB

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