***Supporting Information***

**Modeling of growth kinetics for an isolated marine bacterium, *Oceanimonas* sp. BPMS22 during the production of a trypsin inhibitor**

B.S. Harish and Kiran Babu Uppuluri\*

Bioprospecting Laboratory, School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur 613 401, India.

**Screening of bacteria for extracellular protease inhibitor production**

Microbes were isolated from marine sediments collected from the Arabian Sea in and around Thiruvananthapuram, Kerala, India (8°51' N and 76°88' E). Zobell marine agar 2216 medium with initial pH 7.6 was used for the isolation. Inoculated plates were incubated at 28°C. Distinct colonies were isolated and cultivated The culture supernatant of all the strains was assayed for inhibitor activity and the positive strains were screened by following protease inhibitor assay.

Fifty-five strains from marine sediments were isolated and screened for trypsin inhibitory action. The strains were allowed to grow in production medium and trypsin inhibitor assay was performed for all the strains. Of these only 8 strains showed trypsin inhibitor activity (Table S1). In these isolate designated as BPMS22 had shown maximum inhibitor activity (31.11 ± 0.99%) and was selected as the best isolate for trypsin inhibitor production.

Table S1. Strains showing protease inhibitor activity

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl no.** | **Isolated strain** | **Protease Inhibitory activity (IU/mL)** | **Sl no.** | **Isolated strain** | **Protease Inhibitory activity (IU/mL)** | **Sl no.** | **Isolated strain** | **Protease Inhibitory activity (IU/mL)** |
| 1 | BPM1 | -22.50 | 21 | BPM21 | -21.89 | 41 | BPMS10 | -9.12 |
| 2 | BPM2 | -17.64 | 22 | BPM22 | -9.12 | 42 | BPMS11 | -8.51 |
| 3 | BPM3 | -17.64 | 23 | BPM23 | -39.53 | 43 | BPMS12 | -12.77 |
| 4 | BPM4 | -27.98 | 24 | BPM24 | -16.42 | 44 | BPMS13 | -14.59 |
| 5 | BPM5 | -22.50 | 25 | BPM25 | -11.55 | 45 | BPMS14 | -0.60 |
| 6 | BPM6 | -24.33 | 26 | BPM26 | -13.38 | **46** | **BPMS15** | **6.70** |
| 7 | BPM7 | -13.38 | 27 | BPM27 | -6.69 | **47** | **BPMS16** | **4.26** |
| 8 | BPM8 | -20.68 | 28 | BPM28 | -7.90 | **48** | **BPMS17** | **1.83** |
| 9 | BPM9 | -15.81 | 29 | BPM29 | -7.90 | 49 | BPMS18 | -12.16 |
| 10 | BPM10 | -20.07 | 30 | BPM30 | -11.55 | **50** | **BPMS19** | **5.48** |
| 11 | BPM11 | -16.42 | 31 | BPM31 | -21.29 | 51 | BPMS20 | -3.04 |
| 12 | BPM12 | -27.37 | 32 | BPMS1 | -11.55 | **52** | **BPMS21** | **6.70** |
| 13 | BPM13 | -23.11 | 33 | BPMS2 | -20.07 | **53** | **BPMS22** | **7.91** |
| 14 | BPM14 | -13.99 | 34 | BPMS3 | -12.77 | 54 | BPMS23 | -4.25 |
| 15 | BPM15 | -10.34 | 35 | BPMS4 | -10.34 | **55** | **BPMS24** | **-6.69** |
| 16 | BPM16 | -15.20 | 36 | **BPMS5** | **0.61** |  |  |  |
| 17 | BPM17 | -22.50 | 37 | **BPMS6** | **1.22** |  |  |  |
| 18 | BPM18 | -18.85 | 38 | BPMS7 | -8.51 |  |  |  |
| 19 | BPM19 | -27.98 | 39 | BPMS8 | -13.38 |  |  |  |
| 20 | BPM20 | -18.24 | 40 | BPMS9 | -10.94 |  |  |  |

Identification of BPMS22 by 16S rDNA sequencing

Genomic DNA was isolated from the samples using Chromous Genomic DNA isolation kit. The ~ 1.5 Kb and ~1.3 Kb 16S rDNA fragment was amplified using high fidelity PCR polymerase and the PCR polymerase was sequenced bi-directionally. Forward and reverse primer sequence which was used for amplification was 5’- CAGRCCYAAMACWTRCRARTM -3’ and 5’- GGMGRATGYGTWMARGRC -3’ respectively.

PCR cycling Parameters: (Name of the Thermal Cylcer: ABI2720)

PCR Amplification conditions

Template (PCR product) 1 μL

Forward Primer 1 μL

Reverse Primer 1 μL

dNTPs (10mM) 1 μL

10X Taq Assay Buffer 2.5 μL

Taq DNA Polymerase Enzyme (3U/ μL) 0.25 μL

Water 18.25 μL

Total reaction volume 25 μL

PCR cycle conditions

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 94°C | 94°C | 50°C | 72°C | 72°C |
| 5 min | 30 sec | 30 sec | 1.5 min | 15 min |
|  | 35 cycles |  |

Amplicon of ~1.5 KDa was obtained (Figure S2).



