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Three New Synthetic Algal Culture Media to Grow Them All †

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† Dedicated to the memory of Harold Charles Bold (1909–1987), whose basal culture medium (BBM) revolutionized the cultivation of chlorophyte algae from freshwater and terrestrial environments.

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ABSTRACT: Three new synthetic algal culture media are described that have been used to cultivate ~12,000 diverse strains of (micro)algae, one culture medium for marine and brackish-water algae (ASP-MEL (Artificial Seawater Provasoli-MELKONIAN)), and two culture media for freshwater/terrestrial algae (SFM (Synthetic Freshwater Medium) and W-MEL (Waris-MELKONIAN)). The genesis of the three media since their original formulation and the rationale for modifications of these media over the past 50 years are outlined. A complex trace element mix derived from an enriched natural seawater culture medium (L1) is used in all three media, and allows the omission of soil water extract from one freshwater culture medium (W-MEL). It is suggested that the inclusion of selenite renders soil extract in algal culture media superfluous. Prospects and limitations of the three synthetic algal culture media as general-purpose media for large collections are discussed.

Keywords: Algae; Synthetic culture media; ASP-MEL; SFM; W-MEL; Culture collections; Selenite

1. Introduction

Over the past five decades, the authors have isolated thousands of algal strains from a diverse range of habitats (freshwater, marine, and terrestrial) and geographic locations. Algae are here defined as organisms with permanent oxygenic photosynthesis and without an embryo (a young sporophyte generation enclosed by parental tissue). In this life form definition, they include both bacteria (cyanobacteria) as well as eukaryotes. During this time, we developed a new culture medium and substantially modified two existing media to eventually devise three synthetic general-purpose media, in which we currently grow ~6000 algal strains, and which have also been adopted by several public algal culture collections. The range of cultivated algae includes cyanobacteria and all lineages of eukaryotic algae, micro- as well as some macroalgae. In this communication, we describe the genesis of these culture media, the rationale of their chemical composition, and the range of algae grown. We encourage colleagues and public algal repositories to test and, if appropriate, use these new culture media for stock cultures and smaller-scale experimental setups. For an excellent general treatise of algal culturing techniques, we refer to [1].

In addition to the composition of the culture medium, many other factors determine the success of algal cultivation. While limitations of space do not permit an in-depth discussion, a few factors should be outlined. Chemicals: Chemicals for preparation of culture media should be of the highest purity grade available (*i.e.*, ACS (American Chemical Society) grade). This is especially important for synthetic marine media for which impurities in sodium chloride may exert a major detrimental effect on algal growth. Water: While historically the purity of water was one of the limiting factors in algal cultivation, nowadays ultrapure water (e.g., of the Milli-Q type (water purified using a Millipore Milli-Q lab water system)) with an electrical resistivity of 18.2 MΩ·cm, a TOC (total organic carbon) < 10 ppb, and a bacterial count <10 CFU (colony forming units)/mL is the laboratory standard and has largely replaced double distillation. Ultrapure water should be used promptly and not stored; food-grade containers are preferred. Plasticware: Although traditionally and often still in practice, algae are grown in glass tubes and flasks, for stock cultures in larger collections, plasticware such as multi-well cell culture plates may be preferable for reasons of cost-efficiency and to save space. All cell culture microplates are nowadays made of high grade, virgin polystyrene but differ somewhat in optical properties as well as coatings. Since some algae may be sensitive to plasticizers, the choice of microplate should take the absence of stripping agents, biocides, plasticizers, or other chemical additives, softeners, or heavy metals into consideration. Temperature and Light Conditions: Temperature and light conditions should be supportive of algal growth. For stock cultures, both parameters should be as low as possible to allow extension of serial transfer intervals and to control bacterial growth in the often xenic cultures. In our experience, most algae grow at a temperature range of 16–22 °C and light intensities between 3–10 µmol photons $m^{-2} \cdot s^{-1}$ (white light LEDs). Some algae require higher temperatures and/or higher light intensities (e.g., some dinoflagellates and prasinophytes), others are psychrophiles (obligate cryophilic) requiring very low temperatures for growth (<10 °C). Inoculum: Some microalgae, especially large-celled species of euglenophytes, dinoflagellates, and cryptophytes, among others, will not divide when inoculated as single cells into the culture medium. This can often be overcome by isolating several cells (5–10) of a population into a small volume of culture medium to foster equilibration of diffusible substances (e.g., glycolate) between the cell and the external medium. Once a culture is established, single cells can often be more readily isolated. Similarly, serial transfer of the same algae into fresh culture medium may require a larger volume (and thus number of cells) to be transferred. Axenicity: Microbial cultures should consist of only a single species. However, traditionally and in practice, most algal strains in the major public culture collections are xenic, *i.e.*, they contain bacteria and possibly other organisms (fungi, protists). This relates to the fact that manual isolation of single algal cells/filaments from suspensions usually fails to recognize and remove bacteria. Bacterial contaminants in algal cultures are usually mixtures of species that derive from the natural sample (the 'phycosphere') and laboratory contaminants that may accumulate over time during subculturing. In general, the numerical ratio of bacteria to algal cells is magnitudes higher in cultures than in the natural environment, suggesting the need to isolate algal cells directly to establish axenic cultures, e.g., by fluorescence-activated cell sorting [2]. Fungal contaminants are usually detrimental to algal growth and are often responsible for the failure to establish a culture. Methods to obtain axenic cultures in the authors' laboratory have been previously summarized [3,4].

