

Fig. 1. Schematic representation of the algal ADHEs. The scheme is produced from the multiple sequence alignment of *Polytomella* sp. ADHEs with their counterparts in *E. coli* and *C. reinhardtii* shown in Supplementary Fig. S1. Signatures for CoASH-dependent aldehyde dehydrogenase (ALDH) and iron-containing alcohol dehydrogenase (Fe-ADH) are indicated by blue and red boxes, respectively. Yellow boxes refer to NAD-binding sites, vertical dotted lines to the linker sequences. The vertical black arrow indicates the position of the residue critical for the assembly of *E. coli* ADHE into tetramers (F670). Hatched boxes indicate the *N*-termini which likely serve as intracellular targeting sequences.

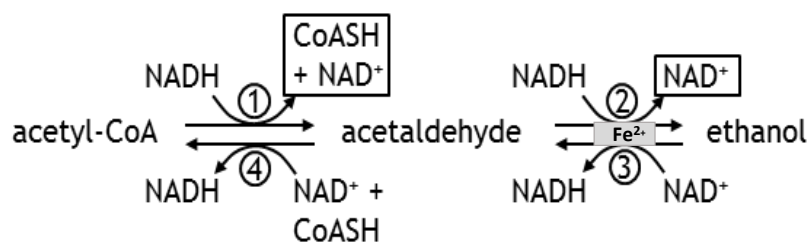


Fig. 2. Schematic representation of the enzymatic reactions typically catalyzed by aldehyde-alcohol dehydrogenases. ADHE is a bidirectional and bifunctional enzyme which converts acetyl-CoA to ethanol with acetaldehyde as intermediate. The individual enzymatic reactions (1-4) can be assessed based on the presence of substrates, cofactors (CoASH, NADH) and iron center (Fe²⁺).

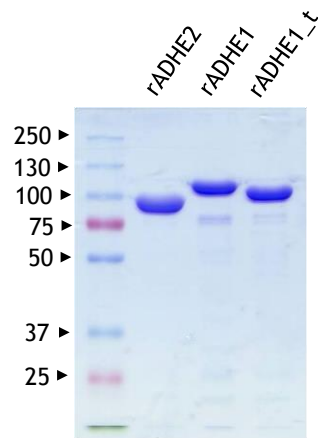


Fig. 3. SDS-PAGE analysis of recombinant *Polytomella* ADHEs. Proteins purified by affinity chromatography (10 μ G) were separated on a 10% acrylamide gel and stained with Coomassie Brilliant Blue R. rADHE2, recombinant ADHE2; rADHE1, recombinant ADHE1; rADHE1_t, recombinant ADHE1 lacking its 41 carboxy-terminal residues. The gel is representative of three independent protein purifications.

