# Supplemental Material

**Table S1.** Inhibition effect between *Haloferax* sp. strain Q22 and other haloarchaea.

| Indicator strain | Diameter of inhibition zone (mm) |
|---|---|
| *Halorubrum saccharovorum* CGMCC1.6259 | 37±1.5 |
| *Halorubrum aidingense* CGMCC 1.2670 | 28±2.0 |
| *Halorubrum litoreum* CGMCC 1.8280 | 45±1.5 |
| *Haloparvum sedimenti* DYS4 | 40±1.5 |
| *Haloarcula hispanica* ATCC 33960 | 22±1.5 |
| *Halorhabdus utahensis* DSM 12940 | 30±1.5 |
| *Halobacterium noricense* DSM15987 | 45±2.0 |

Note: All the strains were cultured in liquid AS-168 medium (see **MATERIALS AND METHODS**). 200 µL of each indicator cell suspension at logarithmic-phase were spread onto AS-168 agar plate. After dry, a piece of sterile circular filter paper (6 mm in diameter) was placed onto each agar plate. Then, 10 µL of cell suspensions of *Haloferax* sp. strain Q22 was dropped onto sterile filter paper. After 7 days cultivation, diameter of inhibition zone was measured and recorded. These data were derived from three biological replicates. Average value plus/minus variation was shown.

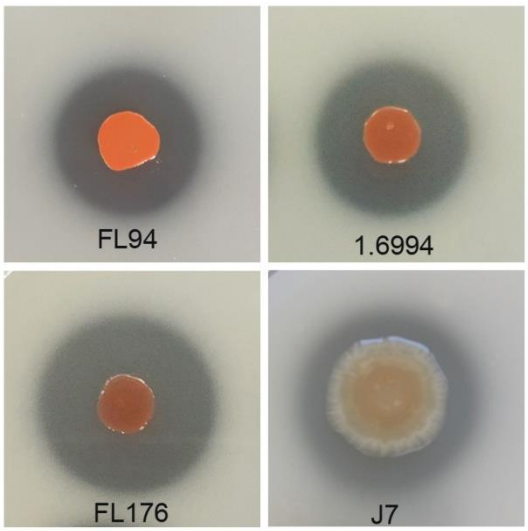


**Fig. S1. Proteolytic activity detection on skim milk agar plates.** Cell suspensions (10 μL for each) were spotted onto skim milk agar plate. The proteolytic zone was observed after cultivation for two weeks. FL94, *Haloarchaeobius* sp. strain FL94; 1.6994, *Halococcus saccharolyticus* CGMCC 1.6994; J7, *Natrialba* sp. strain J7; and FL176, *Haloarchaeobius* sp. strain FL176.

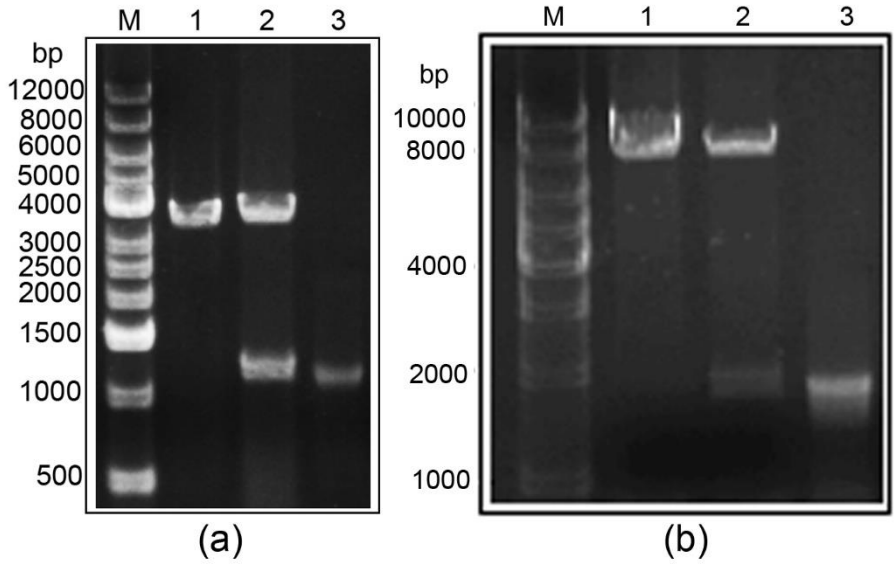


**Fig. S2. Plasmids construction for gene knock-out and complementation study.** (a) Construction of hlyR4-gene-knock-out vector; lane 1: plasmid pHFX digested with HindIII; lane 2: plasmid pHFX-UDR4 digested with HindIII; lane 3: PCR products of the linked up- and down-stream fragment of hlyR4. (b) Recombinant plasmid used to generate the complementation strain of hlyR4-deficient strains; lane 1: plasmid pWL502 cut by KpnI; lane 2: pWR4 cut by BamHI and KpnI; lane 3: PCR products of the entire hlyR4 with its native promoter. DNA ladder is shown on the left.

