**Supplementary material**

**Fungal Methane Production Under High Hydrostatic Pressure in Deep Subseafloor Sediments**

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**Table S1.** Transcriptome quality control analysis

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample\_ID** | **Raw\_reads** | **Raw\_bases** | **Clean\_reads** | **Clean\_Bases** | **Q20%** | **Q30%** |
| d1\_01M1 | 61225688 | 9245078888 | 59924560 | 8923556763 | 98.59 | 95.67 |
| d1\_01M2 | 57660352 | 8706713152 | 57093920 | 8492669667 | 98.63 | 95.76 |
| d1\_01M3 | 78692462 | 11882561762 | 77966734 | 11574682555 | 98.72 | 96.03 |
| d1\_15M1 | 58292626 | 8802186526 | 57372462 | 8546726611 | 98.41 | 95.32 |
| d1\_15M2 | 57596182 | 8697023482 | 57148878 | 8523100243 | 98.38 | 95.23 |
| d1\_15M3 | 65583050 | 9903040550 | 64891218 | 9628476854 | 98.4 | 95.33 |
| d1\_35M1 | 62930370 | 9502485870 | 62248622 | 9273294662 | 98.38 | 95.28 |
| d1\_35M2 | 50448150 | 7617670650 | 49998534 | 7475352356 | 98.3 | 95.02 |
| d1\_35M3 | 49151190 | 7421829690 | 48754856 | 7270718571 | 98.46 | 95.42 |
| d3\_01M1 | 50533726 | 7630592626 | 48120520 | 7154045635 | 98.48 | 95.36 |
| d3\_01M2 | 57806778 | 8728823478 | 55059002 | 8197060519 | 98.6 | 95.7 |
| d3\_01M3 | 57385564 | 8665220164 | 56966126 | 8507516649 | 98.71 | 95.98 |
| d3\_15M1 | 61646368 | 9308601568 | 61022202 | 9085820646 | 98.47 | 95.54 |
| d3\_15M2 | 65798042 | 9935504342 | 65238428 | 9738132671 | 98.39 | 95.29 |
| d3\_15M3 | 56952974 | 8599899074 | 56250886 | 8366846120 | 98.46 | 95.49 |
| d3\_35M1 | 61443184 | 9277920784 | 60730442 | 9069017317 | 98.4 | 95.34 |
| d3\_35M2 | 47487154 | 7170560254 | 46292670 | 6938107353 | 98.36 | 95.23 |
| d3\_35M3 | 47088886 | 7110421786 | 46439164 | 6913881206 | 98.39 | 95.33 |
| d5\_01M1 | 57971786 | 8753739686 | 57444428 | 8573357523 | 98.74 | 96.03 |
| d5\_01M2 | 46008078 | 6947219778 | 45531714 | 6851055859 | 98.58 | 95.59 |
| d5\_01M3 | 52839130 | 7978708630 | 52454630 | 7886413231 | 98.78 | 96.16 |
| d5\_15M1 | 61865750 | 9341728250 | 61253746 | 9122127602 | 98.42 | 95.33 |
| d5\_15M2 | 50901704 | 7686157304 | 50333004 | 7484406434 | 98.51 | 95.6 |
| d5\_15M3 | 49558750 | 7483371250 | 49052310 | 7267314405 | 98.51 | 95.66 |
| d5\_35M1 | 44510344 | 6721061944 | 43941122 | 6551168123 | 98.5 | 95.58 |
| d5\_35M2 | 43622492 | 6586996292 | 43113272 | 6448029277 | 98.56 | 95.7 |
| d5\_35M3 | 49276522 | 7440754822 | 48618848 | 7253078402 | 98.52 | 95.66 |

Note: d1\_01, d3\_01, d5\_01 respectively represent strains grown at 0.1MPa for one, three and five days ; d1\_15, d3\_15, d5\_15 respectively represent strains grown at 15 MPa for one, three and five days;d1\_35, d3\_35, d5\_35 respectively represent strains grown at 35 MPa for one, three and five days;Q20: The percentage of bases in clean reads with a Phred value >20; Q30: The percentage of bases in clean reads with a Phred value >30.

**Table S2.** qRT-PCR primers

|  |  |
| --- | --- |
| **Primer ID** | **Sequence (5'-3')** |
| mct1-F | ATTCCTGGGATATTGCATGG |
| mct1-R | GTCGAATATGCGAGTTAGCTC |
| mct2-F | CTCCGGGACCAGAGACAGTT |
| mct2-R | AGCCAGGGGTGATGTTCTTG |
| dh1-F | GAATCTCGCCGCTACCAACT |
| dh1-R | GACGAGAACGCTGATCCAGTA |
| dh2-F | GTTGGCCGGACTCAACACTA |
| dh2-R | GTTCACCAACAAGCGCACAT |
| dh3-F | TCACTCCCCAGCCCAACC |
| dh3-R | GCTTGTCCGTCTCGCCG |
| dh4-F | GGCGGCACCAAATCGAATAC |
| dh4-R | TTGTACCAGTTGAGCGGACC |
| dh5-F | TTCCAGAAGGGCGGTCTGA |
| dh5-R | GGCTTCGTGACTTTGAGGGT |
| ms-F | CATCCCCAGCGGTGATTTCT |
| ms-R | GGCCCATCGCAAAGTAGACA |
| act-F | GTCCGCCCTCGAGAAGAGTTA |
| act-R | TTGTACGTCGTCTCGTGGATA |



**Figure S1.** *S. commune* 20R-7-F01 cell activity under high hydrostatic pressure

**Table S3.** Intracellular O2- and OH· levels of *S. commune* 20R-7-F01 under different stress conditions

|  |  |  |
| --- | --- | --- |
|  | **O2- ( μmol/g )** | **OH· ( μmol/g )** |
| **0.1MPa** | **15MPa** | **35MPa** | **0.1MPa** | **15MPa** | **35MPa** |
| 1d | 0.21 ± 0.01 | 0.24 ± 0.02 | 0.34 ± 0.03 | 0.13±0.01 | 0.19±0.02 | 0.13±0.01 |
| 3d | 0.33 ± 0.05 | 0.26 ± 0.01 | 0.37 ± 0.01 | 0.16±0.02 | 0.16±0.03 | 0.14±0.03 |
| 5d | 0.25 ± 0.01 | 0.31 ± 0.01 | 0.35 ± 0.02 | 0.17±0.03 | 0.21±0.02 | 0.25±0.04 |

**Table S4.** Intracellular ROS levels of *S. commune* 20R-7-F01 under different stress conditions

|  |  |  |
| --- | --- | --- |
|  |  **CdCl2** | **H2O2**  |
| **0.75mM** | **1.5mM** | **3mM** | **0.75mM** | **1.5mM** | **3mM** |
| 1d | 1.13 ± 0.21 | 2.31 ± 0.52 | 2.92 ± 0.55 | 1.81 ± 0.31 | 2.54 ± 0.46 | 3.17 ± 0.65 |
| 3d | 2.23 ± 0.18 | 4.78 ± 0.27 | 5.42 ± 0.46 | 2.74 ± 0.25 | 5.23 ± 0.60 | 7.12 ± 0.33 |
| 5d | 3.99 ± 0.16 | 6.82 ± 0.37 | 8.15 ± 0.32 | 4.95 ± 0.45 | 8.65 ± 0.61 | 10.29 ± 0.47 |



**Figure S2.** *S. commune* 20R-7-F01 cell activity under different stress conditions



**Figure S3.** Alterations in the biomass of *S. commune* 20R-7-F01 under varying stress conditions.