

Original Research – *Supplementary Materials*

Culturomics remains a highly valuable methodology to obtain rare microbial diversity with biotechnological potential from two Portuguese salterns

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Author Contributions

These should be presented as follows: EA, MFC and OML designed the research study. EA performed the research. EA, MFC and OML analyzed the data. EA, MFC and OML wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

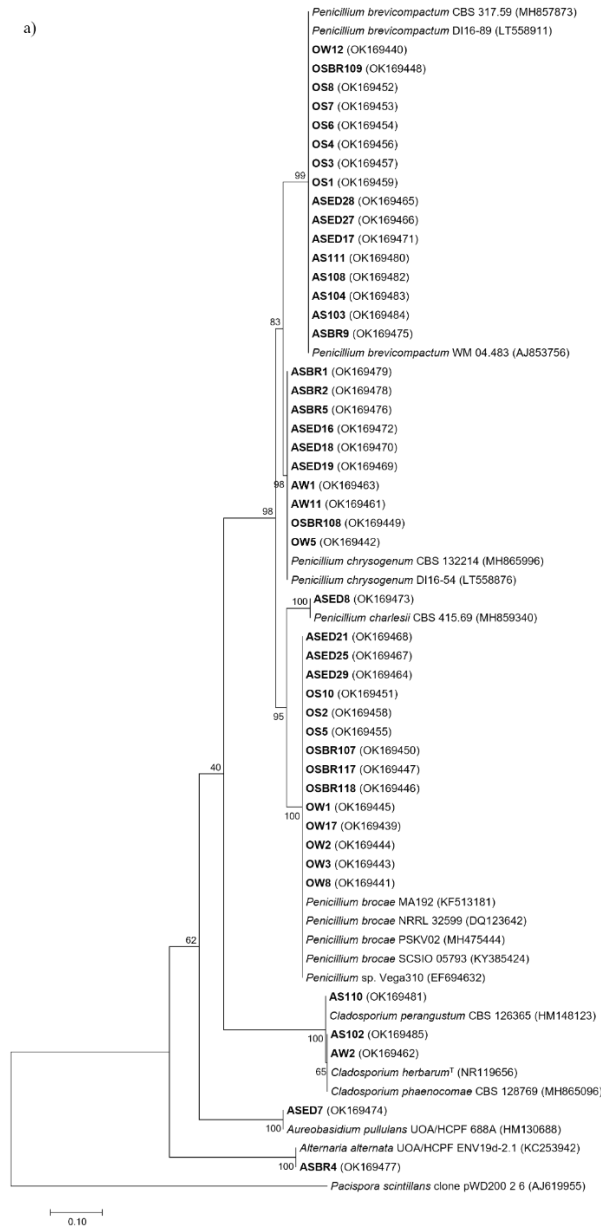


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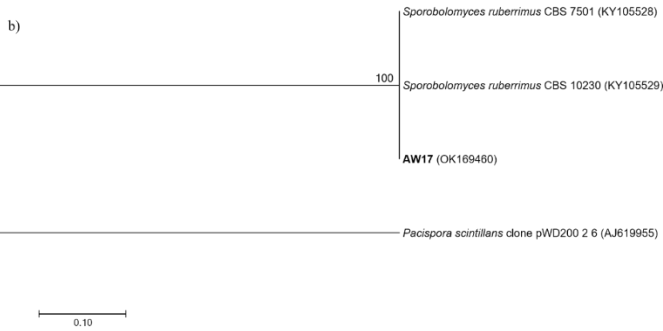
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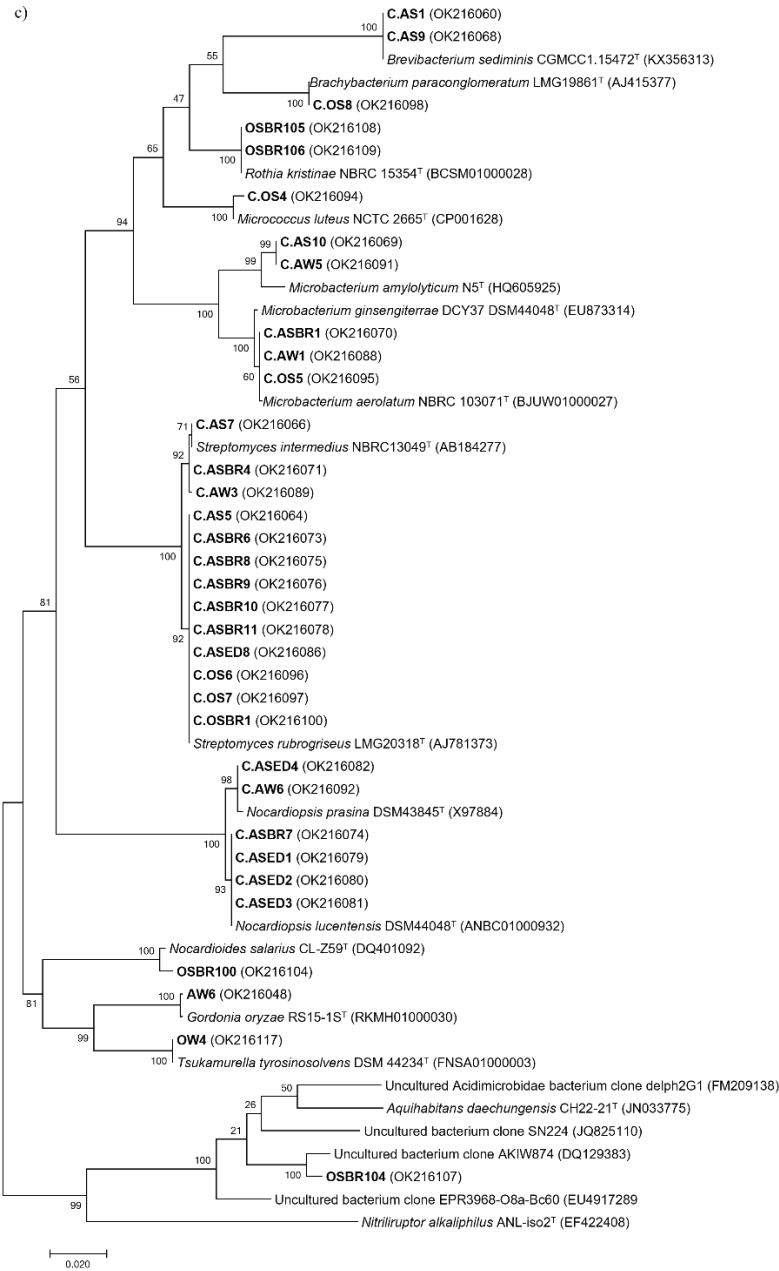
Supplementary Materials



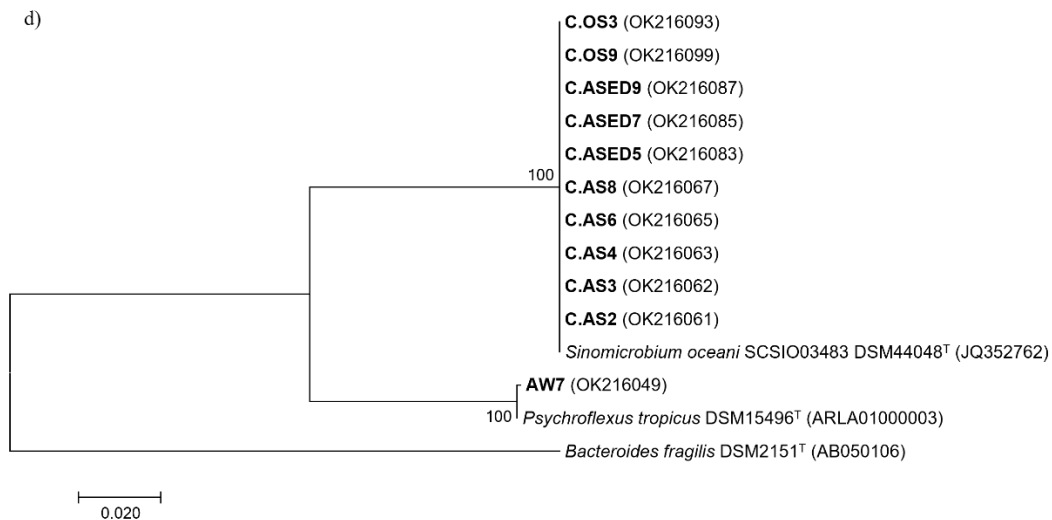
Supplementary Fig. 1. Phylogenetic framing of all strains isolated from the two Portuguese Salterns. Maximum-Likelihood (ML) phylogenetic trees constructed with MEGA 7 software [1], using 16S rRNA gene sequences of the saltern isolates and of the phylogenetically closest sequences obtained in the EzBioCloud webserver. Bootstrap values were calculated based on 1000 replications and the numbers at each branch represent the bootstrap support in percentage for each cluster. The trees are phyla-grouped, and a different outgroup was used for each phylum: a) phyla Ascomycota and b) Basidiomycota with *Pacispora scintillans* (AJ619955) as outgroup; c) phylum Actinomycetota with *Nitriliruptor alkaliphilus* (EF422408) as outgroup; d) phylum Bacteroidota with *Bacteroides fragilis* (AB050106) as outgroup; e) phylum Bacillota with *Clostridium butyricum* (AJ458420) as outgroup; f) phylum Gemmatimonadota with *Chloroflexus aurantiacus* (D38365) as outgroup; g) phylum Planctomycetota with *Verrucomicrobium spinosum* (X90515) as outgroup; h) phylum Pseudomonadota with *Burkholderia cenocepacia* (AF148556) as outgroup; and i) phylum Rhodothermota with *Rhodothermus marinus* (AF217494) as outgroup. Bar, 0.10 substitutions per nucleotide position in a) and b); Bar, 0.020 substitutions per nucleotide position in c) to f) and h); Bar, 0.050 substitutions per nucleotide position in g) and i).



