

Further characterization of *Lactobacillus* GG peptides NPSRQERR and PDENK

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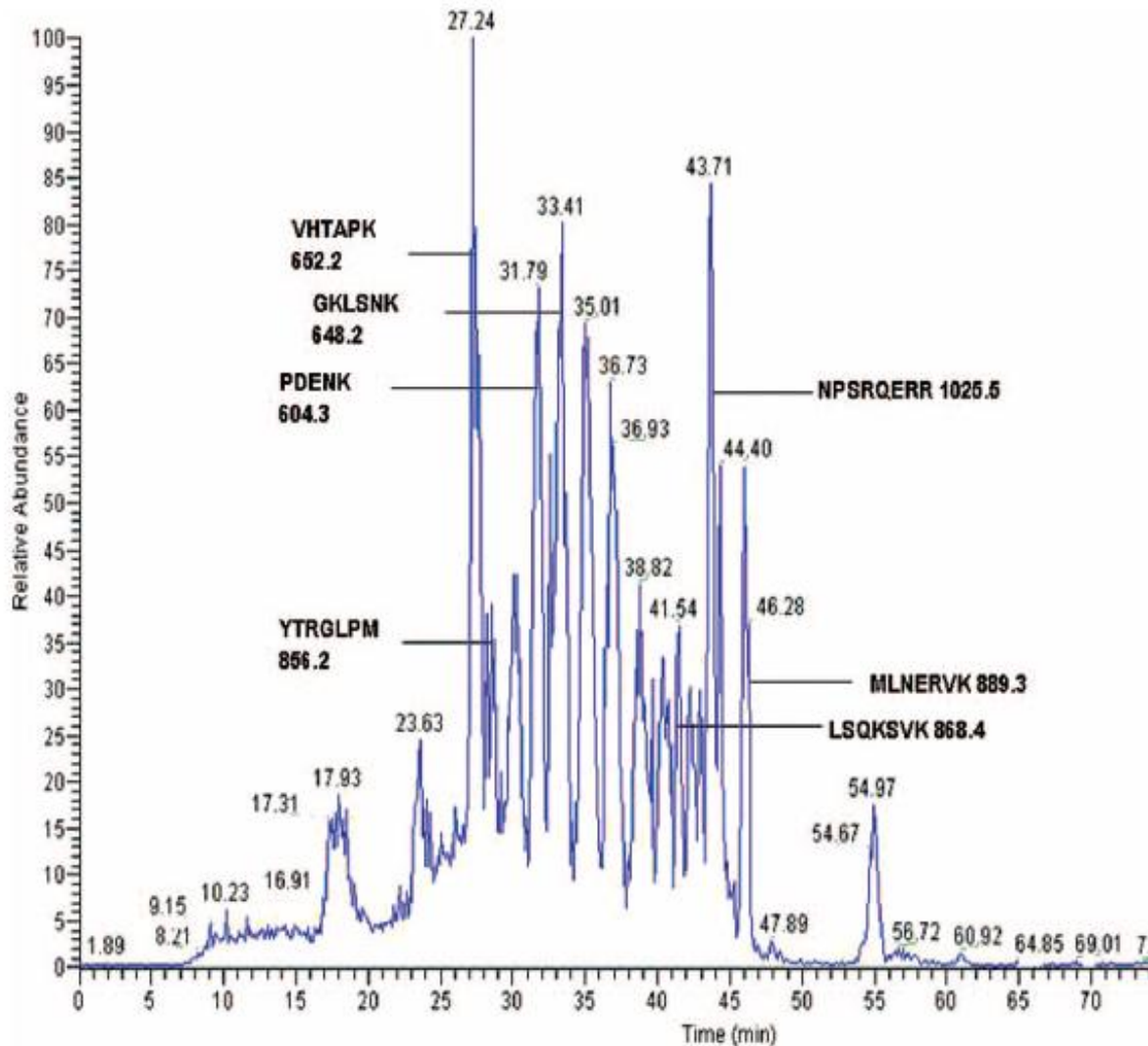
Introduction

- Probiotics are non-pathogenic bacteria that are claimed to prevent and treat several inflammatory diseases, including diarrhea, inflammatory bowel diseases, genitourinary tract infections, cancer, and respiratory infections.
- Lactobacillus GG is one of the most used probiotic isolated in 1983 from a healthy subject and patented in 1985.
- Several hypothesis have been proposed to explain the antibacterial effects of probiotics, including their capability to compete for nutrients, the establishment of a microenvironment in which pathogenic microorganisms are not able to survive, and/or elaboration of toxins lethal for pathogenic bacteria.
However, their mechanism of action has not yet been completely established.

