Our Ref: PP130504CN-US-KRS
PCT/CN2012/071825

CLAIM Amendment according to PCT Article 28/41

1 (Orignal). A preparation method of a novel recombinant antibacterial polypeptide medicine comprises the steps as follows:

(1) Preparing Escherichia coli strain comprising recombinant plasmid and freezing for conserving,

(2) Enlarging cultivation of the strain in liquid production medium,

(3) Inducing the strain to express the recombinant antibacterial polypeptide and getting purified polypeptide,

characterized in that, said liquid production medium comprises, in w/v%, disodium hydrogen phosphate 0.4%-0.7%, potassium dihydrogen phosphate 0.1%-0.6%, ammonium chloride 0.05%-0.2%, calcium chloride 0.0005%-0.001%, magnesium sulfate 0.5%-2.5%, peptone 1%-3%, yeast extract powder 0.5%-1%, glucose 0.1%-0.5%, sodium chloride 0.2%-0.8% and water of the rest.

2(Orignal). The preparation method of claim 1, wherein said liquid production medium comprises, in w/v%, disodium hydrogen phosphate 0.68%, potassium dihydrogen phosphate 0.3%, ammonium chloride 0.1%, calcium chloride 0.001%, magnesium sulfate 0.02%, peptone 2.5%, yeast extract powder 0.75%, glucose 0.2%, sodium chloride 0.6%, and water of the rest.

3(Orignal). The preparation method of claim 1, wherein said enlarging cultivation comprises three stages, with parameters of 220 rpm, 37℃, and 3-8 hours in each stage.

4(Orignal). The preparation method of claim 1, wherein said inducing the strain to express the recombinant antibacterial polypeptide means treating bacteria liquid out of step (2) as follows: with stirring rate at 220 rpm, maximum oxygen flow volume, 30℃ for 2~4 hours; 42℃ for 0.5 hours; and 37℃ for 1~2 hours, and

IPTG at a final concentration of 0.5mM is added at 42℃.

5(Orignal). The preparation method of claim 1, wherein before enlarging cultivationof step (2), the strain is treated as follows:

(1) The strain is thawed at 4℃ and recovered overnight in an LB liquid medium containing 50 μg/ml of AMP at 220 rpm, 37℃, and then coated onto LB solid medium to cultivate single colonies.

(2) Single colony is picked and cultivated in 1.5 ml of LB liquid medium at 220 rpm, 37℃ for 5-8 hours, and then transferred into 100 ml of LB liquid medium and cultivated at 220 rpm, 37℃ for 5-8 hours;

the compositions and their ratios in said LB, in w/v, are sodium chloride of 1%, peptone of 1%, yeast extract of 0.5%, agar of 0.8~1%, and water of the rest.

6 (Orignal). The preparation method of claim 1, wherein said Escherichia coli refer to engineered Escherichia coli BL-21 containing recombinant plasmid of pBHC-SA1, pBHC-SA2, pBHC-SA3, pBHC-SA4, pBHC-SE or pBHC-PA.

7 (New). A culture medium, comprises compositions in w/v% as following: disodium hydrogen phosphate 0.4%-0.7%, potassium dihydrogen phosphate 0.1%-0.6%, ammonium chloride 0.05%-0.2%, calcium chloride 0.0005%-0.001%, magnesium sulfate 0.5%-2.5%, peptone 1%-3%, yeast extract powder 0.5%-1%, glucose 0.1%-0.5% and sodium chloride 0.2%-0.8%.

8. (New). The culture medium of claim7, comprises compositionss in w/v% as following: disodium hydrogen phosphate 0.68%, potassium dihydrogen phosphate 0.3%, ammonium chloride 0.1%, calcium chloride 0.001%, magnesium sulfate 0.02%, peptone 2.5%, yeast extract powder 0.75%, glucose 0.2% and sodium chloride 0.6%.

CLAIMS

1. A preparation method of a recombinant antibacterial polypeptide drug, comprising:

(1) preparing Escherichia coli strain comprising recombinant plasmid, and freezing conserving,

(2) large-scale culturing in liquid production medium, and

(3) inducing expressing and purifying the recombinant antibacterial peptide;

characterized in that, said liquid production medium comprises, in w/v%, disodium hydrogen phosphate 0.68%, potassium dihydrogen phosphate 0.3%, ammonium chloride 0.1%, calcium chloride 0.001%, magnesium sulfate 0.02%, peptone 2.5%, yeast extract powder 0.75%, glucose 0.2%, sodium chloride 0.6%, and water the rest.

2. The preparation method of claim 1, wherein said large-scale culture is divided into three stages, with working parameters of 220 rpm, 37°C, and 3-8 hours in all different stages.

3. The preparation method of claim 1, wherein said induction expression of the recombinant peptide refers to treating the bacteria liquid of step (2) as follows: stirring rate 220 rpm, maximum oxygen, 30°C, 2~4 hours; 42°C, 0.5 hours; and 37°C, 1~2 hours, with the addition of IPTG at a final concentration of 0.5mM at 42°C.

4. The preparation method of claim 1, wherein before the enlarge cultivation in said step (2), the strain is treated as follows:

(1) The strain is thawed at 4°C and recovered overnight in an LB liquid medium containing 50μg/ml of AMP at 220 rpm, 37°C, and then plated onto LB solid medium to cultivate single colonies,

(2) Single colony is picked and cultivated in 1.5ml of LB liquid medium at 220 rpm, 37°C for 5-8 hours, and then transferred into 100 ml of LB liquid medium and

cultivated at 220 rpm, 37°C for 5-8 hours;

the composition and mass-volume ratio of said LB are sodium chloride 1%, peptone 1%, yeast extract 0.5%, agar 0.8~1%, and water the rest.

5. The preparation method of claim 1, wherein said Escherichia coli refer to engineered Escherichia coli BL-21 containing recombinant plasmid of pBHC-SA1, pBHC-SA2, pBHC-SA3, pBHC-SA4, pBHC-SE or pBHC-PA.