Supplementary Materials

Microbial Fermentation of the Water-Soluble Fraction of Brewers' Spent Grain for the Production of High-Value Fatty Acids

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S.1 Materials and General Methods

All air and moisture-sensitive reactions were carried out using dry solvents and under a static atmosphere of nitrogen. BSG was provided by the Brewery "L'Orso Verde" (Busto Arsizio, Italy). Unrefined sea salt was purchased from Sosalt S.p.A. (Trapani, Italy). Riboflavin was purchased from Health Leads UK Ltd., (Horeb, UK). The other reagents and the employed solvents were purchased from Merck (Merck Life Science S.r.l., Milan, Italy) and used without further purification.

Reference standard samples of C_{12}-C_{20} branched-chain fatty acids (BCFAs) were synthesized in our laboratory by modification of the procedure described for the synthesis of *iso*-branched-chain aldehydes [1] D-(+)-Galactose, L-(−)-fucose and reference standard samples of palmitoleic, oleic, linoleic, α-linolenic, γ-linolenic, arachidonic (ARA), *cis*-5,8,11,14,17-eicosapentaenoic (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic (DHA) were purchased from TCI (Milano, Italy). (L)-(+)-rhamnose, L-(+)-arabinose, D-(+)-xylose, D-(+)-mannose, D-(+)-glucose and ergosterol were purchased from Merck (Merck Life Science S.r.l., Milan, Italy).

S.2. Microorganisms and Growth Media

Streptomyces jeddahensis (DSM 101878), *Shewanella hanedai* (DSM 6066), *Moritella marina* (DSM 104096), *Trichoderma viride* (DSM 63065) *Chaetomium globosum* (DSM 1962), *Mucor plumbeus* (DSM 62759), *Mucor circinelloides* (DSM 1191), *Rhizopus stolonifer* (DSM 855), *Cunninghamella echinulata* (DSM 1905), *Neurospora crassa* (DSM 894) and *Alternaria alternata* (DSM 1102) were purchased from DSMZ GmbH collection (Braunschweig, Germany).

Aspergillus niger (CBS 626.66), *Aspergillus kanagawaensis* (CBS 424.68), *Cladosporium cucumerinum* (CBS 158.51), *Sodiomyces alcalophilus* (CBS 114.92), *Rhizopus oryzae* (CBS 112.07), *Rhizomucor pusillus* (CBS 354.68), *Entomophthora exitialis* (CBS 180.60), *Pythium ultimum* (CBS 805.95), *Mortierella Isabellina* (CBS 208.32), *Mortierella alpina* (CBS 754.68) and *Conidiobolus heterosporus* (CBS 543.63) were purchased from CBS-KNAW collection from Westerdijk Fungal Biodiversity Institute (Utrecht, Netherlands).

Syncephalastrum racemosus (MUT 2770) was provided by the Turin University Culture Collection (Turin, Italy).

Absidia coerulea (AM93) and *Beauveria bassiana* (AM278) were provided by Wrocław University (Wrocław, Poland).

Crypthecodinium cohnii (CCMP 316) was purchased from the National Center for Marine Algae and Microbiota (East Boothbay, ME 04544 USA).

Streptomyces cavourensis subsp. *cavourensis* (DSM 112466), *Nigrospora oryzae* (MUT 5844) and *Fusarium culmorum* (MUT 5855) were isolated as axenic cultures in our laboratory, then identified through gene sequencing and finally deposited in the DSMZ GmbH collection (Braunschweig, Germany) and Turin University Culture Collection (Turin, Italy), under the collection number given in brackets.

The growth media used in this work are YUM (Yeasts Universal Medium) supplemented with 20 mg/L of riboflavin, PDB (Potato Dextrose Broth), ME (Malt Extract), SABG (Sabouraud Glucose), MB (Marine Broth), GYM (Glucose Yeast extract Malt extract), CRY (*Crypthecodinium*), BSG and MBSG (Marine BSG).

YUM medium composition: yeast extract (3 g/L), malt extract (3 g/L), peptone from soybeans (5 g/L), glucose (10 g/L).

PDB medium composition: potato extract (4 g/L), glucose (20 g/L).

ME medium composition: malt extract (20 g/L), glucose (20 g/L), peptone (2 g/L).

SABG medium composition: soya peptone (10 g/L), glucose (20 g/L), $MgSO_4 \cdot 7H_2O$ (1 g/L), KH_2PO_4 (1 g/L), trace elements solution (10 mL).

MB medium composition: soya peptone (6 g/L), meat extract (2 g/L), yeast extract (1 g/L), unrefined sea salt (20 g/L), $MgCl_2 \cdot 6H_2O$ (7 g), Na_2SO_4 (3 g/L), $CaCl_2$ (1 g/L), KCl (0.5 g/L), $SrCl_2 \cdot 6H_2O$ (0.1 g/L), NaBr (0.1 g/L), $NaHCO_3$ (0.1 g/L), soil extract (50 mL), trace elements solution (10 mL).

GYM medium composition: glucose (4 g/L), yeast extract (4 g/L), malt extract (10 g/L).

CRY medium composition: glucose (10 g/L), soya peptone (1 g/L), yeast extract (3 g/L), NaOAc (1 g/L), unrefined sea salt (20 g/L), $MgCl_2 \cdot 6H_2O$ (5 g/L), $MgSO_4 \cdot 7H_2O$ (2 g/L), $CaCl_2$ (1 g/L), KCl (0.5 g/L), $SrCl_2 \cdot 6H_2O$ (0.1 g/L), NaBr (0.1 g/L), Fe(III) citrate (0.1 g/L), soil extract (50 mL), trace elements solution (10 mL).