Background

We have previously described that seven small peptides isolated from *Lactobacillus* GG conditional media exert broad spectrum anti-bacterial activities, including activities against antibiotic-resistant strains. Among them, peptide NPSRQERR and PDENK showed higher anti-bacterial activity.

Identification of Antibacterial Peptides from LGG CM by LC-MS/MS Analysis

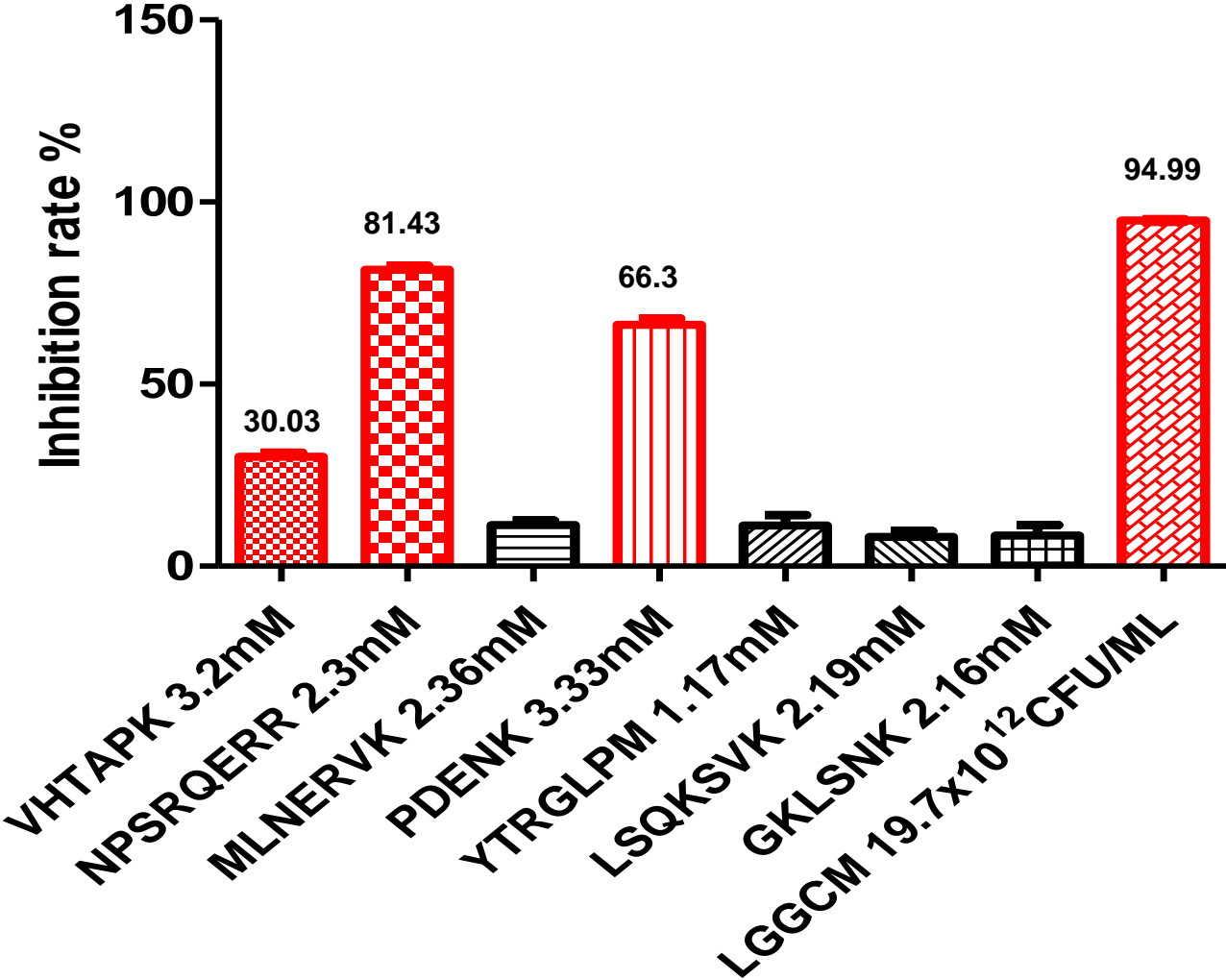


Seven peptides:

