Biondi et al. *Hot springs cyanobacteria endowed with biological activities for cosmetic applications: evaluation of on-site collected communities and isolated strains*. Suplementary Materials

Extraction Protocol

1) weighing of the lyophilised sample;

2) addition of chloroform with a ratio of 50-60 mL per gram of sample organic matter and manual stirring;

3) extraction overnight at room temperature (25-27 °C);

4) filtration on paper to separate the sample solids and washing with half of the solvent volume used for extraction;

5) drying of the sample solid residue under the cabinet;

6) addition of water:ethanol 70:30 at the same ratio as for chloroform and manual stirring;

7) extraction overnight;

8) filtration on paper with half of the solvent volume used for extraction;

9) solvent evaporation under vacuum with the water bath set at 35°C;

10) resuspension of the dry residue of chloroform extracted sample in methanol (lipophilc extracts) and of ethanol:water 70:30 extracted sample in the extraction solvent (hydrophilic extract) to reach an extract concentration of 100 g of extracted material per liter for bioglea and isolated strain biomasses and 1000 g of extracted material per liter for mud samples (about 100 g of extracted of organic matter per liter).

Table S1. Relation between isolated strains and on-site communities. Cyanobacterial and microalgal strains isolated from samples collected from different thermal spring sites and relevance to or correspondence with components of the phototrophic population of the sampling site.

strain	genus/species	correspondence to natural sample populations
BIOG1	*Leptolyngbya* sp.	Minor component of Saturnia pool bioglea
BIOG2	*Leptolyngbya* sp.	Major component of Saturnia pool bioglea (cf. *Leptolyngbya* sp. 1)
BIOG3	*Chroococcidiopsis* sp.	Minor component of Saturnia pool bioglea
BIOG4	*Leptolyngbya* sp.	Minor component of Saturnia pool bioglea and mat
BIOG5	*Spirulina* cf. *labyrinthiformis*	Major component of Saturnia pool and canal bioglea
BIOG6	*Oscillatoria* sp.	Component at low densities of Saturnia pool bioglea (*Oscillatoria* sp. 1)
BIOG7	*Leptolyngbya* sp.	Minor component of Saturnia pool bioglea
TSAT1	*Chroococcus* sp.	Major component of Saturnia mat
TSAT2	*Synechococcus* sp.	Component of Saturnia pool bioglea, occasionally at high densities, and of thermal mud populations
TPET1	*Oscillatoria* sp.	Component of Terme di Petriolo bioglea
TPET2	*Leptolyngbya* sp.	Major component of Terme di Petriolo bioglea
BdR1	*Leptolyngbya* sp.	Major component of Bagno di Romagna oxygenic phototrophic community of cultivated bioglea
BdR2	*Leptolyngbya* sp.	Minor component of Bagno di Romagna oxygenic phototrophic community of cultivated bioglea
BdR3	*Nannochloris* sp.	Major component of Bagno di Romagna oxygenic phototrophic community of cultivated bioglea and of thermal mud
Dx1	*Chroococcus minutus*	Component of Dax cultivated bioglea
Dx2	*Phormidium* sp.	Component of Dax cultivated bioglea
Dx3	*Chlorogleopsis* sp.	Component of Dax cultivated bioglea
Dx4	*Tolypothrix* sp.	Component of Dax cultivated bioglea

