BACILLUS PROBIOTICS AS ANTIMICROBIALS

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INTRODUCTION

Antibiotic resistance in pathogens was identified as a serious health threat, which is associated with increased morbidity and mortality worldwide. The emergence of multiresistant pathogens requires the development of new approaches to their control. Bacteria of the *Bacillus* genus are known as potent producers of a wide variety of antimicrobial compounds. These bacteria are also reputed to promote health benefits on the host. Our previous study showed beneficial effects of probiotic strain *Bacillus subtilis* 3 (BS) in prevention and treatment of bacterial infections in animal models (1,2) and in clinical trials (3,4). **The main goal** of this study was to evaluate efficacy of *B. subtilis 3* probiotic strain against influenza virus.

MATERIALS AND METHODS

Antiviral activity probiotic strain : *in vitro* was tested in Madin-Darby canine kidney (MDCK cells); *in vivo* - on mice orally treated with *B. subtilis* 3 before intranasal infection with influenza virus.
Active peptide was isolated from nutrient broth after cultivation of *B. subtilis* 3 strain.
Purification of peptide was performed by Beckman System Gold HPLC with further analysis in 12% polyacrylamide.
Molecular mass and amino acid sequence of purified peptide was determined with MALDI/TOF MS (Ultraflex II, Bruker, Germany).
Identification of peptides was performed using Mascot program (Matrix Science, USA) searching against a NCBI database.
The selected peptide sequence TVAAPSVFIFPPSDEQLK was synthesized by Metabion GmbH (Planegg, Germany) at the highest available purity (90%), using an automated synthesizer (Applied Biosystem 433A).
Cytotoxicity of P18 peptide was analyzed by MTT assay on MDCK cells.
Antiviral activity of isolated peptide was tested on mice before and after influenza infection. Control groups received PBS and Tamiflu.

RESULTS

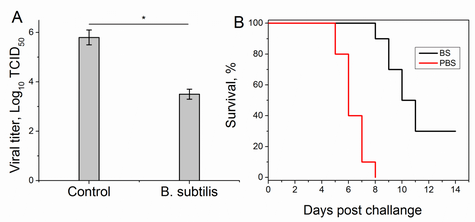

Fig.1 Antiviral activity of *B. subtilis in vitro* (A) and *in vivo* (B).

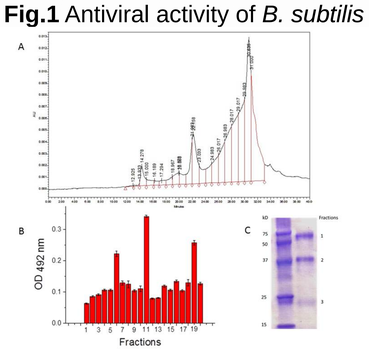

Fig. 2 Isolation and characterization of *B. subtilis* peptides.
A- HPLC separation of the protein fractions from *B. subtilis* 3;
B- Protein fractions interaction with antibodies to peptides from *B.subtilis* 3;
C- Gel electrophoresis analysis of fraction 11.

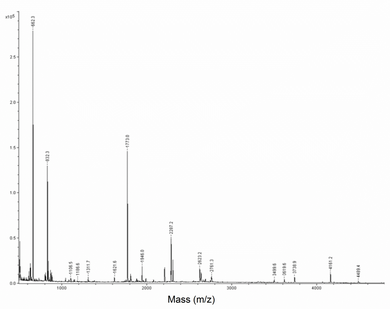

Fig. 3 MALDI-TOF mass spectrum of fraction 3 (Fig.2C).

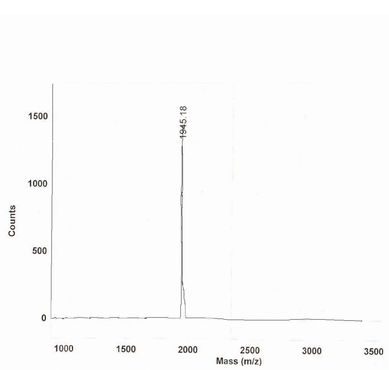

Fig. 4 MALDI-TOF mass spectrum of the chemically synthesized peptide P18.

RESULTS

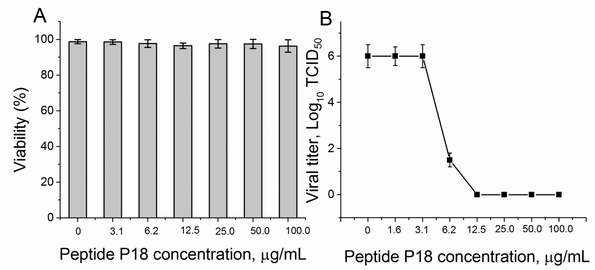

Fig. 5 Characterization of peptide P18.
A-Cytotoxicity of P18 peptide analyzed by MTT assay on MDCK cells; B- Antiviral activity on monolayer of MDCK cells.

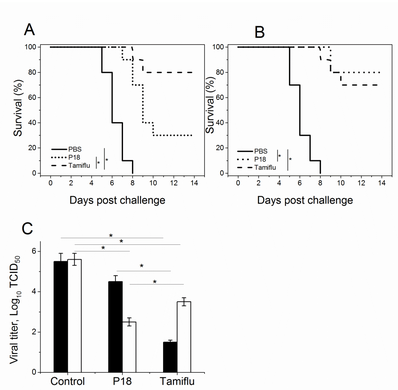

Fig. 6 Efficacy of peptide P18 *in vivo*. Mice were treated with PBS, P18 or Tamiflu before infection with influenza virus (A) or after infection (B). On day 4 postinfection, the lungs from three mice in each group before infection (solid bars) and post-infection (open bars) were removed, and viral titers were evaluated in each supernatant by TCID50 analysis in MDCK cells (C), *p<0.05.

CONCLUSION

Among *Bacillus* bacteria, *B. subtilis* is the most productive species of antimicrobial compounds. In this study, we analyzed the activity of probiotic strain *B. subtilis* 3 against influenza virus. The antiviral effect of this strain has been demonstrated *in vitro* and *in vivo*. New peptide P18 produced by probiotic strain was isolated, purified, chemically synthesized, and characterized. Cytotoxicity studies demonstrated no toxic effect of P18 on Madin-Darby canine kidney (MDCK) cells, even in the highest tested concentration (100 μg/mL). Complete inhibition of influenza virus *in vitro* was observed at concentrations 12.5 – 100 μg/mL. Protective effect of P18 in mice was comparable with Tamiflu. Further study will assess the potential of peptide P18 as antiviral compound and as a promising candidate for the development of new antiviral vaccines.

ACKNOWLEDGMENTS

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