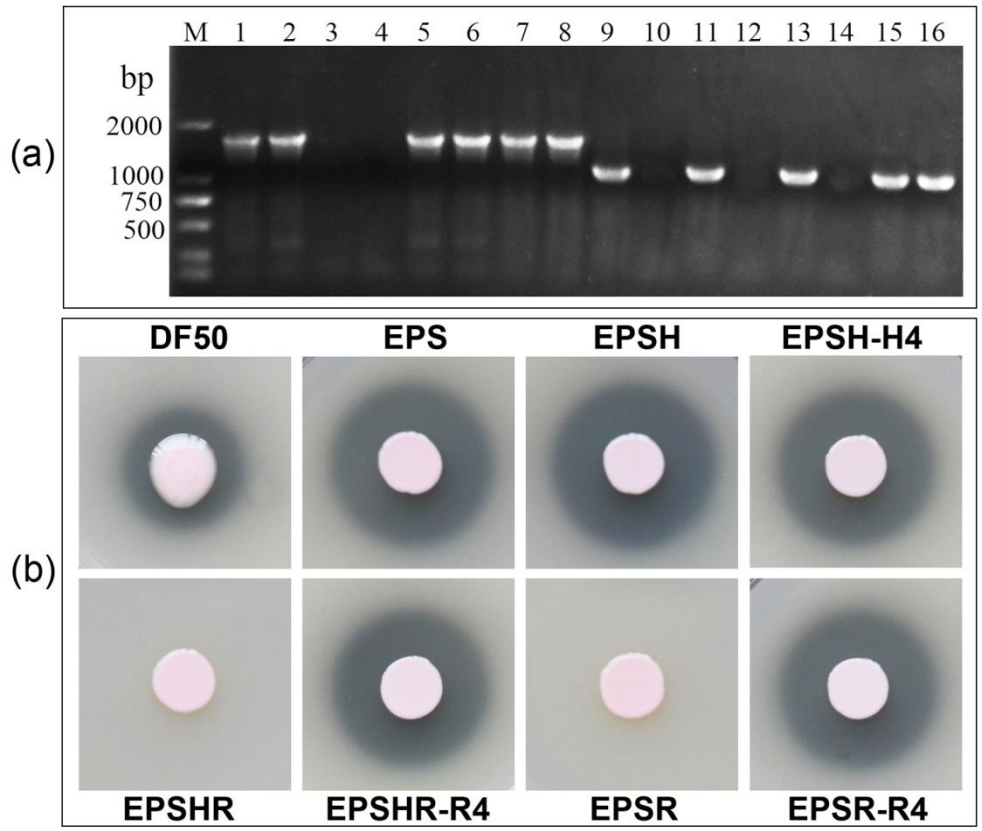


**Fig. S3. Verification of eight *Haloferax mediterranei* ATCC 33500 derivatives.** (a) PCR verification of these *Haloferax mediterranei* ATCC 33500 derivatives; lane 1, 9: strain EPS; lane 2, 10: strain EPSH; lane 3, 11: strain EPSR; lane 4, 12: strain EPSH; lane 5, 13: strain EPSR-R4; lane 6, 14: strain EPSHR-R4; lane 7, 15: strain DF50; lane 8, 16: strain EPSH-H4; DNA ladder is shown on the left. (b) The proteolytic activities of these strains were checked on skim milk agar plates.