VHTAPK
GKLSNK
MLNERVK
PDENK
LSQKSVK
YTRGLPM
NPSRQERR

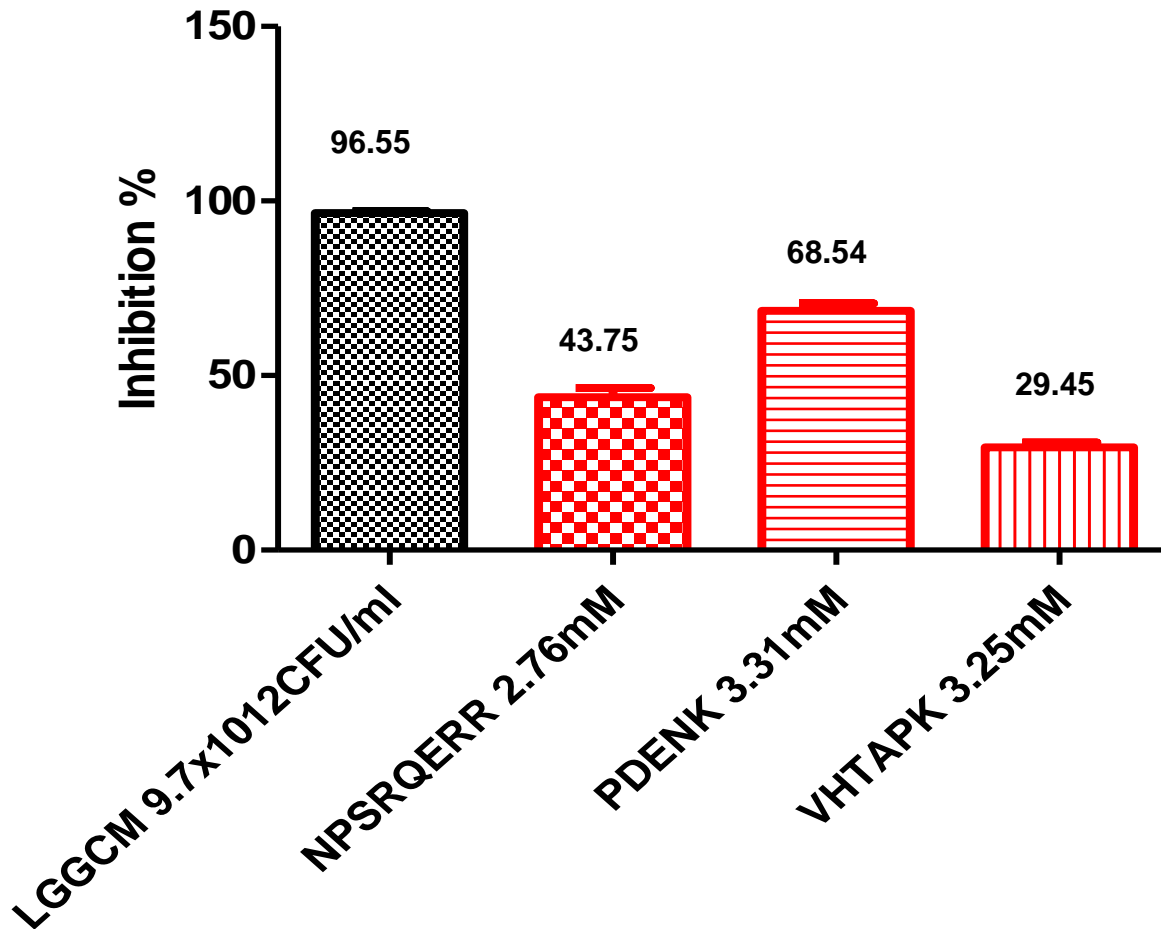
↓
Peptides
synthesized

LGCM and 7 Synthetic Peptides Relative Inhibition Activities on *E. coli* growth.



