# Antimicrobial Activity of *Brassica rapa* Nectar Lipid Transfer Protein

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## Abstract

In plants, there are several large families of antimicrobial peptides (AMPs) defined by sequence similarity. The lipid transfer protein (LTP) family is defined by a conserved signature of eight cysteines and has a compact structure with a flexible lipid-binding hydrophobic cavity. The antimicrobial activity of LTPs varies greatly among plant species. An LTP from *Brassica rapa* (variety R-o-18) nectar (neLTP) was tested for antimicrobial activity. Using a microplate reader assay, the amount of neLTP needed to inhibit growth of pathogen strains by 50% ($IC_{50}$) was calculated. neLTP was most effective against *Trichoderma viride* and *Bipolaris oryzae* with $IC_{50}$ values of 0.78 µM and 1.71 µM, respectively. Additionally, both *Colletotrichum trifolii* and *Alternaria solani* had $IC_{50}$ values of less than 4.0 µM. Using a spread plate assay, the $IC_{50}$ value against bacterial pathogens was calculated. neLTP had a $IC_{50}$ value of 34.8 µM against *Pseudomonas syringae* pv. *tomato*. The broad activity of neLTP at such low biological values indicates that it is a potent defensive protein in flowers.

## Introduction

- AMPs provide an ancient, innate form of immunity conserved in all multicellular organisms.
- LTPs are compact, cationic peptides that have antimicrobial activity.
- LTPs are classified as pathogenesis-related (PR) proteins.

## Objectives

- Determine if the heterologously expressed neLTP is biologically active.
- Assess the ability of neLTP to inhibit both fungal and bacterial growth.
- Evaluate neLTP for potential transgenic expression to develop plant lines with increased disease resistance.

## Methods

**Microplate Assay**

A microplate reader assay was used to monitor fungal growth inhibition as previously described (1).

- Spore suspensions were filtered, and densities were determined microscopically.
- In a 96-well microplate, each well contained half strength potato dextrose broth, approximately 2000 spores, and concentrations of neLTP peptide up to 300 µg/mL in a total volume of 100 µL.
- After 48 h at 25 °C in the dark, absorbance of the wells was measured at 595 nm to quantify the inhibition of fungal growth.
- From these values, the amount of neLTP needed to inhibit growth of the pathogens strains by 50% ($IC_{50}$) was calculated.

**Spread-plate Assay**

A spread-plate assay was used to monitor bacterial growth inhibition as previously described (2).

- Bacteria were grown out to an $OD_{600}$ value of 0.1.
- 200 µL of bacteria was incubated with shaking for 3 hours with concentrations of neLTP peptide up to 300 µg/mL.
- Bacteria were serially diluted, and 100 µL was plated onto NBY plates.
- After 48 hours of incubation, the bacterial colonies were counted.
- From these values, the neLTP $IC_{50}$ values were calculated.

## Results

MGKNNTTILIIAMVLTTAMIMEEAKSYPI
**C**NTDTNDLQK**C**SPAATGNNPPTPGPD**C**
**C**AVAKSADLE**C**L**C**PYLSLSGIDPSKIKSVL
AS**C**GVGNPS**C**LS

**Figure 1.** Amino acid sequence of *Brassica rapa* nectar LTP (neLTP). Cysteines are in bold, and a putative signal peptide required for secretion from the cell is underlined.

neLTP (Fig. 1) was cloned into the pET21a expression vector and heterologously expressed in SHuffle T7 *E. coli* cells. Protein expression was induced by IPTG and was purified using the Thermo Scientific HisPur Cobalt Purification Kit. neLTP was tested *in vitro* against economically important pathogens. neLTP visibly suppressed fungal growth (Fig. 2). This inhibition lead to low $IC_{50}$ values and high biological activity against numerous fungal plant pathogens (Table 1). Also to a lesser extent, neLTP reduced bacterial growth and colony formation of *P. syringae* pv. *tomato*.

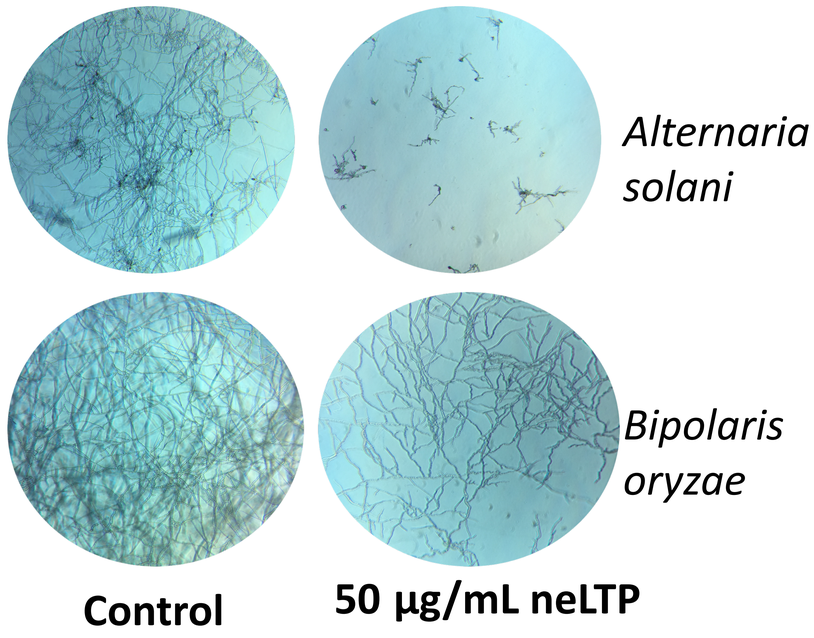


**Figure 2.** Multiple fungal pathogens were pre-incubated with neLTP from 0 to 300 µg/mL and monitored for growth over 48 hours. Examples of growth inhibition of *Alternaria solani* and *Bipolaris oryzae* pre-incubated with 50 µg/mL neLTP (~5 µM) are shown.

**Table 1**. Activity of *Brassica rapa* neLTP against fungal and bacterial pathogens.

| Microbe | $IC_{50}$ (µM) | Host |
|---|---|---|
| *Alternaria solani* | 3.73 | Tomato and Potato |
| *Bipolaris oryzae* | 1.72 | Rice |
| *Colletotrichum trifolii* | 2.98 | Alfalfa |
| *Fusarium oxysporum* | 14.4 | Wide Host Range |
| *Fusarium graminearum* | 25.5 | Wheat and Barley |
| *Trichoderma viride* | 0.79 | Cultivated Mushrooms |
| *Pseudomonas syringae* pv. *tomato* | 34.8 | Tomato |

## Conclusions

- The heterologous expression of *Brassica rapa* neLTP in an *E. coli* expression system lead to the production of biologically active neLTP peptide.
- neLTP has high biological activity of against both fungal and bacterial pathogens.
- Transgenic expression of this antimicrobial LTP has the potential to lead to increased broad-spectrum resistance to economically important plant diseases.

## References

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