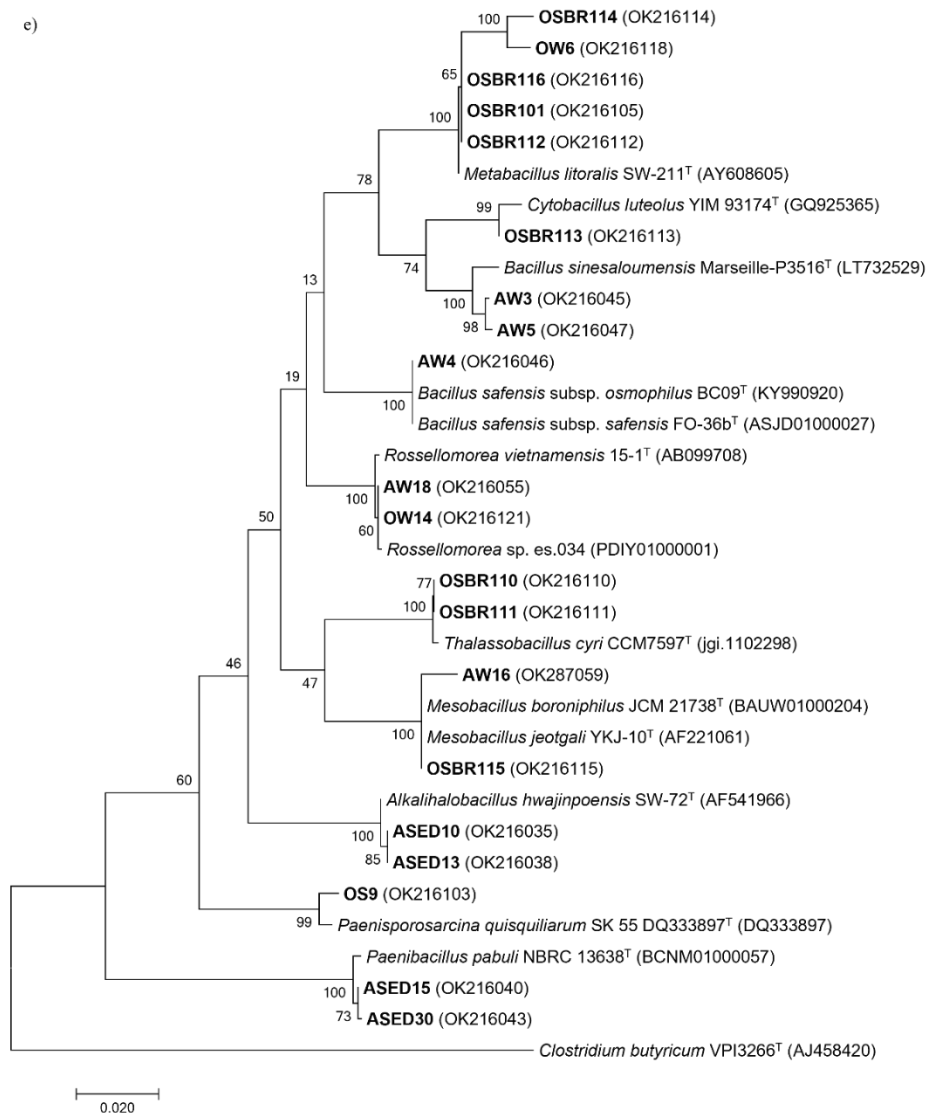
Supplementary Fig. 1. (cont.) Phylogenetic framing of all strains isolated from the two Portuguese Salterns.