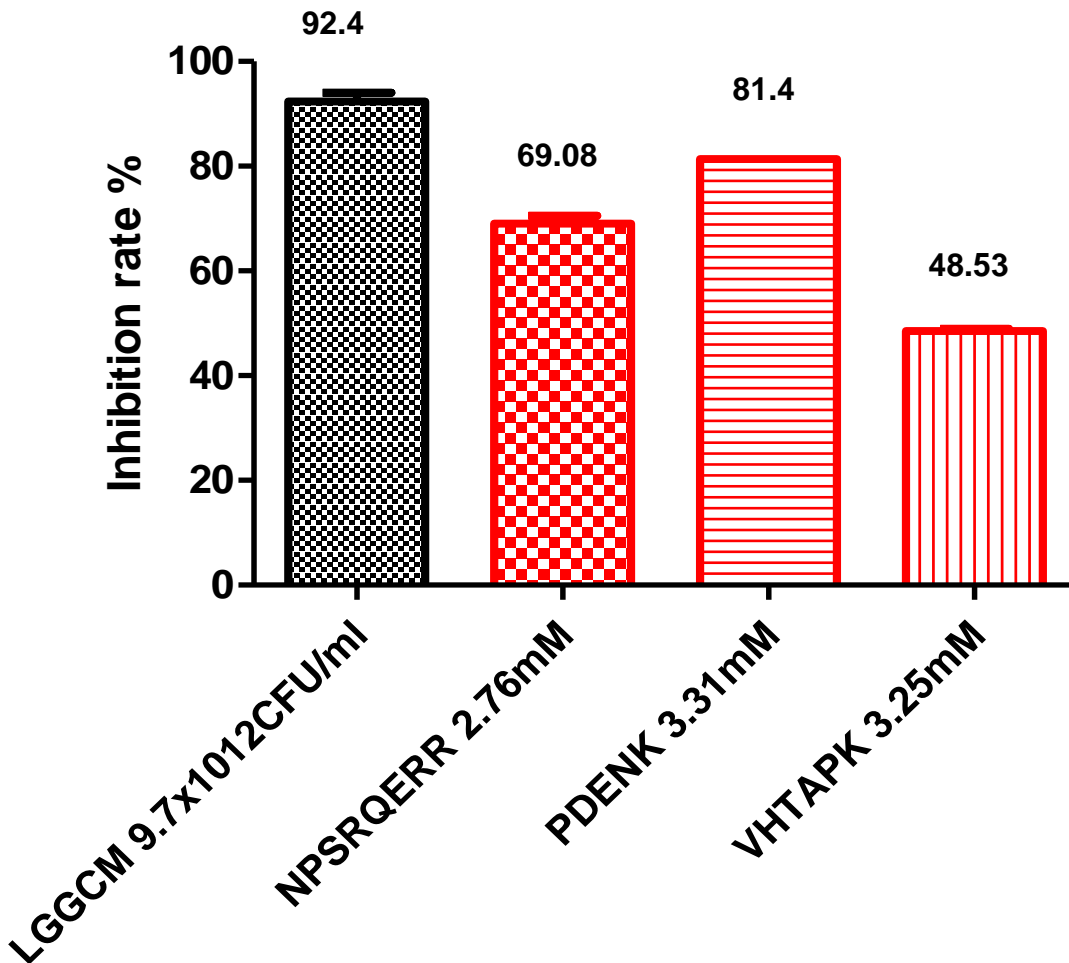
Growth inhibition assay: Mean *E. coli* record at A₆₀₀ in the presence of different peptides (n=3) or LGGCM (positive; n=7). Readings are normalized with media alone

Peptides NPSRQERR, PDENK and VHTAPK inhibition on *Kanamycine-resistant E. coli SM10 λ pir* growth.