Figure S1. PCR- amplified 16S-rDNA amplicon loaded on 1% agarose gel L- 500 bp DNA Ladder, 1- 16S-rDNA amplicon

Sequencing was done using ABI3130 Genetic Analyzer using Big Dye Terminator version 3.1”.

The Sequencing mix Composition and PCR Conditions are as follows:

10μl Sequencing Reaction

 Big Dye Terminator Ready Reaction Mix : 4μL

 Template (100ng/μL) : 1μL

 Primer (10pmol/λ) : 2μL

 Milli Q Water : 3μL

PCR Conditions: (25 cycles)

Initial Denaturation : 96°C for 5 min

Denaturation : 96°C for 30 sec

Hybridization : 50°C for 30 sec

Elongation : 60°C for 1.30 min

Instrument and Chemistry Details

Sequencing Machine : ABI 3130 Genetic Analyzer

Chemistry : Big Dye Terminator version 3.1” Cycle sequencing kit.

Polymer & Capillary Array : POP\_7 polymer 50 cm Capillary Array.

Analysis protocol : BDTv3-KB-Denovo\_v 5.2

Data Analysis : Seq Scape\_ v 5.2

Software Reaction Plate : Applied Biosystem Micro Amp Optical 96-well reaction plate

*Aligned sequence data*

CAGGCCTAACACATGCAAGTCGAGCGGTAACAGAGGGTAGCTTGCTACTTTGCTGACGGGCGGCGGACGGGTGAGTAATGCTTGGGGATCTGCCCGGTCGAGGGGGATAACCGTTGGAAACGACGGCTAATACCGCATACGCCCTACGGGGGAAAGCAGGGGACCTTCGGGCCTTGCGCGATTGGATGAACCCAAGCGAGATTAGCTTGTTGGTGAGGTAACGGCTCACCAAGGCGACGATCTCTAGCTGGTCTGAGAGGATGACCAGTCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGTGGTGAGGAAAGGTAAGTGACTAATACTTGCTTACTGTGACGTTAACCACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATAACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGCCAGATGTGAAAGCCCCGGGCTCAACCCGGGAACTGCATTTGGAACTGGCAGACTAGAGTCTTGGAGAGGGGGGGTAGAATTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGGAAGGAATACCAGTGGCGAAGGCGGCCCCCTGGCCAAAGACTGACGCTCAGGTGGCGAAAGCGTGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTTGAAGTCTGTGCCATTTGAGCGCGGGTTTCGGAGCTAACGCGTTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACCCTTGACATACAGCGAAGTTTTCAGAGATGAATTCGTGCCTTCGGGAACGCTGATACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTTTTTGCCAGCGATTCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGGCCCTTACGGGTAGGGCTACACACGTGCTACAATGGCGCGTACAGAGGGCAGCGAACTTGCGAGAGTAAGCGAATCCAAAAAAGCGCGTCGTAGTCCGGATCGGAGTCTGCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACATCCGGCC

The sequence data was aligned and analyzed to identify the bacterium to the closest neighbors.

Phylogenetic analysis (Figure S2) showed that the strain BPMS22 formed a robust clade with genera *Oceanimonas* sp. Hence it was named as *Oceanimonas* sp. BPMS22. The sequenced data have been submitted to the DDBJ/EMBL/GenBank databases with accession number KU860462.



Figure S2. Phylogenetic position of strain BPMS22 (Geneious version 9.1 created by Biomatters. Available from [http://www.geneious.com](http://www.geneious.com/))

**Production and purification of trypsin-inhibitor**

*Oceanimonas* sp. BPMS22 was inoculated in the modified PI production medium and incubated for 60 h at an initial pH of 7.0. The cells were separated by centrifuging the broth at 12000 × g for 10 minutes. The extracellular protein PI was concentrated and purified from the supernatant by a series of purification stages, ammonium sulfate precipitation, anion exchange chromatography using Q FF column, size exclusion chromatography using Sephacryl S-100 column and affinity chromatography using trypsin- sepharose column. Molecular mass and purity of trypsin inhibitor were checked by native-PAGE.

The extracellular broth was centrifuged to remove the cells. Proteins were precipitated from this cell-free supernatant by salting out with 70% ammonium sulfate. After dialysis, the sample was loaded on to a Q FF column (Figure 5.A). Positive fractions were collected and loaded into S-100 column. The fractions with protease inhibitor activity were further purified using trypsin-agarose affinity chromatography. Approximately 30 KDa protein-protease inhibitor was purified to homogeneity after affinity chromatography (Figure 5.C).

**Figure S3: Purification of PI**- Chromatograms after Anion exchange (A) and gel filtration (B) chromatography. (C) Native-PAGE for each purification steps- After anion exchange, gel filtration and affinity chromatography (Showing from left to right).