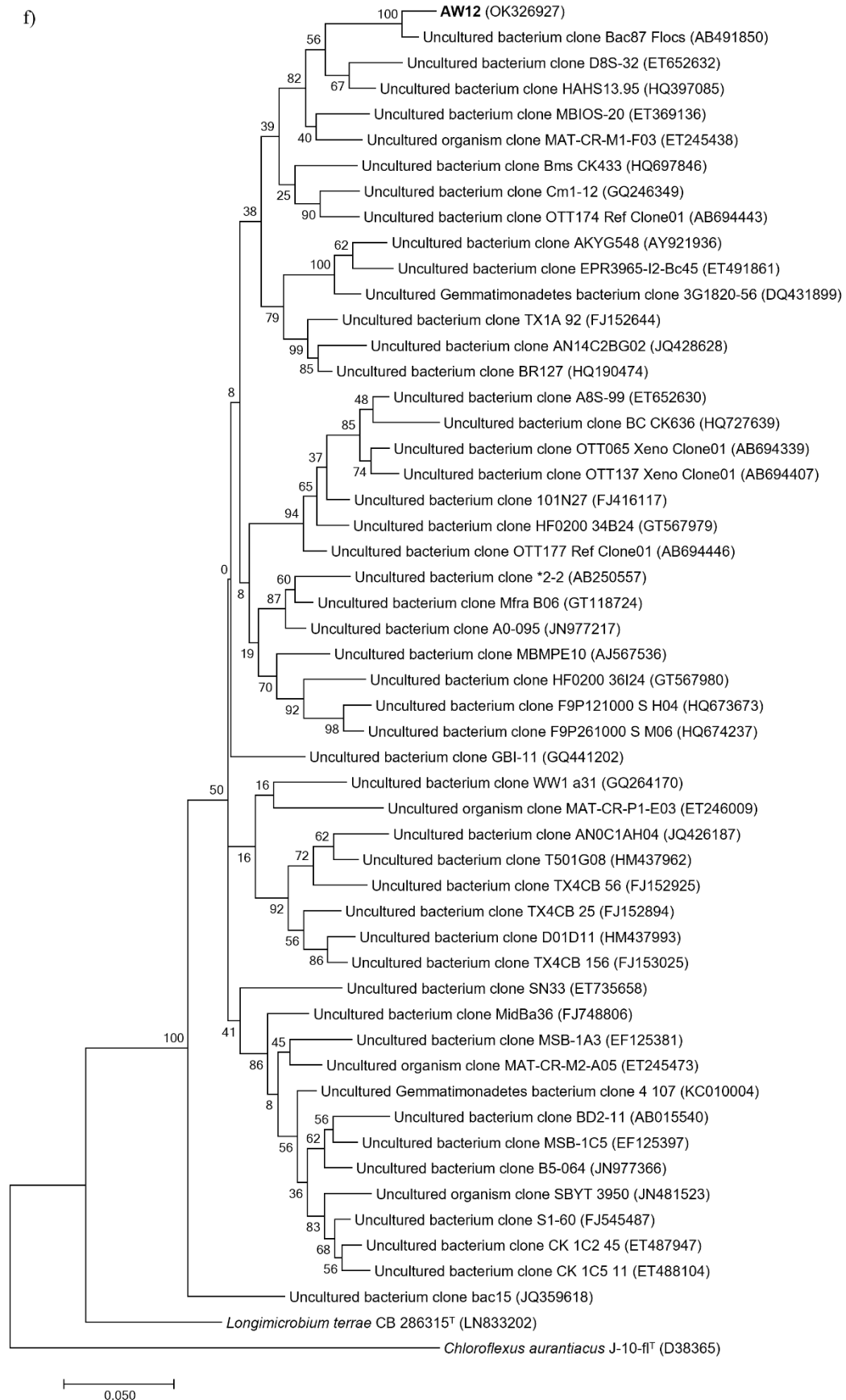
Supplementary Fig. 1. (cont.) Phylogenetic framing of all strains isolated from the two Portuguese Salterns.