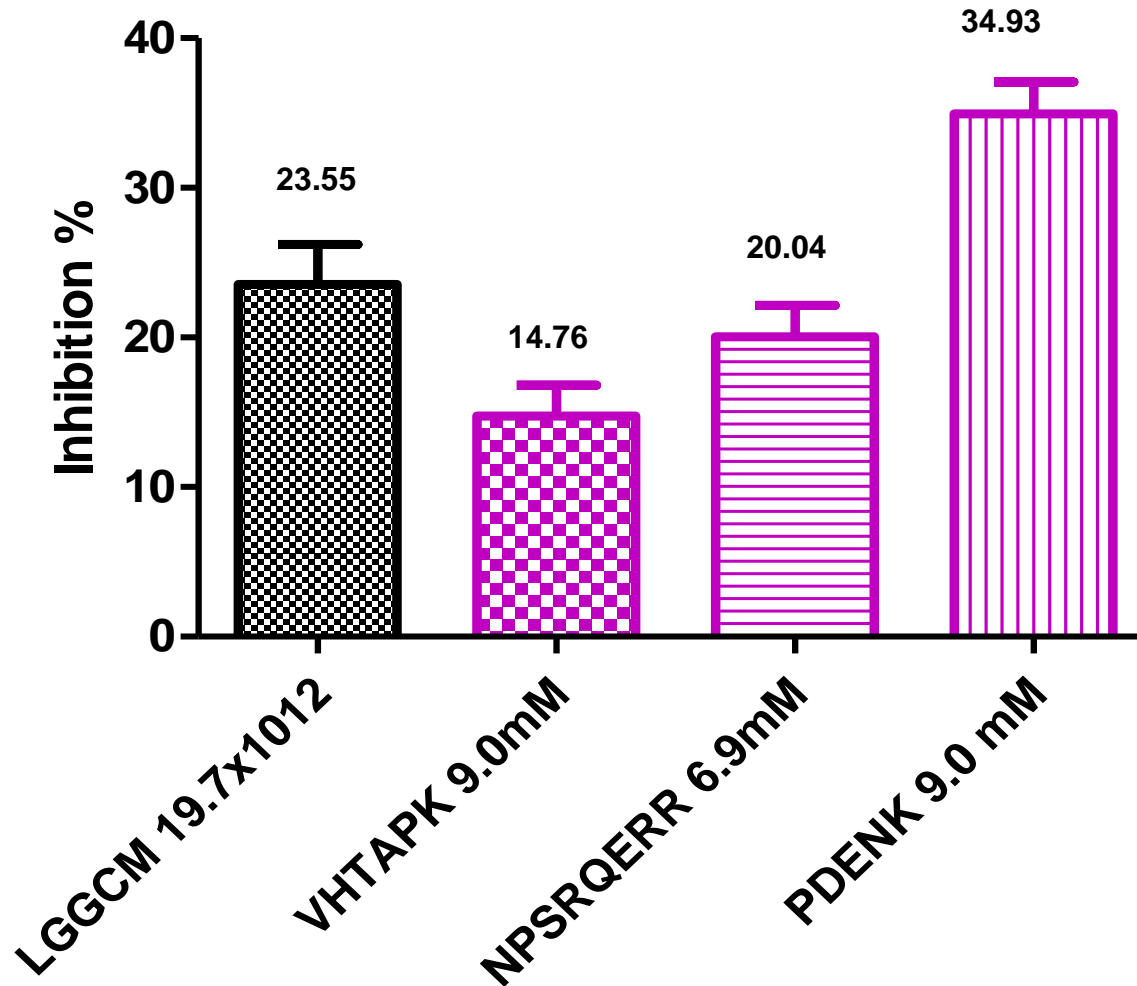
Readings are normalized with media alone
Data show Average (n \geq 3) \pm SE .
LGGCM(9.7X10¹² CFU/ML) as positive control.

Peptides NPSRQERR, PDENK and VHTAPK inhibition on *Tetracycline-resistant E. coli* TOPO10 growth.



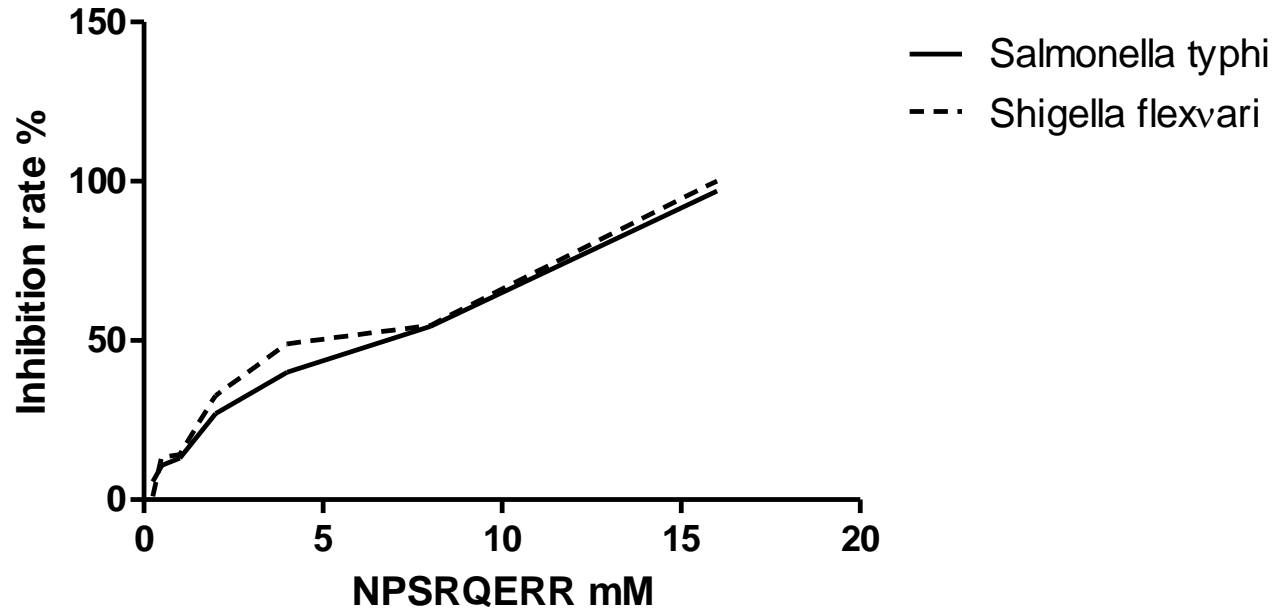
Readings are normalized with media alone
Data show Averages (n≥3)±SE.
LGGCM(9.7X10¹² CFU/ML) as positive control.

