

High hydrostatic pressure adaptive strategies in an obligate piezophile

Pyrococcus yayanosii

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Supplementary Data

Table S1 General characteristics of the 4 species of *Pyrococcus* studied, *P. abyssi* GE5, *P. furiosus* DSM 3638, *P. horikoshii* OT3 and *P. yayanosii* CH1¹

| | | <i>Pyrococcus abyssi</i> GE5 | <i>Pyrococcus furiosus</i> DSM 3638 | <i>Pyrococcus horikoshii</i> OT3 | <i>Pyrococcus yayanosii</i> CH1 |
|------------------------|--------------------------|------------------------------|-------------------------------------|----------------------------------|---------------------------------|
| Genomic features | Size (bp) | 1,768,562 | 1,908,256 | 1,738,505 | 1,716,818 |
| | Predicted CDS | 2,051 | 2,179 | 2,061 | 1,915 |
| | GC % | 45 | 41 | 42 | 52 |
| | CDS % | 92.86 | 90.41 | 91.94 | 89.91 |
| | Mean CDS length (bp) | 863 | 842 | 843 | 828 |
| | rRNA | 5 | 4 | 4 | 4 |
| | tRNA | 46 | 46 | 47 | 46 |
| | arCOG % | 72.94 | 72.28 | 72.84 | 74.57 |
| Geographical features | Origin | Vulcano Island, Italy | North Fiji Basin, SW Pacific Ocean | Okinawa Trough, Pacific Ocean | Ashadze, Mid- Atlantic Ridge |
| | Depth (m) | 0 | 2,000 | 1,395 | 4,100 |
| Physiological features | Morphology | Cocci | Cocci | Cocci | Cocci |
| | Metabolism | Strictly anaerobic | Strictly anaerobic | Anaerobic | Strictly anaerobic |
| | Size (µm) | 0.8-2 | 0.8-2.5 | 0.8-2 | 0.6-1.4 |
| | Temperature range (°C) | 67-102 | 70-103 | 80-102 | 80-108 |
| | Optimum temperature (°C) | 96 | 100 | 98 | 98 |
| | pH range | 4-8.5 | 5-9 | 5-8 | 6-9.5 |
| | Optimum pH | 6.8 | 7 | 7 | 7.5-8 |
| | NaCl range (% w/v) | 0.7-2.5 | 0.5-5 | 1-5 | 2.5-5.5 |
| | Optimum NaCl (% w/v) | 3 | 2 | 2.4 | 3.5 |
| | Pressure range (MPa) | 0.1-50 | 0.1-45 | 0.1-40 | 20-120 |
| Optimum Pressure (MPa) | 20 | 0.1 | 0.1 | 52 | |
| Doubling time (min) | 33 | 37 | 32 | 50 | |

Table S2 : Predicted pathways of amino acid biosynthesis. -§ is missing the last enzyme of the pathway.

| Amino Acid | Predicted pathways of amino acid biosynthesis | | | |
|------------|---|--------------------|----------------------|---------------------|
| | <i>P. abyssii</i> | <i>P. furiosus</i> | <i>P. horikoshii</i> | <i>P. yayanosii</i> |
| Cys | + | + | - | + |
| Leu | -§ | -§ | - | - |
| Lys | + | + | + | - |
| Met | - | + | + | - |
| Phe | - | + | - | - |
| Trp | + | + | - | - |
| Val | -§ | -§ | - | - |
| Ala | + | + | + | + |
| Asn | + | + | + | + |
| Asp | + | + | + | + |
| Glu | + | + | + | + |
| Gln | + | + | + | + |
| Gly | + | + | + | + |
| His | - | + | - | - |
| Ile | -§ | -§ | - | - |
| Pro | - | - | - | - |
| Ser | + | + | + | + |
| Thr | + | + | + | + |
| Tyr | - | + | - | - |