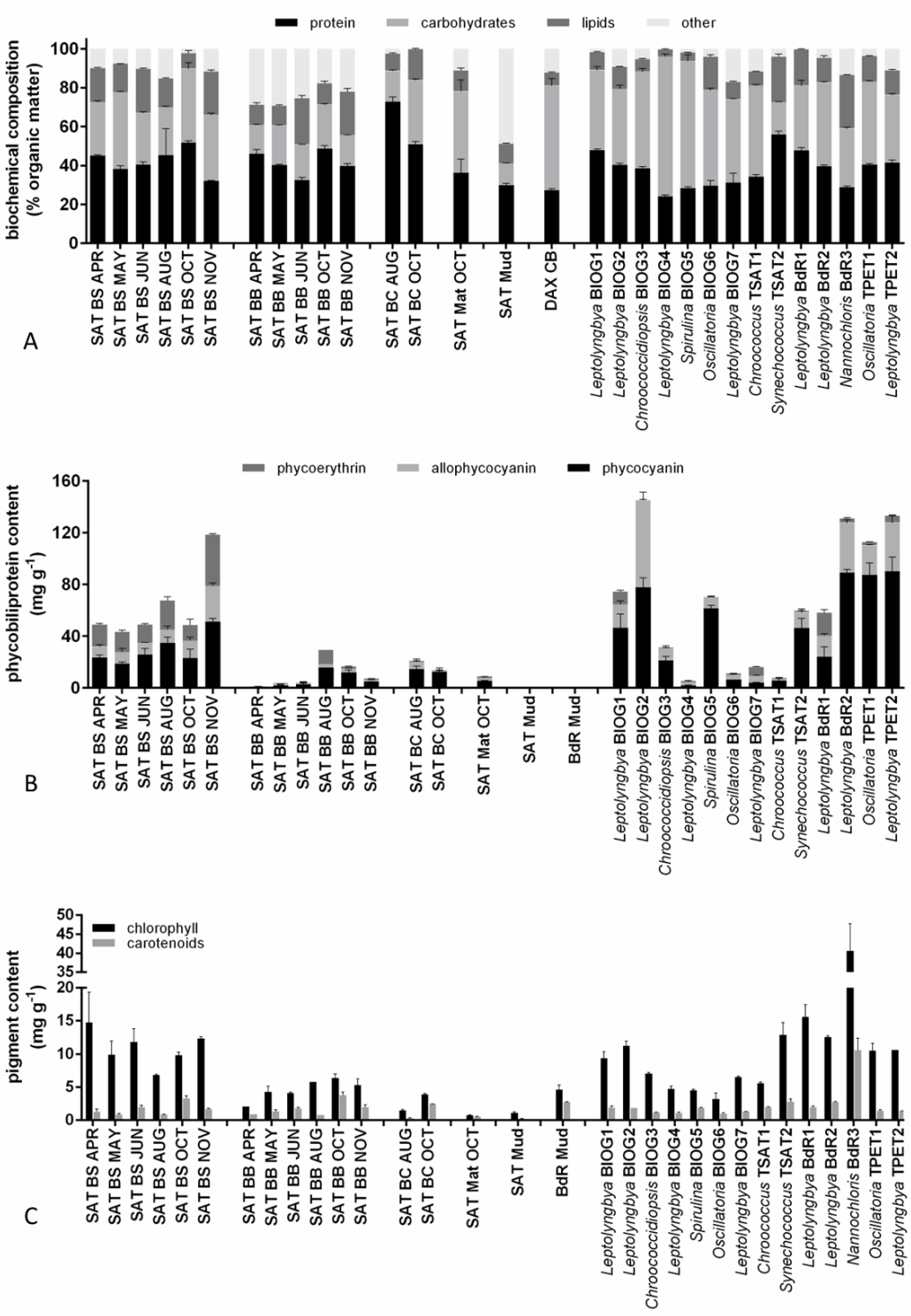

Figure S1. Biochemical composition (A), phycobiliprotein (B), and chlorophyll and carotenoid (C) content in hot spring microbial communities samples and of the isolated strains. Sample names indicate sampling spa (SAT, Saturnia spa; BdR, Bagno di Romagna spa; DAX, Dax thermal area), location of sampling within the spa (BS, surface *bioglea*; BB, bottom *bioglea*; BC, canal *bioglea*; Mat, coriaceous microbial mat; Mud, thermal mud; CB, cultivated *bioglea*) and, where needed, month of sampling (APR, April; JUN, June; AUG, August, OCT, October; NOV, November).

