Farnesol and geraniol loaded nanocapsules enhance the susceptibility of mcr-1 *Escherichia coli* to colistin

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Introduction

- The use of colistin, has been limited in the past because of its nephrotoxicity. Nowadays, colistin has become one of last antibiotic yet active against some multiresistant Gram negative bacteria. But, the discovery in 2015 of a transferable plasmid-encoded colistin resistance gene MCR-1, raised an important public health problem. Also, to keep this ATB as a last resort ATB a way to tackle this mcr-1 mechanism would be very useful. Non-antibiotic drugs, like terpenes (TPs), combined to ATB have shown effectiveness against resistant bacteria.
- The aim of the study was to propose an effective combination between colistin and terpene alcohols against mcr-1 Gram negative bacteria. Interesting results were obtained using geraniol and farnesol encapsulated within lipid nanocapsules (LNCs).

Materials/Methods

➢ *Formulation LNCs*

Lipophilic geraniol (LogP: 2.9) and farnesol (LogP: 4.84) were encapsulated within LNCs (Fig. 1). LNCs were formulated using the phase inversion method to facilitate the aqueous dispersion.

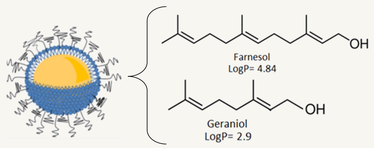

Fig.1: (a) Schematic representation of a LNC

➢ *Checkerboard titration*

The diminution of colistin MIC in the presence of farnesol and geraniol was determined by checkerboard titration using *E.coli* J53 strain and its mcr-1 transconjugant. Potency (EC50) and Efficacy (Emax) were determined for each terpene/colistin combinations, using the following Emax model (Equation 1).

$$\widehat{MIC} = MIC_0 - \frac{(MIC_0 - MIC_\infty) * C_{terpene}^{\gamma})}{EC_{50}{}^{\gamma} + C_{terpenes}^{\gamma}} \qquad Emax = \frac{MIC_0}{MIC_\infty}$$

➢ *Kill-time experiments*

Time-kill studies were performed with an inoculum of 5.10^5 CFU/mL. Colistin concentration was 1/8 of the MIC value e.g. 0.031 mg/L for *E.coli* J53 and 1mg/L for its mcr-1 transconjugant. Based on checkerboard results, farnesol concentrations tested were; 10, 30 and 60 mg/L and geraniol concentrations were 60, 100 and 200 mg/L.

Materials/Methods

➢ *Determination of the permeability of the outer membrane (OM) - 1-N- phenylnaphtylamine (NPN) uptake.*

NPN is a nonpolar dye that fluoresces strongly in lipophilic environments, such as the lipid bilayer of the IM or the inner leaflet of the OM. Its uptake by *E. coli* J53 mcr-1 cells was measured in the presence of colistin alone at various concentrations (0 - 32 mg/L) or supplemented with 30 mg/L of farnesol or 30 or 90 mg/L of geraniol. NPN uptake (%) = $(F_{obs} - F_0)/(F_{100} - F_0) \times 100$ where F_{obs} was the observed fluorescence at a given colistin concentration, F_0 was the fluorescence of NPN with *E. coli* cells in the absence of colistin, and F_{100} was the fluorescence of NPN measured in the presence of 32 mg/L of colistin in the absence of terpene.

➢ *Determination of the permeability of the inner membrane (IM) - propidium iodide (PI) uptake.*

PI is a dye which penetrates only damaged *IM*. Its interaction with double-stranded DNA amplifies its fluorescence. A protocol similar to that used for NPN uptake was used.

Results & Discussion

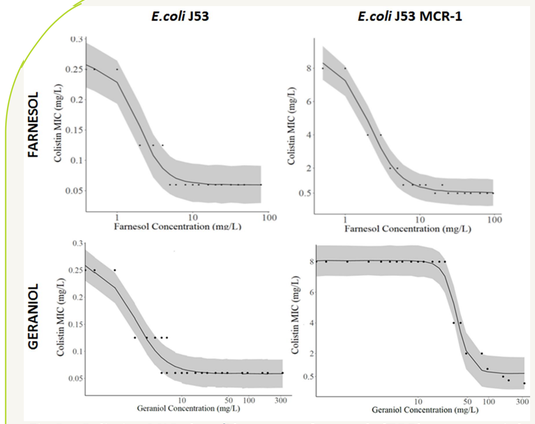

Fig.2: Colistin MIC (mg/L) versus farnesol (TOP) or geraniol (bottom) concentrations (mg/L) measured for E.coli J53 (left) and the corresponding mcr-1 transconjugant *E.coli* J53 mcr-1 (right). The shaded area is a 95% confidence interval on the fitted values (lines).