Table S4 Real time RT-PCR validations and operon regulation during High pressure stress. Relative gene expression changes data from microarray analysis and quantitative real-time RT-PCR assays

| Locus | Description | Coordinates | | Microarray | | qRT-PCR | |
|-------------|-------------------------------------|-------------|-----------|------------|-------|---------|-------|
| | | | | 20-52 | 80-52 | 20-52 | 80-52 |
| PYCH_00020 | NiFe hydrogenase II subunit | 1 016 | 2 029 | -3,96 | -3,19 | -3,62 | -5,52 |
| PYCH_00030 | cytochrome-c3 hydrogenase | 2 016 | 2 882 | -2,96 | -2,83 | -3,97 | -3,89 |
| PYCH_01430 | 30S ribosomal protein | 132 937 | 133 569 | 2,26 | 3,64 | 1,9 | 2,4 |
| PYCH_r10 | ARNr 5S | 164 226 | 164 347 | -3,27 | -1,15 | -2,69 | -1,16 |
| PYCH_05460 | hypothetical protein | 452 227 | 452 766 | 2,21 | -2,54 | 2,82 | -2,15 |
| PYCH_06240 | ribulose-1,5-biphosphate synthetase | 524 514 | 525 272 | 2,2 | 2,13 | 3,25 | 1,95 |
| PYCH_06250 | Thiamine biosynthesis protein | 525 353 | 526 645 | 5,3 | 7,05 | 3,18 | 2,25 |
| PYCH_08570 | Transposase | 755 521 | 756 726 | -1,55 | 1,19 | -1,05 | 1,24 |
| PY0867_0868 | Intergenic Space | 765 083 | 765 369 | 4,5 | 8,91 | 2,35 | 2,45 |
| PYCH_09590 | <i>Cas 4</i> | 847 106 | 847 621 | 2,23 | 2,96 | 12,34 | 8,94 |
| PYCH_11050 | formate hydrogenlyase II subunit | 974 097 | 975 539 | -9,25 | -9,87 | -12,35 | -4,08 |
| PYCH_11070 | formate hydrogenlyase II subunit | 976 760 | 978 814 | -5,64 | -4,94 | -27,78 | -3,76 |
| PYCH_11420 | NADH dehydrogenase subunit | 1 004 080 | 1 005 255 | 2,08 | 2,38 | 1,75 | 2,11 |
| PYCH_11930 | alanine aminotransferase | 1 051 537 | 1 051 764 | 1,01 | 1,21 | 1,15 | 1,46 |
| PYCH_17100 | maltodextrin transport - permease | 1 522 525 | 1 523 466 | 2,04 | 3,04 | 1,71 | 1,9 |
| PYCH_17110 | maltodextrin transport - permease | 1 523 463 | 1 524 704 | 1,92 | 2,7 | 1,26 | 2 |