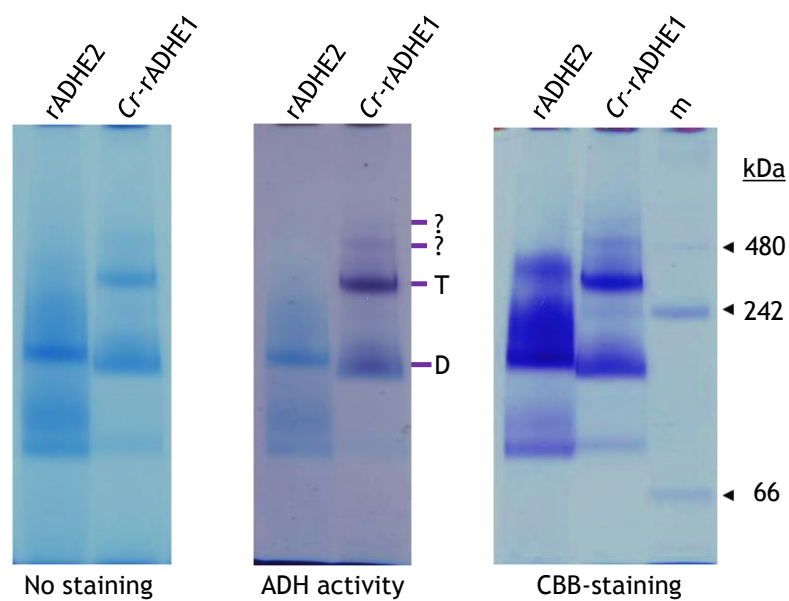


Fig. 4. Blue Native PAGE analysis of recombinant algal ADHEs. Purified ADHEs (30 μ G) were analyzed on a 3-12% BN-PAGE. rADHE2, recombinant *Polytomella* ADHE2; Cr-rADHE1, *C. reinhardtii* ADHE as control. m, markers. Putative oligomeric states of Cr-rADHE1 are indicated: D, dimer; T, tetramer. Gel bands were photographed before and after staining for alcohol dehydrogenase (ADH activity) or after staining with Coomassie Brilliant Blue R (CBB-staining). The ADH activity must be evaluated by comparison with the left panel (no staining). The gels are representative of two or three independent protein preparations.

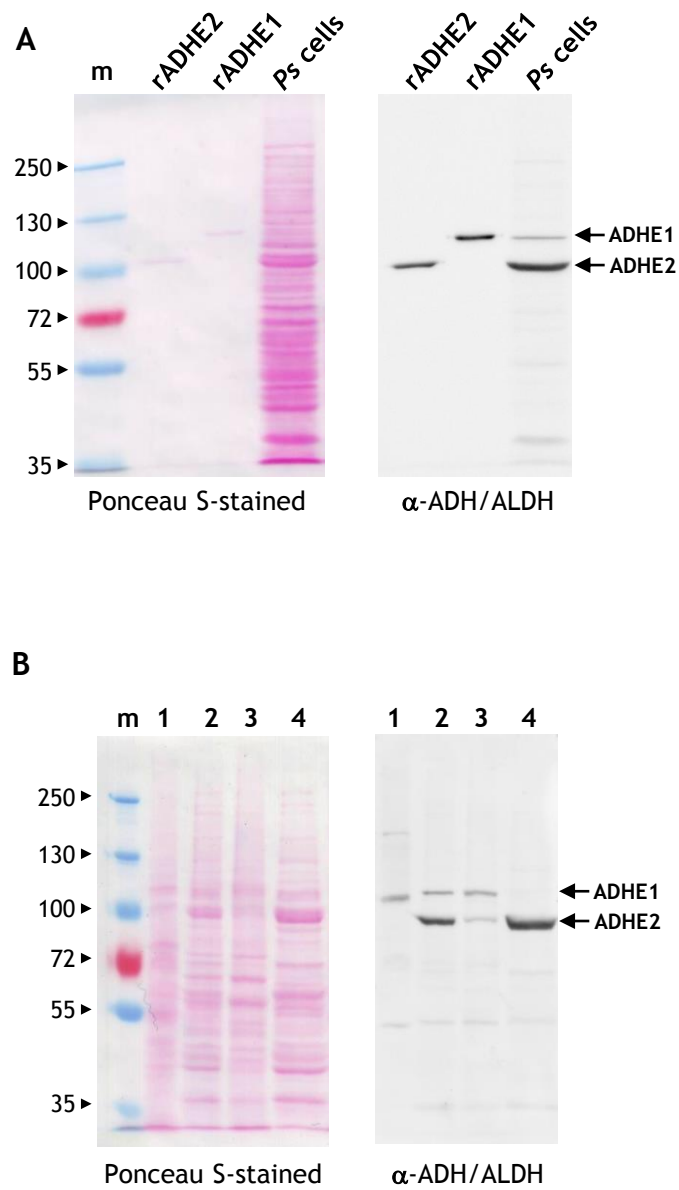


Fig. 5. Accumulation levels of *Polytomella* sp. ADHEs evaluated by immunoblotting experiments. Proteins were separated on a 8% SDS-acrylamide gel, transferred onto nitrocellulose membranes and stained with Ponceau S. The blots were probed with anti-ADHE serum (α -ADH/ALDH). **A.** Calibration of the amounts of ADHE proteins in *Polytomella* sp. Recombinant ADHEs (0.25 μ G); *Ps* cells, *Polytomella* cells grown on ethanol at pH 6.0 (40 μ G). **B.** ADHEs in cells grown on acetate at pH 6.0. 1, *C. reinhardtii* chloroplasts (control); 2, *Polytomella* sp. cells; 3, *Polytomella* sp. particulate fraction and 4, *Polytomella* sp. soluble fraction.