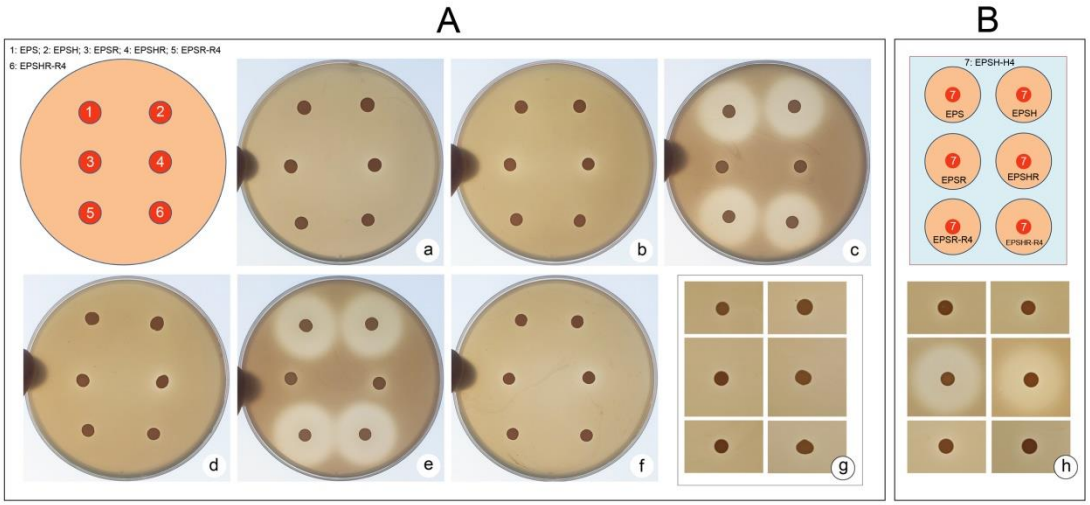


**Fig. S4. Antagonistic activity between each other of *Haloferax mediterranei* ATCC 33500 derivatives.** Schematic diagrams are shown on the right and left of the top row. Cell suspensions of each *Haloferax mediterranei* strain dropped on the agar plates are numbered as showed on the top (1-7). (A) Growth of the same six strains, EPS, EPSH, EPSR, EPSHR, EPSR-R4 and EPSHR-R4, on the indicator plates of strains EPS (a), EPSH (b), EPSR (c), EPSHR (d), EPSR-R4 (e), EPSHR-R4 (f) and EPSH-H4 respectively. (B) Growth of strain EPSH-H4 on six indicator plates of strains EPS, EPSH, EPSR, EPSHR, EPSR-R4 and EPSHR-R4 respectively (h); the indicator strains in the plates are shown in the schematic diagram. Clear inhibition zones are present.