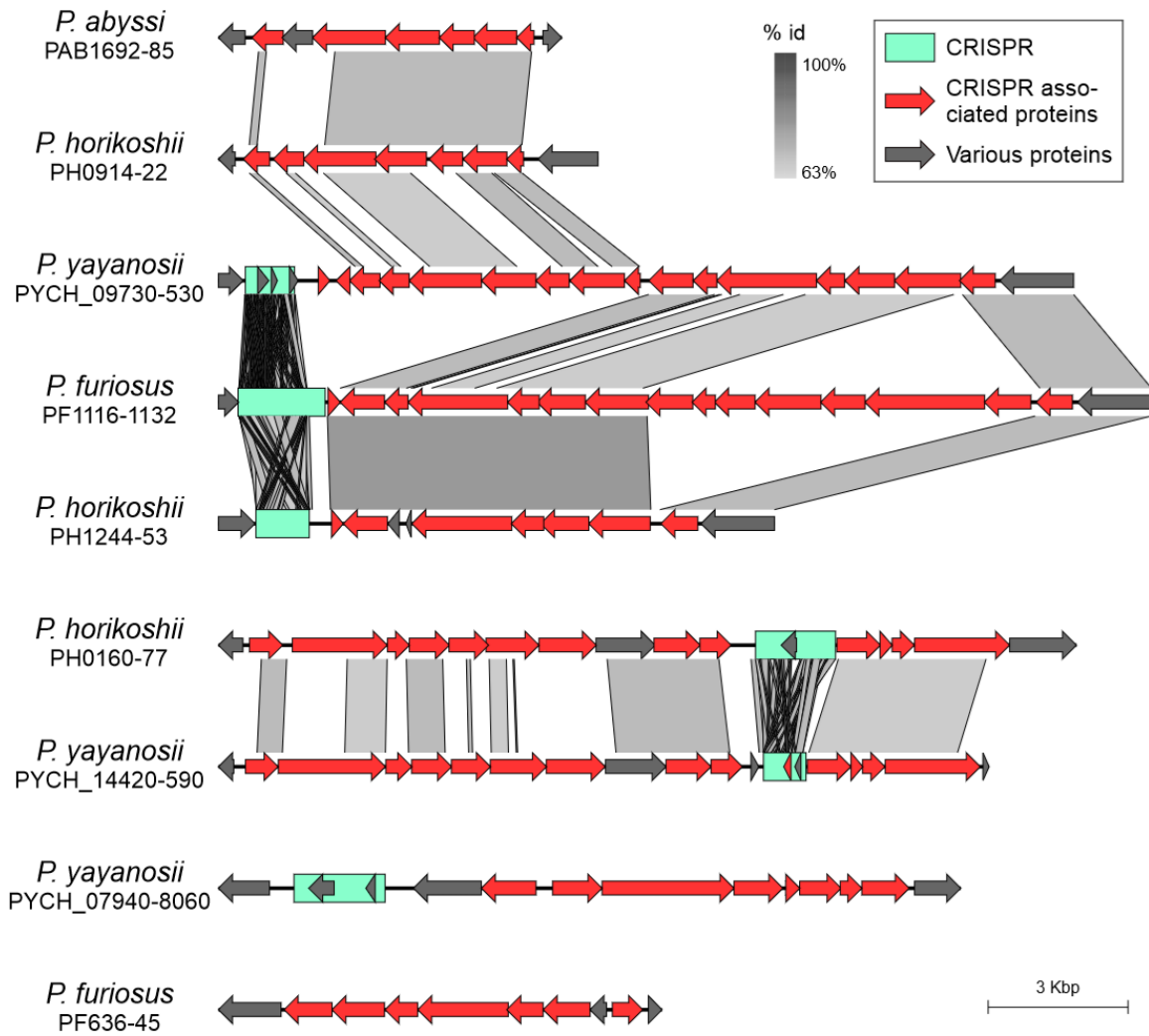


Figure S1 : CRISPR/cas alignements in *Pyrococcus*. The alignments were done with the Easyfig software (blastn, e-value 10^{-4})².

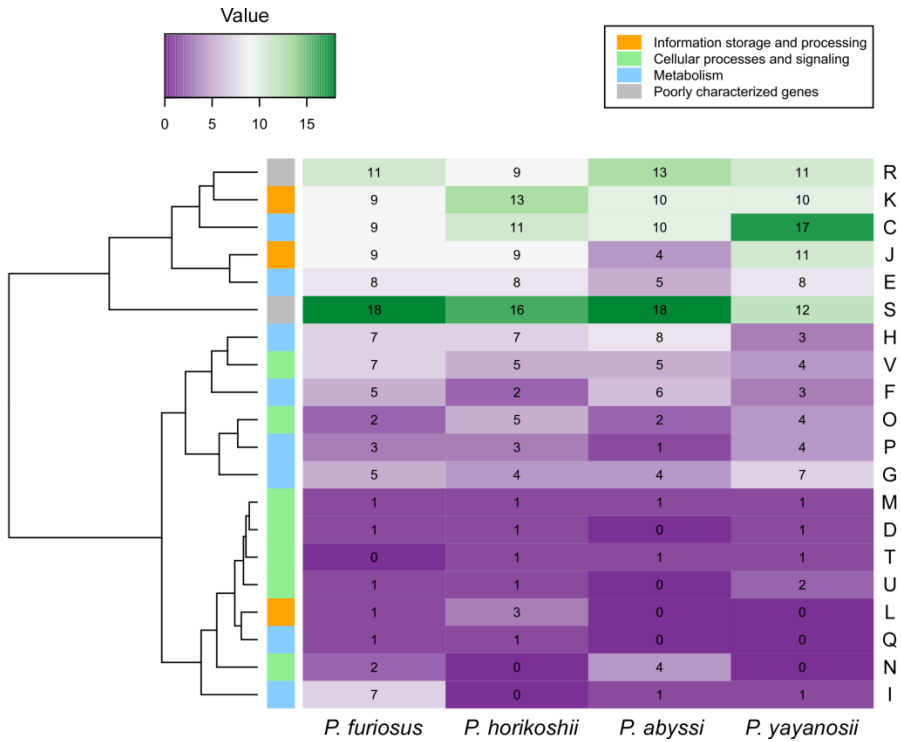


Figure S2: Heatmap representing the proportion of highly expressed genes by arCOG categories done using the R script “gplots”³.