The compositions of BSG and marine BSG media (MBSG) are described in paragraph 2.1.

Trace elements solution: $FeCl_3$ (50 mM), $CaCl_2$ (20 mM), $MnCl_2$ (10 mM), $ZnSO_4$ (10 mM), $CoCl_2$ (2 mM), $CuCl_2$ (2 mM), $NiCl_2$ (2 mM), Na_2MoO_4 (2 mM), Na_2SeO_3 (2 mM), H_3BO_3 (2 mM).

Soil extract: 500 g of freshly collected garden soil was suspended in one liter of deionized water and the obtained heterogeneous mixture was sterilized at 121 °C for one hour. After cooling, the solid was removed by centrifuge. The supernatant was collected, the pH was adjusted to 7 and the resulting clear solution was sterilized (121 °C, 15 min.).

YUM was used for the pre-growth of *B. bassiana*. PDB was used for the pre-growth of *T. viride*, *C. globosum*, *M. plumbeus*, *M. circinelloides*, *R. stolonifer*, *C. echinulate*, *C. cucumerinum*, *R. oryzae*, *R. pusillus*, *P. ultimum*, *S. racemosus*, *N. crassa* and *A. coerulea*. ME was used for the pre-growth of *A. niger*, *A. kanagawaensis*, *S. alcalophilus*, *M. Isabellina*, *M. alpina*, *N. oryzae A. alternata* and *F. culmorum*. SABG was used for the pre-growth of *E. exitialis* and *C. heterosporus*. MB was used for the pre-growth of *S. hanedai* and *M. marina*. CRY was used for the pre-growth of *C. cohnii*. GYM was used for the pre-growth of *Streptomyces jeddahensis.*

Either BSG or MBSG media were used for screening experiments whereas only BSG medium was used for fermentation in bioreactor.

S.3 General Analytical Methods and Analysis of BSG Medium

S.3.1 General analytical methods. The following analytic techniques have been employed to determine the BSG medium and microbial fatty acids composition.

Thin layer chromatography (TLC): Merck silica gel *60* F_{254} plates (Merck Millipore, Milan, Italy).

Column chromatography: silica gel.

Melting points were measured on a Reichert apparatus (Reichert, Vienna, Austria), equipped with a Reichert microscope, and are uncorrected.

Mass spectra were recorded on a Bruker ESQUIRE 3000 PLUS spectrometer (ESI detector) (Billerica, MA, USA) or by GC-MS analyses.

GC-MS analyses: An HP-6890 gas chromatograph equipped with a 5973 mass detector and using an *HP-5MS* column (30 m × 0.25 mm, 0.25 μm film thickness; Hewlett Packard, Palo Alto, CA, USA) was used with the following temp. program: 60 °C (1 min) – 6°/min – 150 °C (1 min)– 12°/min – 280 °C (5 min); carrier gas: He; constant flow 1ml/min; split ratio, 1/30.

S.3.2 Determination of BSG medium protein content. A sample of BSG medium was prepared as described in paragraph 2.1 omitting the addition of ammonium sulfate and yeast extract. The spectrophotometric determination of the protein content was performed according to the Bradford protocol [2], using bovine serum albumin (BSA) as the standard protein. The analysis indicated the presence of 0.43 g/L of proteins, corresponding to 1.6 % (w/w) of the starting BSG_D.

S.3.3 Determination of BSG medium polysaccharide content. A sample of BSG medium was prepared as described in paragraph 2.1 omitting the addition of ammonium sulfate and yeast extract. Hence, the concentration of a portion (150 mL) of this extract, followed by drying in high vacuum and weighting of the residue, allowed establishing that about 28% (w/w) of the starting BSG_D has been extracted. Considering that, cellulose and lignin are not soluble in neutral water and about 1.6% of the dry extract is made of proteins, the remaining 26.5% of the extracted biomass takes account of polysaccharides. The composition of this fraction, in terms of monosaccharides ratio, was determined by a two-step analytic procedure that proved to be very reliable [3], even for the analysis of samples containing complex mixtures of sugars. Accordingly, the polysaccharides were completely hydrolyzed using mineral acid (1 M H_2SO_4 at reflux, for 2 hours). Hence, sulfuric acid was quenched by the addition of sodium hydroxide and the solution was treated with $NaBH_4$ (1 g, 26.4 mmol) stirring at r.t. for 2 h. Then, glacial acetic acid (10 mL) was carefully added, keeping the temperature under 30 °C by external cooling. The solvent (water) and the excess of acetic acid were removed by evaporation under reduced pressure and the obtained powder was treated with pyridine (30 mL) and acetic anhydride (30 mL) stirring at reflux for 1 h. Hence, the reaction was concentrated to the dryness under a reduced pressure and the residue was partitioned between ethyl acetate (70 mL) and water (100 mL). The aqueous phase was extracted again with ethyl acetate (50 mL) and the combined organic phases were washed in turn with saturated $NaHCO_3$ aq. (100 mL) and with brine (100 mL). The resulting solution was dried (Na_2SO_4) and concentrated in vacuo. The residue contained the alditol acetates of glucose and of the hemicellulose monosaccharides, whose relative composition was determined by GC-MS analysis. The

reference standards of the alditol acetates were prepared starting from the corresponding monosaccharides, following the reduction/acetylation protocol described above.

The analysis indicated the presence of glucose, arabinose, xylose and galactose with the following relative ratio:

Glucose:	84.2%
Arabinose:	8.5%
Xylose:	5.8%
Galactose:	1.5%

References

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