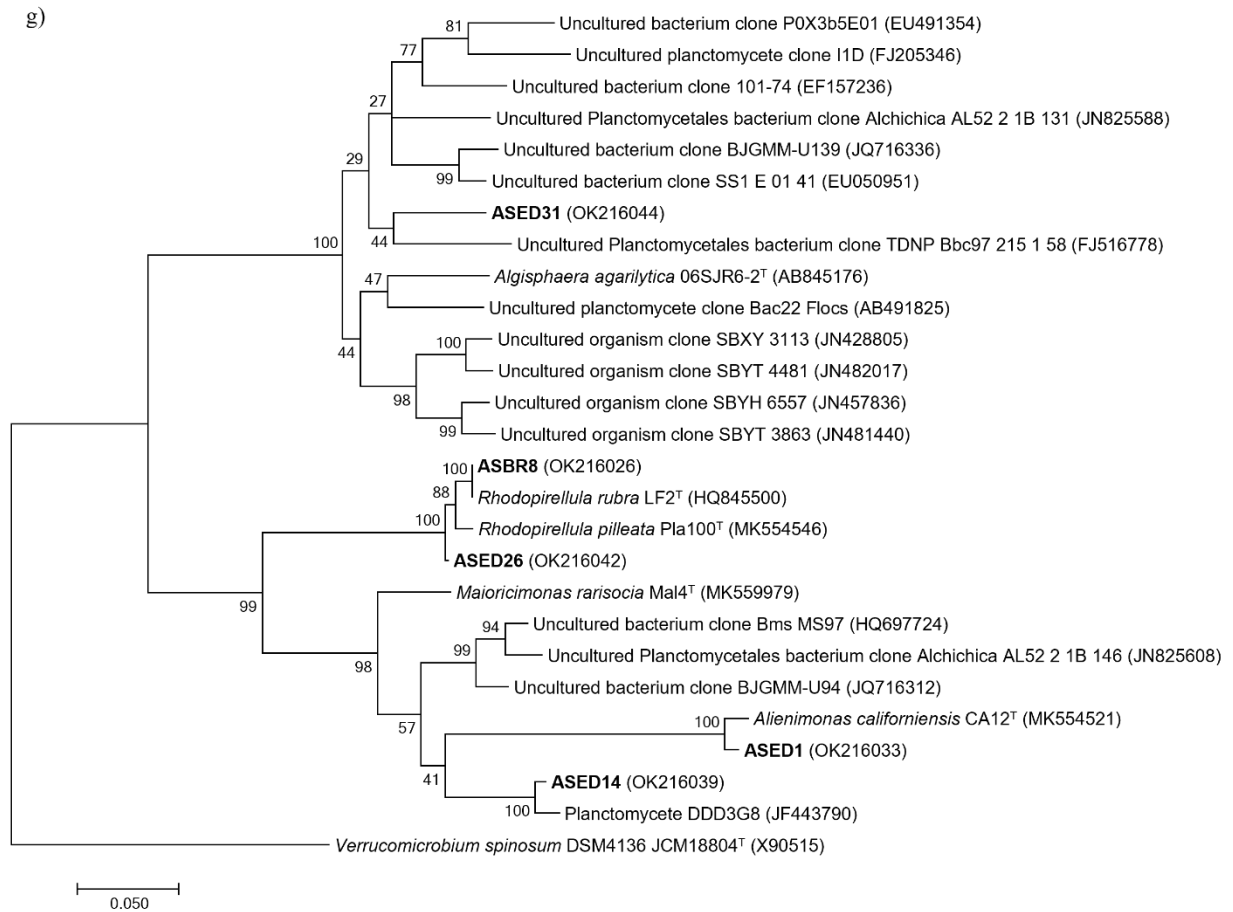
Supplementary Fig. 1. (cont.) Phylogenetic framing of all strains isolated from the two Portuguese Salterns.