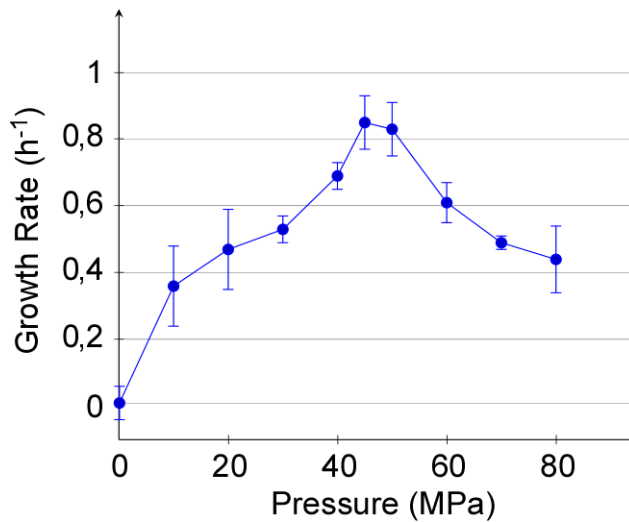


Figure S3: Growth assays of *P. yayanosii*.

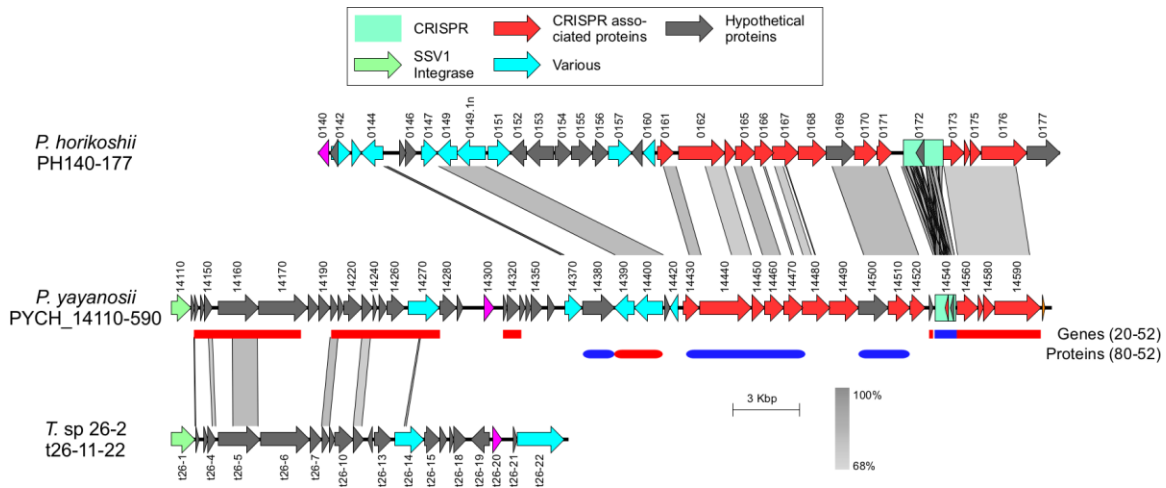


Figure S4: Alignment of a regulated *P. yayanosii* CRISPR-*cas* cluster with part of a plasmid of *Thermococcus* sp. 26-2 and *P. horikoshii*⁴. The arrows represent the genes, the ovals the expression results of RNA and the rectangle the expression results of proteins. The blue color mean the down-regulation on conditions indicated on the left whereas the red one corresponds to the up-regulation. The alignments were done with the Easyfig software (blastn, e-value 10^{-4})².

References

1. Birrien, J.-L. *et al.* *Pyrococcus yayanosii* sp. nov., an obligate piezophilic hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **61**, 2827–2881 (2011).
2. Sullivan, M. J., Petty, N. K. & Beatson, S. A. Easyfig: a genome comparison visualizer. *Bioinformatics* **27**, 1009–1010 (2011).
3. Warnes, G. R. *et al.* gplots: Various R programming tools for plotting data. *R package version 2*, (2009).
4. Soler, N. *et al.* Two novel families of plasmids from hyperthermophilic archaea encoding new families of replication proteins. *Nucleic Acids Res* **38**, 5088–5104 (2010).