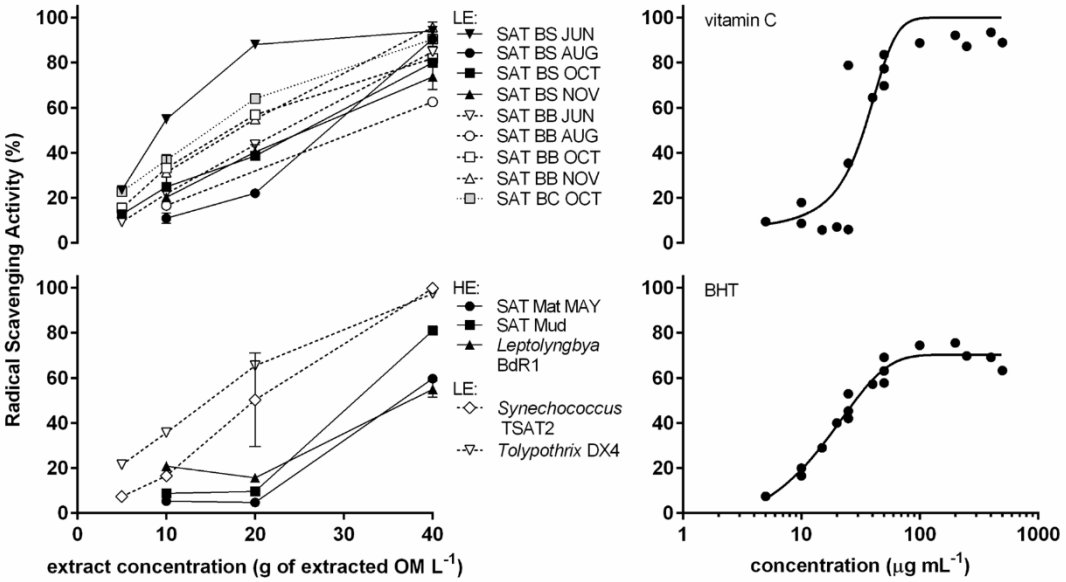

Figure S2. Dose response curves for radical scavenging activity with DPPH assay. Two positive standards, vitamin C and BHT, as well as extracts that showed an activity >50% during the screening were diluted to obtain dose-response curves. OM, organic matter; LE, lipophilic extract; HE, hydrophilic extract. Sample names indicate sampling spa (SAT, Saturnia spa), location of sampling within the spa (BS, surface *bioglea*; BB, bottom *bioglea*; BC, canal *bioglea*; Mat, coriaceus microbial mat; Mud, thermal mud) and, where needed, month of sampling (JUN, June; AUG, August, OCT, October; NOV, November).

Table S2. Equations of the regressions used to calculate IC_{50} values for radical scavenging activity determined with DPPH assay. The IC_{50} values were calculated for the extracts reported in Figure S2. In the regressions, *y* represents radical scavenging activity and *x* extract concentration.

Extract	Linear regression parameters
Lipophilic extracts	
SAT BS JUN	$y = 4.2x+6.9$; $R^2 = 0.97$
SAT BS AUG	$y = 2.3x-23.9$; $R^2 = 0.97$
SAT BS OCT	$y = 1.9x+3.7$; $R^2 = 0.99$
SAT BS NOV	$y = 2.2x-13.0$; $R^2 = 0.86$
SAT BB JUN	$y = 2.3x-1.6$; $R^2 = 1.00$
SAT BB AUG	$y = 1.5x-1.4$; $R^2 = 1.00$
SAT BB OCT	$y = 1.8x+12.8$; $R^2 = 0.95$
SAT BB NOV	$y = 2.1x+11.0$; $R^2 = 1.00$
SAT BC OCT	$y = 1.9x+17.9$; $R^2 = 0.96$
Synechococcus TSAT2	$y = 3.4x-12.9$; $R^2 = 0.99$
Tolypothrix Dx4	$y = 2.1x-14.8$; $R^2 = 0.97$
Hydrophilic extracts	
SAT Mat MAY	$y = 2.7x-50.0$; $R^2 = 0.89$
SAT Mud	$y = 2.6x-26.8$; $R^2 = 0.90$
Leptolyngbya BdR1	$y = 2.0x-23.5$; $R^2 = 1.00$
Positive controls	
Vitamin C	$y = 1.6x-2.9$; $R^2 = 0.97$
BHT	$y = 1.1x+10.5$; $R^2 = 0.88$

Sample names indicate sampling spa (SAT, Saturnia spa), location of sampling within the spa (BS, surface *bioglea*; BB, bottom *bioglea*; BC, canal *bioglea*; Mat, coriaceous microbial mat; Mud, thermal mud) and, where needed, month of sampling (JUN, June; AUG, August, OCT, October; NOV, November).