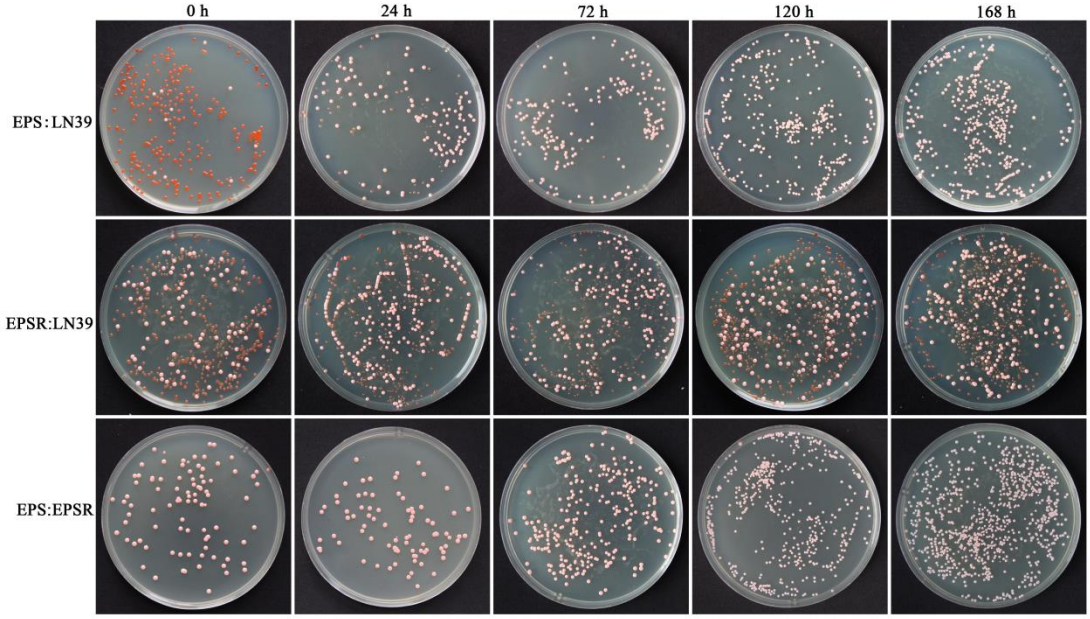


**Fig. S5. Pairwise competitive growth.** Cells of strains EPS and LN39, or EPSR and LN39, or EPS and EPSR, were mixed and inoculated into liquid AS-168 medium supplemented with uracil. At different cultivation time point (0, 24, 72, 120 and 168 h), cell suspensions were spread onto agar plates (supplemented with uracil) under an appropriate dilution using fresh liquid AS-168 medium for colony counting. The colonies of strains EPS and EPSR were pink, while colonies of strain LN39 were red, which facilitating colony counting in EPS and LN39, or EPSR and LN39 systems. The percentage of EPS cells in EPS and EPSR system was calculated based on PCR using *hlyR4* as the marker gene. LN39, *Haloarcula* sp. LN39.

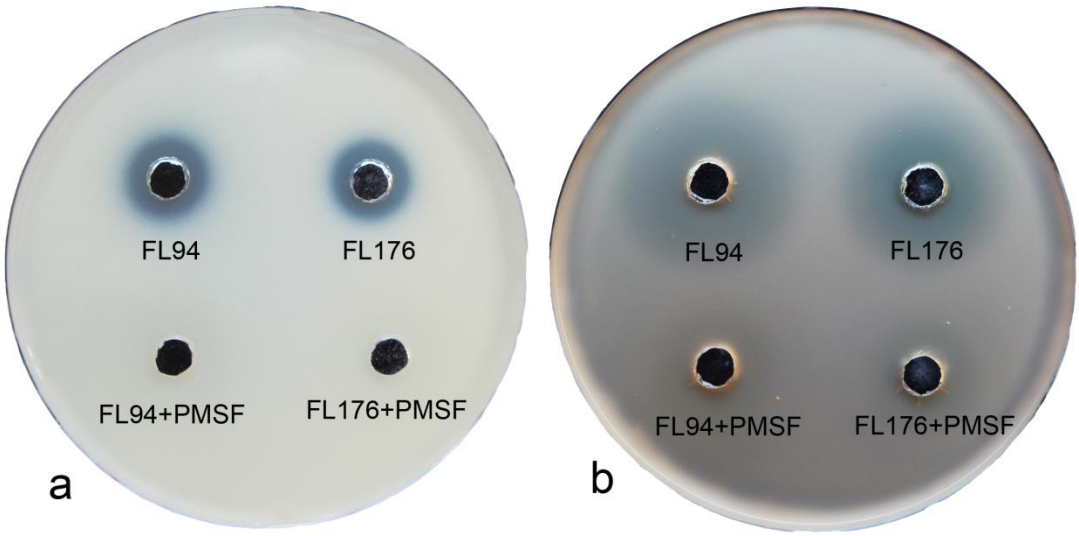


**Fig. S6. Proteolytic activity correlated to inhibition activity.** Cell-free supernatants of *Haloarchaeobius* spp. FL94 and FL176 (top row) or mixed with PMSF (final concentration: 10 mM; bottom row) were dropped into the holes on the skim milk agar plate (a) and the indicator plate of *Halorubrum* sp. FL23 (b). Agar plates were cultivated at 37°C for 1 week.

