ANTIBACTERIALS ALTERNATIVE TO ANTIBIOTICS, WITH HIGH SPECIFICITY AGAINST ESCHERICHIA COLI

DESCRIPTION OF THE TECHNOLOGY

The "Molecular Microbiology" research group at the University of Alicante has developed several modified phage proteins (Poll-N and UK-C) with antibacterial activity highly specific against *E. coli* without the need for previous permeabilization treatments.

Polypeptides (proteins) are a sequence of amino acids that are joined together by peptide bonds.

The polypeptides developed with endolysin activity, Poll-N and UK-C, comprise, respectively:

- An amino acid sequence according to SEQ ID NO: 3 or a derivative thereof (deletion, addition, insertion and/or substitution in this amino acid sequence), and a polycationic tail of amino acids (histidines) at the N-terminal end; and,
- An amino acid sequence according to SEQ ID NO: 4, or a derivative thereof (deletion, addition, insertion and/or substitution in this amino acid sequence), and a polycationic tail of amino acids (histidines) at the C-terminal end.

Once cloned, expressed and purified, the resulting proteins (Poll-N and UK-C) carry a histidine tail at their N-terminal or C-terminal end, being therefore different from the original. This tail not only facilitates its purification, but also favors the endolysin contact to the cell surface, thus improving its lysis efficiency.

The developed polypeptides can be used both as antimicrobial agents to prevent contamination by *E. coli* and in the treatment of diseases (infections) produced by *E. coli*.

MARKET APPLICATION SECTORS

The present invention is framed in the general field of genetic engineering and, in particular, it refers to viral proteins that have been modified by means of the addition of a polycationic tail of amino acids at the N-terminal or C-terminal end, in such a way that they present antibacterial activity against *E. coli* without previous treatments of envelope permeabilization. Both proteins show a high specificity to *E. coli*.

Therefore, the developed polypeptides can be used both as antimicrobial agents against *E. coli* (particularly in food, cosmetics, water contaminated with *E. coli*, etc.), as well as in the treatment of diseases (infections) produced by *E. coli*.

This technology could be applied in biosanitary, veterinary, biotechnological, or agri-food companies interested in antimicrobial treatments alternative to antibiotics to control the growth of *E. coli*.

TECHNICAL ADVANTAGES AND BUSINESS BENEFITS

The main innovative aspect of the modified phage proteins is that they do not require prior treatments of outer membrane (OM) permeabilization. Furthermore, the addition of nucleotides at the ends facilitates their manipulation and subsequent cloning in the appropriate expression vector. Finally, another innovative aspect is the generation of anti-*E. coli* endolysins whose sequence is significantly different from others.

The main advantages of the synthesized peptides, Poll-N and UK-C, are the following:

- The presence of a histidine tail at the N-terminal or C-terminal ends of synthesized peptides facilitates their purification and improves their lysis efficiency.
- They have a high specificity against *E. coli* as, among all bacteria tested, their lytic action is limited to those pertaining to this species without affecting any other belonging to other species, including closely related ones.
- Compared to antibiotics, a lesser probability of resistance occurrence is anticipated.
- Their use is expected to result, at most, in negligible effects on resident microbiota during the treatment of infections.
- Simple formulation, since no previous treatments of permeabilization of the external bacterial membrane are necessary, which in turn facilitates its application.

ANTIBACTERIALS ALTERNATIVE TO ANTIBIOTICS, WITH HIGH SPECIFICITY AGAINST ESCHERICHIA COLI

CURRENT STATE OF DEVELOPMENT

The technology is developed at laboratory scale.

The efficacies of purified Poll-N and UK-C endolysins have been tested by means of spot test experiments against different bacterial strains. Figure 1 (for Poll-N) and Figure 2 (for UK-C) show the appearance of growth inhibition zones produced by lysis.

The results obtained show that both Poll-N and UK-C are capable of directly lysing the majority (92.5% for Poll-N and 91.2% for UK-C) of *E. coli* strains tested (159 in total).

INTELLECTUAL PROPERTY RIGHTS

This technology is protected by patent application, with title "*Viral proteins with antibacterial activity against E. coli* ", application number *P201930890*, and date of application 10/10/2019.

COLABORATION SOUGHT

The research group is looking for biotechnology/pharma sectors companies interested in acquiring this technology for commercial exploitation through patent license agreements, technical cooperation (R&D projects) to adapt the technology to the needs of the company, etc.

RELATED IMAGES

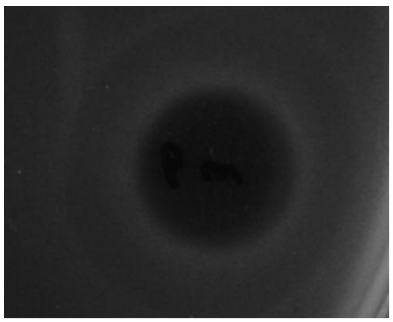

Figure 1: Result of a spot test experiment carried out with Poll-N at a concentration of 16 µg/mL on an E. coli strain. Bacterial growth is visualized as a gray layer on the plate. The dark central area, where the protein solution was loaded, indicates the inhibitory effect in growth produced by the endolysin.

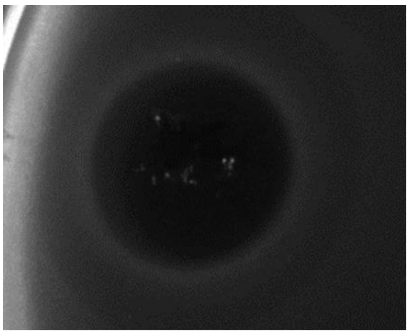

Figure 2: Result of a spot test experiment carried out with UK-C at a concentration of 16 µg/mL on an E. coli strain. Bacterial growth is visualized as a gray layer on the plate. The dark central area, where the protein solution was loaded, indicates the inhibitory effect in growth produced by the endolysin.

CONTACT

Víctor Manuel Pérez Lozano
TTO (University of Alicante)
Phone: +34 965 909 959
Email: areaempresas@ua.es
Web: http://innoua.ua.es/