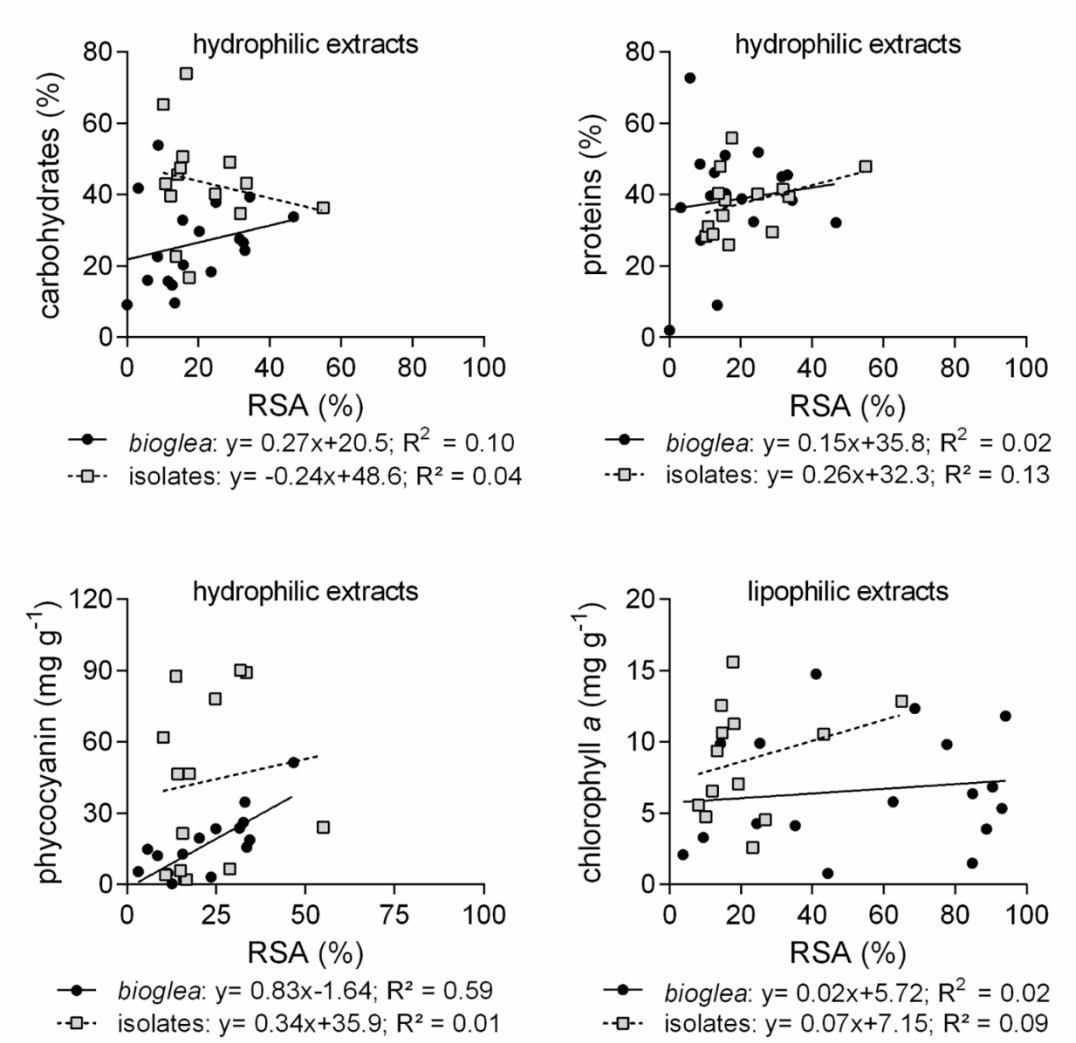

Figure S3. Linear regressions between components of *bioglea*/isolate biomasses and radical scavenging activity (RSA) of lipophilic and hydrophilic extracts. Equations of the interpolated lines are also reported together with the R^2 value. Correlation analysis: hydrophilic extract RSA vs carbohydrate and vs protein–*bioglea*, P>0.05, n =18– isolates, P>0.05, n=14; hydrophilic extract RSA vs phycocyanin– *bioglea*, P<0.001, n=16– isolates, P>0.05, n=13; lipophilic extract RSA vs chlorophyll *a*– *bioglea*, P>0.05, n =17– isolates, P>0.05, n=13.