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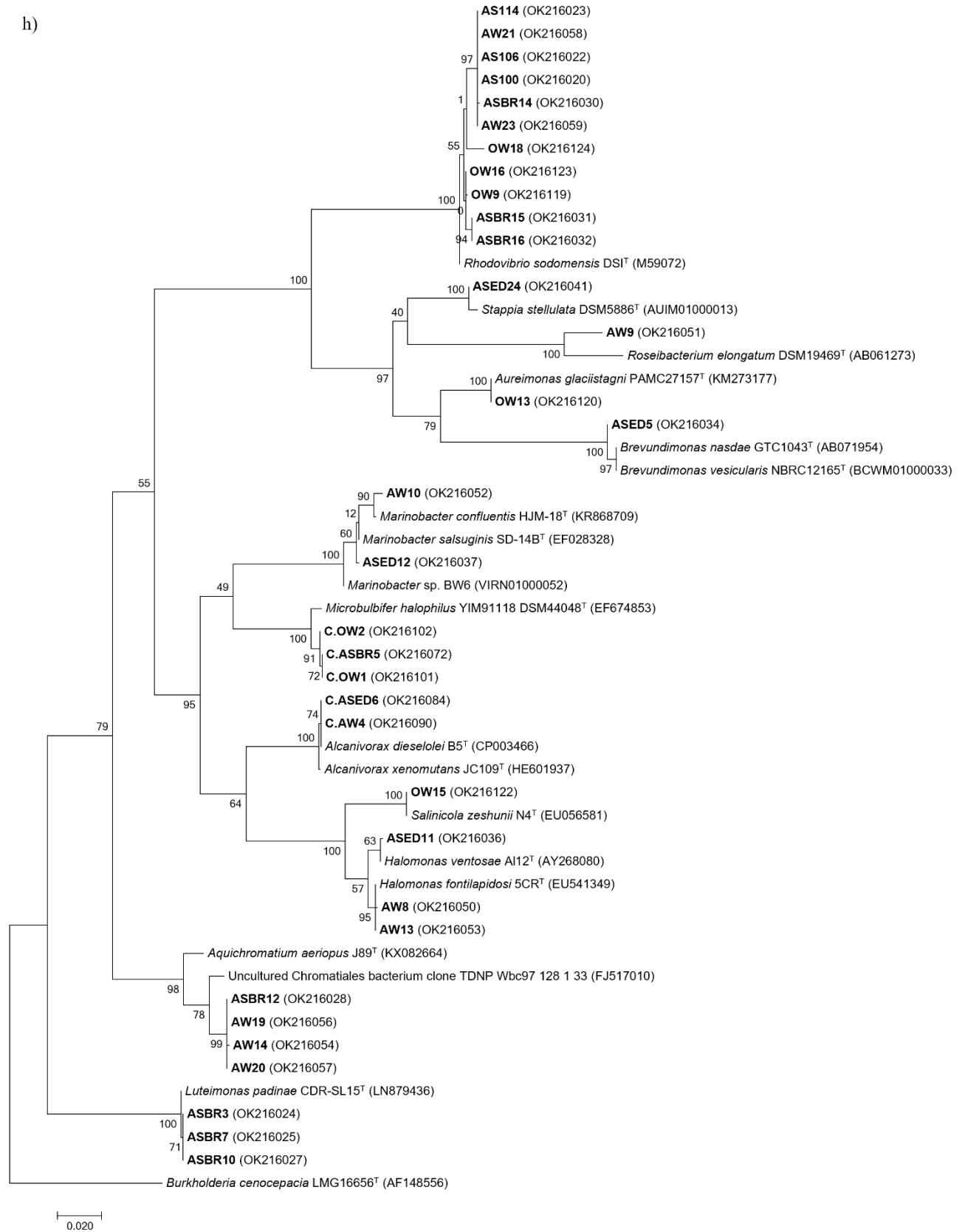


Supplementary Fig. 1. (cont.) Phylogenetic framing of all strains isolated from the two Portuguese Salterns.