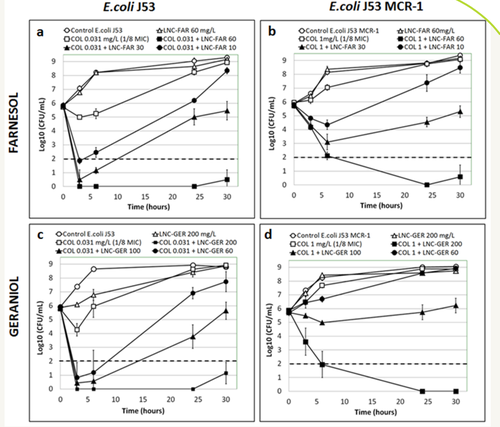

Fig. 3: Time-kill curves obtained for *E.coli* J53 and *E.coli* J53 mcr-1 strains in the presence of a colistin base concentration of 1/8 of the MIC value and farnesol (10, 30, and 60 mg/L - top) or geraniol (60, 100, and 200 mg/L - bottom). The dotted horizontal line indicates the limit of quantification of the *E. coli* concentration.

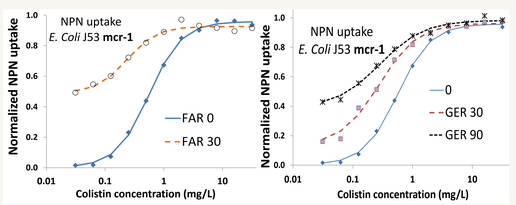

Fig. 4: NPN uptake by *E.coli* J53 mcr-1 versus colistin concentration measured in the presence of farnesol (30 mg/L – left) or geraniol (30 and 90 mg/L – right).

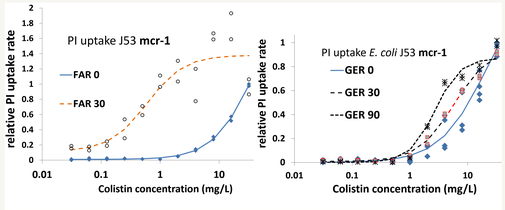

Fig. 5: PI uptake by *E.coli* J53 mcr-1 versus colistin concentration measured in the presence of farnesol (30 mg/L – left) or geraniol (30 and 90 mg/L – right).

Results & Discussion

Geraniol and farnesol were not active alone against these *E.coli* strains, thus the fractional inhibitory concentration (FIC) index could not be used to evaluate the effect of the colistin - terpene combination. Instead, an Emax model was used (Eq. 1).

Farnesol was more potent than geraniol against the mcr-1 strain to reduce the colistin MIC (Fig 2) as shown by the lower EC_{50} values (2.0 ± 0.24 mg/L) for farnesol than for geraniol (35.7± 2.03mg/L). Both were highly effective against the mcr-1 strain, having an Emax≥ 16. Against wildtype *E.coli* J53 strain, similar profiles were found with geraniol or farnesol.

Against the mcr-1 strain, a bactericidal effect was observed for 60 mg/L of farnesol and 1 mg/L of colistin (1/8 of the MIC) after 6 hours of incubation, without bacterial regrowth for up to 30 hours after incubation (Fig 3). Similar results were found with 200 mg/L of geraniol and 1 mg/L of colistin.

Both terpenes were able to increase the NPN uptake in *E.coli*-mcr-1 cells, but farnesol was more potent than geraniol in destabilizing the outer membrane (Fig. 3).

Additionally to its effect on the OM, farnesol was also able to increase the permeabilization effect of colistin on the inner membrane (Fig.4), which could explain the higher potency of farnesol compared to geraniol.

Conclusion

- ✓ Farnesol seems a good adjuvant for restoring colistin activity against mcr-1 E.coli, allowing using low colistin concentrations (1/8 of the MIC), while, preventing bacterial regrowth. The use of a molecule without bactericidal effect could slow down the appearance of resistances.
- ✓ Farnesol potentiates the permeabilization effect of colistin on the outer and inner membranes of *E.coli* mcr-1, thereby reducing the colistin concentration required to kill the bacteria.
- ✓ The Emax model seems a good alternative to quantify the efficacy of the adjuvant effect of a molecule without intrinsic activity when used alone.

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