Peptide NPSRQERR, PDENK and VHTAPK inhibition on *MRSA* growth



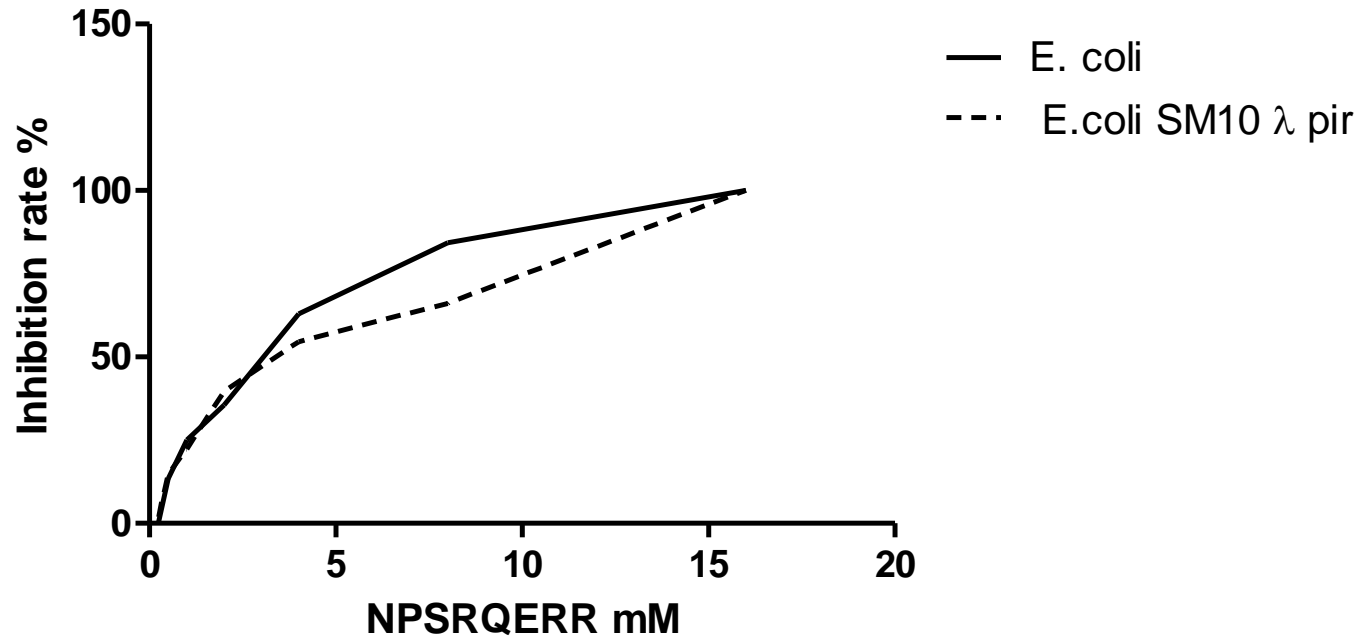
Readings are normalized with media alone
Data show Average (n≥3) ±SE.
LGGCM(19.7X10¹² CFU/mL) as positive control.