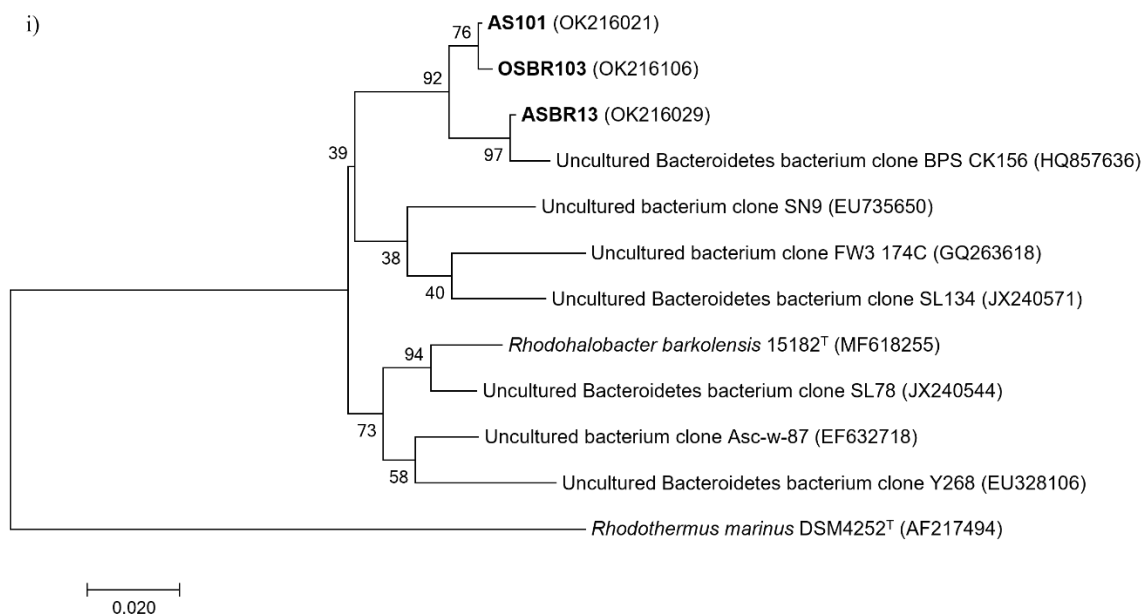


Supplementary Fig. 1. (cont.) Phylogenetic framing of all strains isolated from the two Portuguese Salterns.

h)



Supplementary Fig. 1. (cont.) Phylogenetic framing of all strains isolated from the two Portuguese Salterns.



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Supplementary Table 1. Composition per litre of media used for Planctomycetota and Actinomycetota isolation.

| | Planctomycetota | | | Actinomycetota | | |
|--------------------------------------|-------------------|-------------------|---------------------|------------------|-----------------|------------------|
| | M600 ^a | M607 ^a | M607SW ^b | SCN ^c | M3 ^d | NPS ^e |
| Peptone | 1 g | 0.25 g | -- | -- | -- | -- |
| Yeast Extract | 1 g | 0.25 g | -- | -- | -- | -- |
| 2.5% Glucose | 40 mL | 10 mL | 10 mL | -- | -- | -- |
| No. 6 Vitamins ^f | 10 mL | 10 mL | 10 mL | -- | -- | -- |
| Hutner's Basal Salts ^g | 20 mL | 20 mL | 20 mL | -- | -- | -- |
| 0.1M Tris-HCl (pH 7.5) | 50 mL | 50 mL | 50 mL | -- | -- | -- |
| Soluble starch | -- | -- | -- | 10 g | -- | -- |
| Casein | -- | -- | -- | 0.3 g | -- | -- |
| KNO ₃ | -- | -- | -- | 2 g | -- | -- |
| MgSO ₄ ·7H ₂ O | -- | -- | -- | 0.05 g | -- | -- |
| K ₂ HPO ₄ | -- | -- | -- | 2 g | -- | -- |
| CaCO ₃ | -- | -- | -- | 0.02 g | -- | -- |
| FeSO ₄ ·7H ₂ O | -- | -- | -- | 0.01 g | -- | -- |
| Glucose | -- | -- | -- | -- | 6 g | -- |
| Chitin | -- | -- | -- | -- | 2 g | -- |
| NPS extract ^h | -- | -- | -- | -- | -- | 500 mL |
| Agar | 16 g | 16 g | 16 g | 18 g | 18 g | 18 g |
| Natural Sea Water (3% NaCl) | 880 mL | 900 mL | 900 mL | 700 mL | 1000 mL | 500 mL |
| Distilled Water | -- | 10 mL | 10 mL | 300 mL | -- | -- |

All these media were manually prepared at our laboratory, while for R2A and PDA, BD Difco™, pre-prepared formulations were used.

^a from Lage and Bondoso [2]; ^b This study; ^c adapted from Küster and Williams [3]; ^d from Mincer et al. [4]; ^e adapted from Jensen et al. [5]; ^f from Staley [6]; ^g from Cohen-Bazire et al. [7]; ^h Nutrient-poor sediment (NPS) extract consisted of washing (extracting) 900 ml (wet volume) of sand collected from Matosinhos beach with 500 ml of seawater. The liquid extract was collected and stored at 4 °C until use (adapted from Jensen et al. [5]).

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