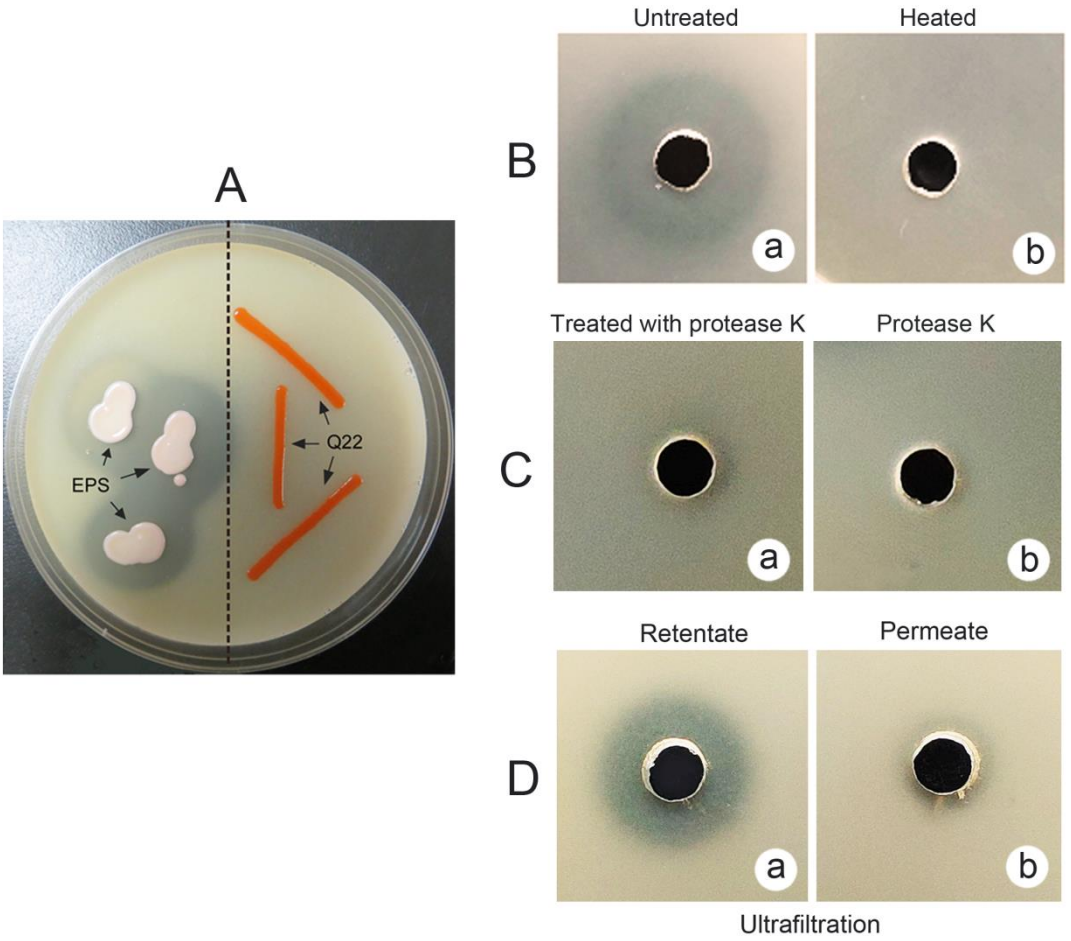


**Fig. S7. Determination of the general biological features of *Haloferax* sp. Q22 supernatants.** A: Strains EPS (left, reddish) and Q22 (right, orange) were dropped (cell suspensions of strain EPS) or streaked (strain Q22) on skim milk agar plate. Agar diffusion experiments were performed to determine inhibition activities of strain Q22 supernatants on *Halorubrum* sp. LN10 indicator plate (B, C and D). B: Cell-free supernatants were directly poured into the holes on the indicator plates (a) or after heating at 85°C for 1 h (b). C: Cell-free supernatants were digested with protease K (20 µg $ml^{-1}$ for 1 h) before inpouring to the holes (a) and used the equal amount of protease K solution as negative control (b). D: Ultrafiltration (MWCO: 50 KD) was used to determine the molecular weight of this antimicrobial substances. The ultrafiltration retentate (a) and the efflux (b) were dropped into the holes on strain LN10 indicator plate. Agar plates were cultivated at 37°C for 1 week.

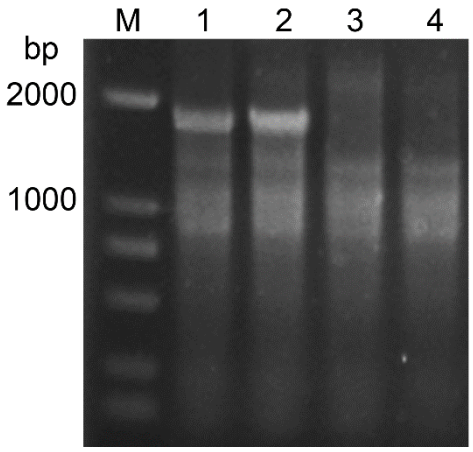


**Fig. S8. Verification of the *Haloarcula hispanica* strains by colony PCR.** Pair of complete *hly*R4 primers named 33500HlyF and 33500HlyF was used. Lanes 1 and 2: cells of *Har. hispanica* strain DF60 used as the PCR template; lanes 3 and 4, cells of *Har. hispanica* strain DF60 containing recombinant plasmid pWR4 harboring *hly*R4 from *Haloferax mediterranei* ATCC 33500 used as the PCR template. DNA ladder is shown on the left.

