Recombinant Lactoferrin-Derived Peptides and Their Application in Enhancing Immunity

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Abstract: In this study, a recombinant lactoferrin-derived peptide was synthesized, which was obtained by constructing an expression vector, transferring said expression vector into an expression host, and expressing it through said expression host in culture. Its antibacterial activity against Escherichia coli, Salmonella cholerae, Salmonella typhimurium, Salmonella enterica and Staphylococcus aureus, and its antiviral activity against influenza virus, measles virus and mumps virus were confirmed by in vitro antipathogenic and antiviral assays, as well as by in vivo assays in mice, which confirmed its ability to promote the proliferation of mouse splenic lymphocytes and to comprehensively promote the secretion of IL-2, IL-4, IL-13 and IL-3 by mouse lymphocytes, The recombinant peptide Ta was shown to have broad-spectrum antibacterial and antiviral activities, as well as immune boosting effects in mice.

Keywords: Recombinant Lactoferrin-Derived Peptide; Lactoferrin Peptide; Immunity

Introduction

Lactoferrin peptide (Lfcin) is a segment of polysaccharide tho released from the N-terminal end of lactoferrin (LF) in an acidic environment by the action of pepsin. It was later found that Lfcin is closely related to the function of lactoferrin, except that it cannot bind iron ions, Lfcin has all the biological activities of lactoferrin, such as antibacterial, antiviral, inhibiting the growth of cancer cells, anti-inflammatory and participating in immune response. Lfcin is free from rare amino acids and exogenous chemicals, making it a healthy and safe product. Lfcin has shown great potential as an alternative to antibiotics due to its powerful biological functions and safety.

LfcinB is derived from amino acids 17 to 41 of bovine lactoferrin and consists of 25 amino acid residues. Although it has immunomodulatory effects, the activation of the body's immune response by LfcinB also requires the mediation of immune chemokines, which bind to specific receptors on the membranes of different immune cells and guide the immune cells to the site of inflammation in order to produce an immunomodulatory effect on the body. The secretion and expression of chemokines The secretion and expression of chemokines themselves require the intervention of defensins, so it is clear that the activation of the immune response by LfcinB is significantly affected and does not directly promote the immune response.[1]

A technology developed by Zhao Ce and Wang Xiaojuan to produce recombinant lactoferrin-derived peptides using budding yeast as an expression host was submitted for patent in October 2022. The technology is divided into several major steps: first, pYC54 is used to transform receptor E. coli DH10B, and LB solid medium containing 100 μg/mL ampicillin is used for culture, and the extracted pYC54 fragment is detected by electrophoresis and recovered. The recombinant expression vector pYC54-Ta was constructed by ligating the target gene fragment obtained by gel recovery with linearized pYC54 using T4 DNA ligase. receptor budding yeast was prepared as the expression host, the expression vector was transformed into said expression host, and cultures of transformants were obtained. Screening of positive transformants, which are budding yeast organisms into which the expression vector

has been transferred and which are capable of successfully expressing said recombinant lactoferrin-derived peptide; collection of said positive transformants, lysis of the organisms and purification to obtain said recombinant lactoferrin-derived peptide.

1. Materials and methods

1.1 Synthesis and cloning of target genes

(1) Amplification and extraction of recombinant plasmid pμC19-L.

The amplified plasmid pμC19-L containing the gene shown in SEQ ID NO.2 was converted into E. coli DHSa competent cells, positive transformants were selected and cultured to amplify the plasmid, and the amplification plasmid pμC19-L was extracted using the Qiagen Plasmid Midi Kit (Sigma-Aldrich).

(2) Extraction and amplification of plasmid pμC19-L.

(3) Amplification of target genes:

After the reaction was finished, it was detected by electrophoresis using 2.2% agarose gel.

Recover the target gene Ta fragment gel.

1.2 Construction of the target gene expression pYC54-Ta vector

Transformation of receptor E. coli cells with the expression vector pYC54.

The preserved competent E. coli cells were removed from -80°C, placed on ice for 10 min, and pYC54 (ZYbscience) was transformed into competent E. coli DH10B (purchased from Shanghai Yaji Biotechnology Co., Ltd.) cells according to the above method, and coated to LB solid medium containing 100 μg/mL ampicillin, and incubated in a constant temperature incubator at 37°C for 16h, and the DH10B-positive transformant colonies carrying the vector pYC54 were to grow. Positive transformants of single colonies were selected for LB liquid culture of 100 μg/mL ampicillin based on 37°C shaking for 8 h, pYC54 was extracted and electrophoresis was detected.

The pYC54 extracted in the previous section was double digested using the restriction nuclease *Eco53kI* and Not *I*.

After the digestion reaction, the digested products were examined by electrophoresis using a 1% agarose gel.

Derived peptide gene fragment linked to pYC54 enzyme.

Transform the recombinant expression vector pYC54-Ta into receptor E.

Coli DH10B cells to obtain positive transformants, inoculate them in LB liquid culture containing 100 μg/mL ampicillin based on shaking at 37°C for 8h, extract the recombinant expression vector pYC54-Ta according to the above method, and perform PCR amplification and identification.

PCR amplification of the recombinant expression vectors pYC54-Ta and pYC54 was performed using F-p: ccagtcacgacgttgtaaaacg and R-p: accatctaattcaacaagaattgggacaac as primers, respectively.

The extracted recombinant expression vector pYC54-Ta was sent to Bio for sequencing and identification and analysis against the pYC54 sequence.

In addition, pEsc-HIS and pLac-EGFP were also constructed as contrasting expression vectors in this study using similar methods as described above.

1.3 Transformation and expression of the recombinant expression vector pYC54-Ta

Preparation of receptor budding yeast.