Results



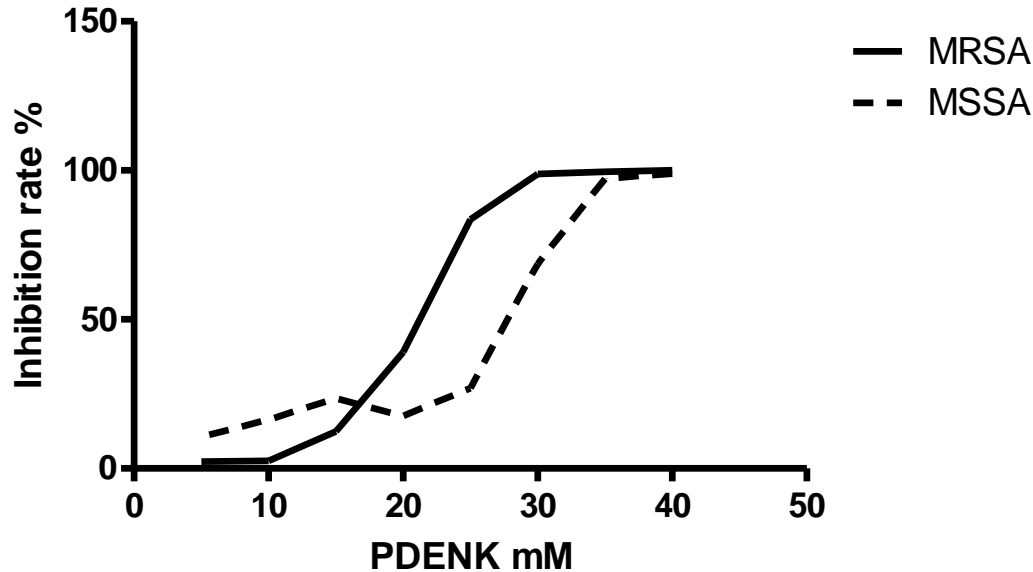
Peptide NPSRQERR inhibits wide type *Salmonella typhi* and wide type *Shigella flexnari* dose-response curve. IC_{50} for *Salmonella typhi* is 7.4 mM, for *Shigella flexnari* is 4.7mM. One colony of *Salmonella typhi* or *Shigella flexnari* incubated in 1.0 ml of LB for 1.0 hour at 37°C. 0.1ml of culture mixed(adjusted bacteria concentration to 10^{3-4} cfu/ml) added to 0.9 ml of LB which has increased concentration of NPSRQERR,incubation for 1 hour at 37°C and then spread 0.1ml culture to LB agar plates, incubation overnight at 37°C, counting colonies next day. Data show 3 times average.

Results



Peptide NPSRQERR inhibits *E.coli* and Kanamycine-resistant *E. coli* SM10 λ pir dose-response curve. IC_{50} for *E. coli* is 2.8 mM, for SM10 is 3.3mM. One colony of *E.coli* or SM10 incubated in 1.0 ml of LB for 1.0 hour at 37°C. 0.1ml of culture mixed(adjusted bacteria concentration to 10^{3-4} cfu/ml) added to 0.9 ml of LB which has increased concentration of NPSRQERR,incubation for i hour at 37°C and then spread 0.1ml culture to LB agar plates, incubation overnight at 37°C, counting colonies next day. Data show 3 times average.