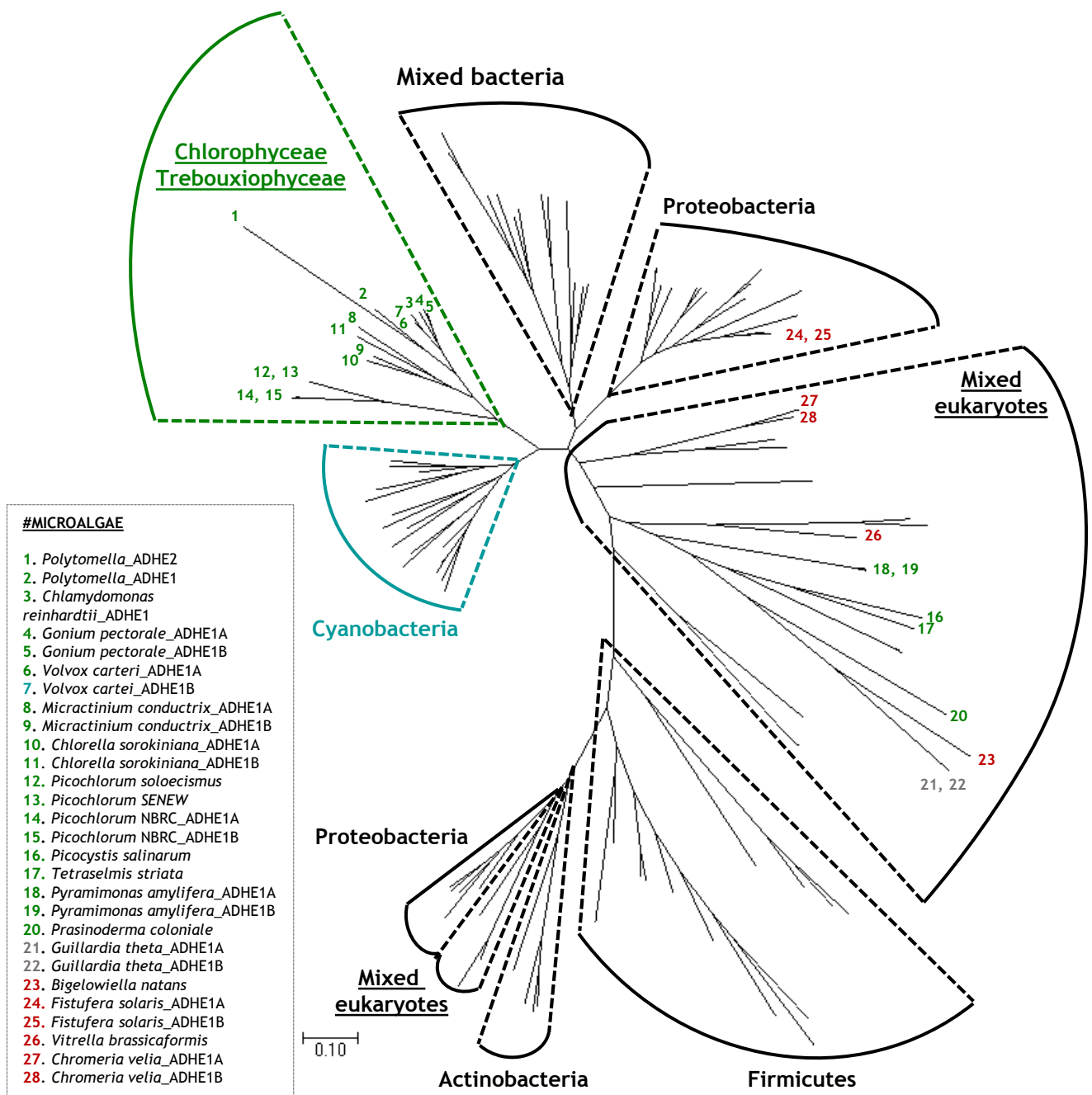


Fig. 6. Maximum-likelihood (ML) phylogeny of ADHE protein sequences reconstructed from an alignment of homologs from 95 organisms. The identifiers of the bacterial and eukaryote ADHE sequences are listed in Supplementary Table S5, The evolutionary history was inferred by using the ML method run with the JTT substitution matrix-based model (Jones et al. 1992). The analysis involved 66 amino acid sequences from bacteria and 39 sequences from eukaryotes. All positions containing gaps and missing data were eliminated. There was a total of 800 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