The budding P. brevis (black yeast) (purchased from Shanghai Yansheng Industrial Co., Ltd. ATCC30142) was inoculated in YPD plates, and incubated in a constant temperature incubator at 30°C for 48~72 h, waiting for single colonies to grow; Single colonies were selected and inoculated in YPD liquid medium, and the bacteria were collected when the OD600 was 0.8 by shaking at 30°C 220r/min, and re-inoculated as seed liquid in 100 mL YPD liquid medium, and cultured at 30°C 220 r/min shaking for 22~24 h to OD600 0. Collect the bacteria by centrifugation at 1500 ×g at 4°C for 5 min at 8 o'clock, and resuspend the bacteria using 100 mL of

sterile deionized water pre-cooled on ice. Resuspend the bacteria with sterile water, 0.68wt% sterile normal saline, and 1M ethylene glycol solution, aliquot, and store temporarily at 4 °C for later use.

Electrotransformation of the recombinant expression vector pYC54-Ta.

Screening of pYC54-Ta-ATCC30142-positive transformants.

Induced expression of positive transformants.

The positive transformants screened by yellow fluorescence were induced, and positive transformants that could efficiently express Ta-derived peptides were obtained.

Purification of recombinant Ta.

Perform mass spectrometric identification of the recombinant peptide Ta

Conduct in vitro inhibition experiments with recombinant peptide Ta.

2. Results

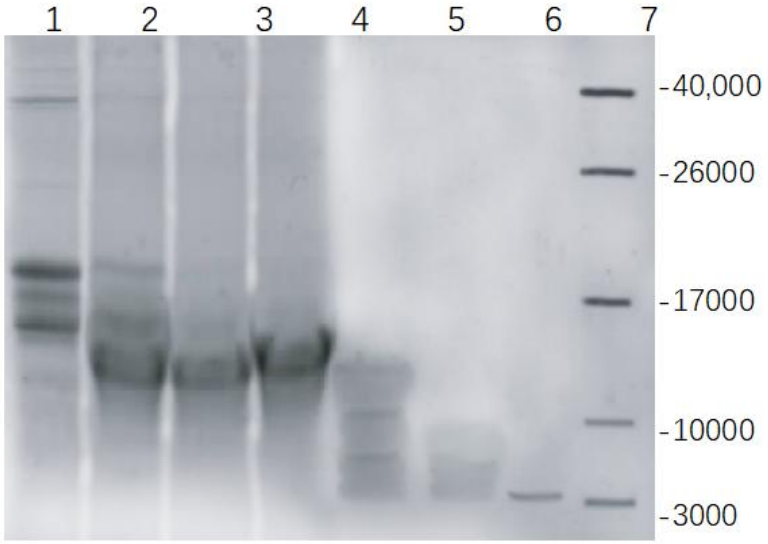

Figure 1: Electropherogram of recombinant peptide Ta after induction of expression

Primary (lane 1) inclusion bodies after washing (lane 2), lysis (lane 3), ethanol graded precipitation (lane 4), desalting (lane 5), ultrafiltration (lane 6) and lyophilisation (lane 7); lane 8 is the Marker.

As shown in Figure 1, after induction of expression, the inclusion bodies were washed, lysed, ethanol graded precipitated, desalted, ultrafiltered and lyophilized to obtain a higher purity of the crude fusion protein. The target polypeptide obtained after the above purification was stained by -SDS-PAGE electrophoresis and Komas Brilliant Blue G250. Various experiments and comparative ratios were active to obtain the recombinant polypeptide Ta. As shown in the figure mass spectra showed that the molecular weight of the purified obtained recombinant polypeptide was similar to the theoretical molecular weight 3236.

Ce Zhao and Xiaojuan Wang improved the existing lactoferrin peptide and adjusted some of its amino acids to obtain lactoferrin-derived peptides with amino acid sequences such as SEQ ID NO.1, and was cloned and expressed by designing its coding gene as shown in SEQ ID NO.2, The recombinant lactoferrin-derived peptide Ta was shown to have antibacterial activity against E. coli, Salmonella cholerae, Salmonella typhimurium, Salmonella enterica and Staphylococcus aureus, and antiviral activity against influenza virus, measles virus and mumps virus, as well as to promote the proliferation of mouse spleen lymphocytes and the secretion of IL-2, IL-4, IL-13 and TNF-α by mouse lymphocytes in vivo. The recombinant protein elongation peptide Ta has comprehensive antibacterial, antiviral and immunity-boosting functions, and has excellent potential for the preparation of related drugs.

3. Concluding remarks

In this study, the amino acid sequence and coding sequence of a lactoferrin-derived peptide were designed, the target gene was synthesized and amplified sequentially, and a suitable expression vector and expression host were constructed to obtain a recombinant derivative peptide Ta, which was confirmed by in vitro anti-pathogenic and anti-viral assays to inhibit Escherichia coli, Salmonella cholerae, Salmonella typhimurium, Salmonella enteritidis and Staphylococcus aureus. The antibacterial activity against E. coli, Salmonella cholerae, Salmonella typhimurium, Salmonella enteritidis and Staphylococcus aureus and the antiviral activity against influenza virus, measles virus and mumps virus have been confirmed by in vivo experiments in mice, as well as its ability to promote the proliferation of mouse splenic lymphocytes and the secretion of IL-2, IL-4, IL-13 and TNF-α by mouse lymphocytes, without the intervention of chemokines and defensins. The study provides the basis for this study. Thus, the recombinant derived peptide Ta has broad-spectrum antibacterial and antiviral activities, and also has the ability to enhance immunity in mice, which is promising for the preparation of relevant antibacterial, antiviral and immunity enhancing drugs.

References

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