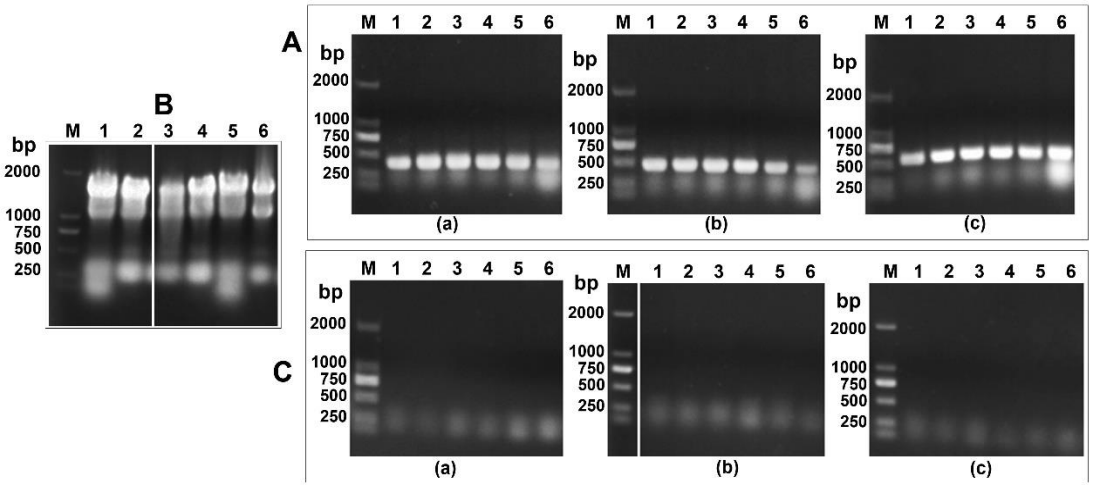


**Fig. S9. Preparation of total RNA for reverse transcription.** Equal amount of cells were harvested for total RNA extraction after cultivation times of 0 h (lane 1), 24 h (lane 2), 48 h (lane 3), 72 h (lane 4), 96 h (lane 5) and 120 h (lane 6), respectively. (A) Total RNAs extracted from different cultivation periods were used for PCR templates; Pairs of primers for amplifying *hal*H4 (a), *hly*R4 (b) and 16S rRNA gene (c) were used to check the PCR system. (B) Total RNAs were treated with RNase-free DNase I; (C) After DNase I digestion, PCR amplification with pairs of primers of *hal*H4, *hly*R4 and 16S rRNA gene were used to check if contaminant chromosomal DNA was removed completely. DNA ladder is shown on the left.

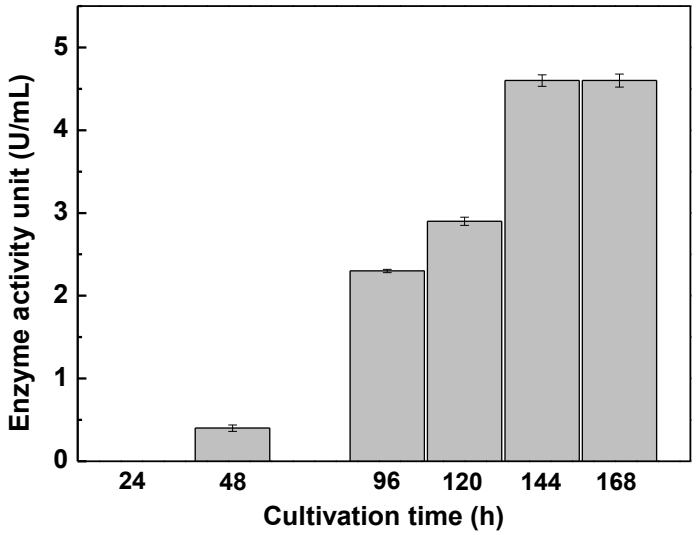


**Fig. S10. Protease activity in strain EPS supernatants at different growth phases.** Cells of strain EPS (1 mL) at logarithmic-phase ($OD_{600}$=1.0) were inoculated into liquid AS-168 medium (100 mL) supplemented with uracil. Supernatants were collected by centrifugation for protease activity detection after cultivation for 24, 48, 96, 120, 144 and 168 h, respectively.