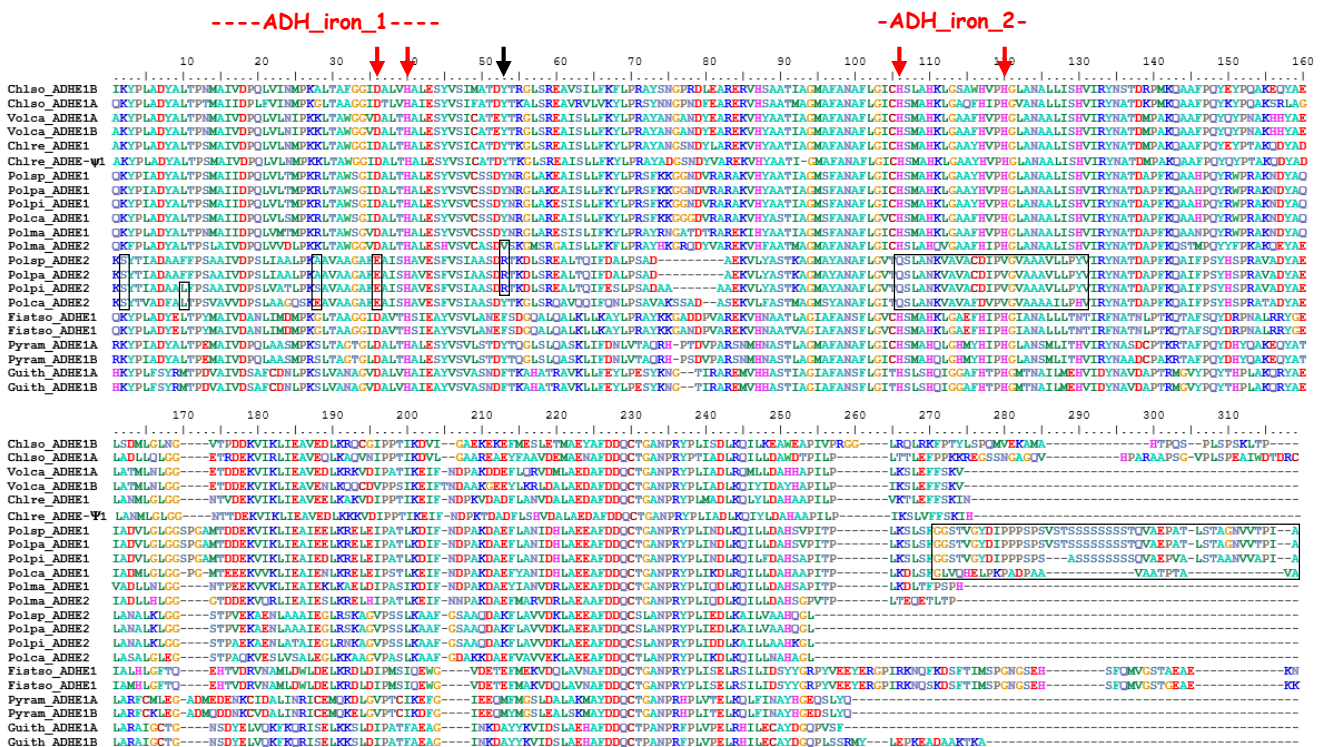


Fig. 7. Multisequence alignment of the Fe-binding residues in the alcohol dehydrogenase domain of selected ADHEs. The divergent residues in the active site of *Polytomella* spp ADHE2s are boxed as well as the C-terminal extension in ADHE1s. The aromatic residue necessary for spiroosome formation is indicated by a black arrow. The position of the iron-binding residues are indicated by red arrows. Comparison of ADHE1 paralogs in other algal species shows a much higher degree of conservation, including between *CrADHE1* and one of the pseudogenes. *Chlre*, *Chlamydomonas reinhardtii*; *Chlso*, *Chlorella sorokiniana*; *Fisso*, *Fistulifera solaris*; *Guith*, *Guillardia theta* CCMP2712; *Polca*, *Polytomella capuana*; *Polma*, *Polytomella magna*; *Polpa*, *Polytomella parva*; *Polpi*, *Polytomella piriformis*; *Pyram*, *Pyramimonas amyliifera* CCMP720; *Volca*, *Volvox carteri*.