Results

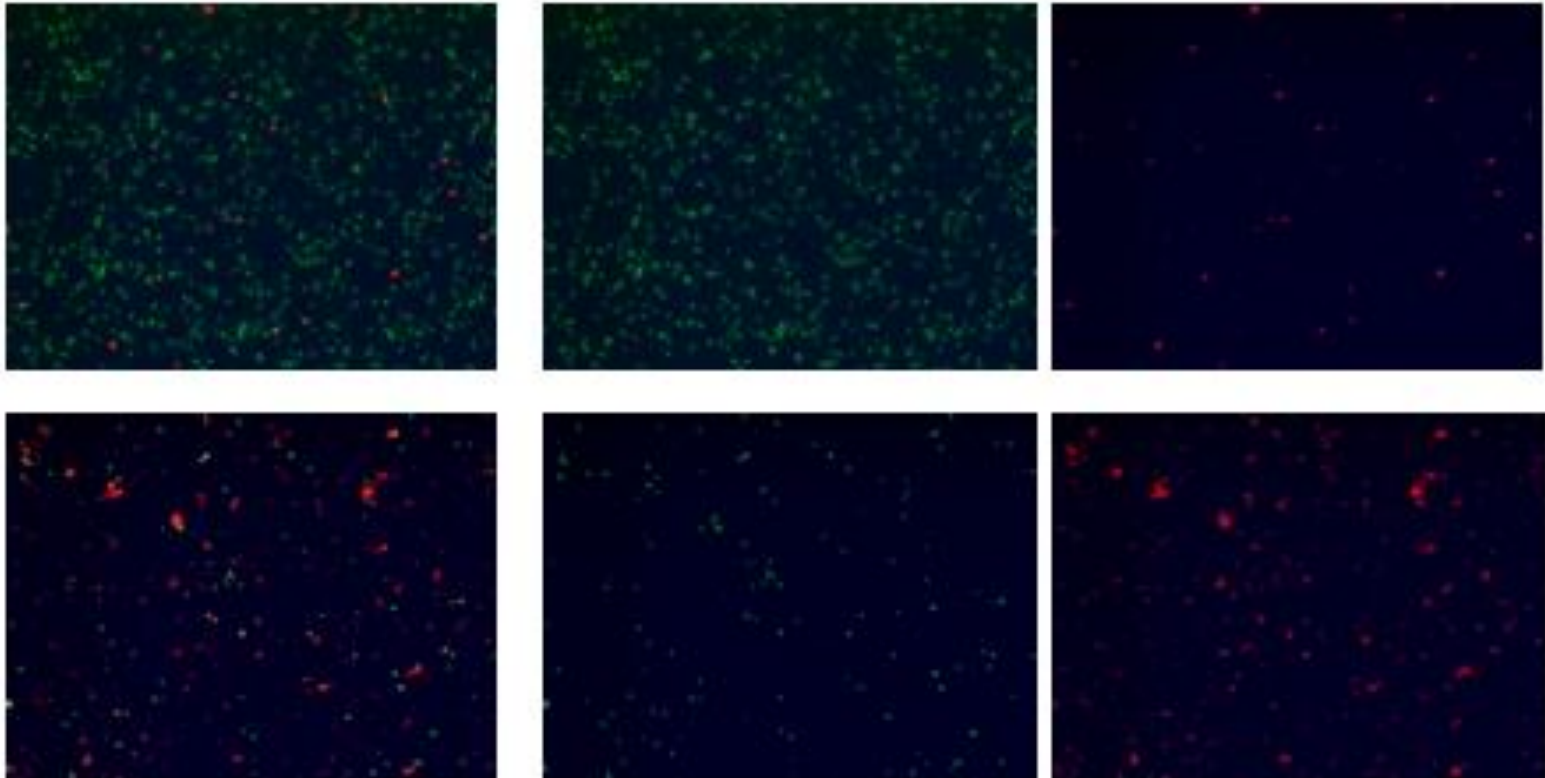


Peptide PDENK inhibits MRSA and MSSA dose-response curve. IC₅₀ for MRSA is 21.3mM, for MSSA is 27.4 mM. One colony of MRSA or MSSA cultured in 1.0 ml of TSB for 1.0 hour at 37°C. Take 100 µl of culture(adjusted bacteria concentration to 10³⁻⁴ CFU/ML)added to 900 µl TSB tube which has increased concentration of peptide PDENK, incubation for 1.0 hour and then spread 100 µl mixture to TSB agar plate with 5% sheep blood. Incubation overnight at 37°C, counting colonies next day. Data show 4 times average for MRSA experiments, 3 times average for MSSA experiments.

MRSA methicillin resistant *staphylococcue aureus*

MSSA methicillin sensitive *Staphylococcus aureus*

Results



Fluorescent microscopy pictures of Methicillin sensitive *S. aureus* (G+), control (top figures) and peptide PDENK (Negative charged) treated (bottom figures).

(Left) is combined picture, (Middle) is only for green light, and (Right) is only for red light.

Conclusions

- Peptide NPSRQERR inhibits the growth of *E. coli* and kanamycine-resistant *E. coli* SM10 λ *pai*, wide type *Salmonella typhi*, and wide type *Shigella flexnari* in a dose-dependent manner;
- Peptide PDENK inhibits the growth of both MRSA(Methicillin-resistant *Staphylococcus aureus*) and MSSA(Methicillin-sensitive *Staphylococcus aureus*).
- Despite current literature negates the possibility that negatively charged antimicrobial peptides can exert anti-bacterial activity, we have demonstrated that an LGG-derived negatively charged peptide (PDENK) exerts bacteriocidal activity on methicillin-sensitive *Staphylococcus aureus* by destroying its membrane.