To what extent the diversity of algae in research collections and public repositories reflects the diversity of algae in the natural environment is an open question and relates to the choice of culture media used to isolate algae (as well as sampling the diversity of algal habitats). It may be argued that, if a specific culture medium is used for the isolation of algae, one selects for those algae that thrive in this medium. Algae having different requirements for the composition of culture media might be lost. While it cannot be denied that not all attempts to isolate algae yield viable cultures (in our estimation, direct isolation of algae from natural samples fails in ~10% of the cases), the reasons, as outlined above, can be manifold and are not necessarily related to the chemical composition of the isolation media. One of the reasons soil extract is often included in culture media recipes is to provide a broad range of nutrients/trace

metals/vitamins/chelators to minimize failures in both isolation and maintenance of algae. Since some environmental parameters are usually recorded upon sampling (such as electrical conductivity, pH, and temperature), these could be taken into consideration when devising media and conditions for algal isolations. If the goal is to isolate the largest diversity of algae from a natural sample, several different culture media in different dilutions (1:2, 1:4), with different pH values, different nutrient compositions such as the source of nitrogen (nitrate *vs*. ammonium or combinations of both or complete lack of combined nitrogen for isolation of nitrogen-fixing cyanobacteria), different phosphate concentrations, addition of silicate, etc. may be used. In addition to directly isolating cells from a natural sample, we have found it useful to set up enrichment cultures using the same approach. Enrichment cultures may be set up with a few drops of culture medium added to the natural sample (which may or may not be filtered to exclude predators) and/or with a few drops of the natural sample added to the culture medium.

The advantage of using enrichment cultures over direct isolations is that algae that grow in enrichments are likely to grow in the same culture medium after isolation. Furthermore, algae that are very rare in the natural sample and are thus often overlooked, may become prominent in enrichments, an example being the streptophyte alga *Mesostigma viride*, which, on several occasions, was found to be enriched in media with added silicate, while it was not seen in the respective natural sample (although no requirement for silicate is known for this flagellate).

2. Results

Before we introduce the new algal culture media, we would like to stress that these media are not suitable for large scale cultivation of algae in commercial applications because some components are too cost intensive, such as ultrapure water, buffers, chelators, and ACS-grade chemicals (especially sodium chloride for marine culture media). For many commercial applications, various types of wastewaters, natural seawater, commercial fertilizers, and pH buffering by carbon dioxide are to be preferred [5–12]. Although a great variety of different algae can be grown in the proposed culture media, some specialists may require additional modifications in the composition of the culture media that essentially result in new formulations. The widely used cyanobacterium *Limnospira* (*Arthrospira*) *platensis*, for example, does not grow sustainably in the three culture media introduced here.

In reviewing the genesis of the three algal culture media, we were amazed to see that most culture media currently in use (as summarized in [1]) are modifications of earlier versions, which, in turn, derive from even older formulations dating back to the pioneers of algal cultivation. The history of algal cultivation since Beijerinck and Miquel [13,14] has been aptly summarized [15]. It must be recognized that those working with algal cultures today are “standing on the shoulders of giants”. The trailblazers of algal cultivation include, among others, Pringsheim, Vischer, Bold, Provasoli, and Guillard.

2.1. ASP-MEL

This synthetic seawater medium is used for the cultivation of marine algae and was originally derived from the ASP-series of artificial seawater media developed by Provasoli and colleagues [16], more specifically the ASP12 culture medium [17,18]. ASP12 more closely approximates seawater with respect to salinity and Calcium and Magnesium concentrations than other ASP media. The first modification of an ASP medium in the authors’ laboratory took place in 1986, when Tris buffer (Tris(hydroxymethyl)aminomethane) was replaced by HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (ASP2 became ASP-H; [19]). When enriched media based on natural seawater (Erdschreiber and f/2 [18] as well as L1 [20]) were phased out in the authors’ laboratory around the turn of the last century, they were replaced by a modified ASP12, which contained HEPES (3 mM) as the pH buffer and the trace element mix of the L1 culture medium [20]. Most recently, K_2CrO_4 was omitted

from the L1 trace element mix to yield the final ASP-MEL culture medium (Table 1). For brackish water algae, the amount of sodium chloride is reduced to 308 mM (ASP18-MEL).

Table 1. ASP-MEL.

Component	Stock Solution	Addition per 1 L Culture Medium	Molar Concentration in Final Medium
HEPES	238.31 g·L^{-1} dH_2O	3 mL	3.00 mM
NaCl		weigh and add 28 g	479.00 mM
KCl	60.00 g·L^{-1} dH_2O	11.6 mL	9.40 mM
$MgSO_4 \cdot 7H_2O$		weigh and add 7 g	28.40 mM
$MgCl_2 \cdot 6H_2O$		weigh and add 4 g	19.70 mM
$CaCl_2 \cdot 2H_2O$	370.00 g·L^{-1} dH_2O	4 mL	10.00 mM
$NaNO_3$	100.30 g·L^{-1} dH_2O	1 mL	1.18 mM
$K_3PO_4 \cdot 3H_2O$	12.50 g·L^{-1} dH_2O	1 mL	47.00 µM
Na_2-Glycerophosphate	6.85 g·L^{-1} dH_2O	1 mL	31.70 µM
$Na_2SiO_3 \cdot 9H_2O$	28.42 g·L^{-1} dH_2O	5.3 mL	528.00 µM
NTA (Titriplex I)	10.00 g·L^{-1} dH_2O	10 mL	523.00 µM
Trace Elements	See Table 4	1 mL	See Table 4
Vitamins	See Table 5	1 mL	See Table 5

Note: J. McLachlan [18]: Growth media—marine. In: Handbook of Phycological Methods, Culture Methods and Growth Measurements; ed.: Janet R. Stein; pp. 25–51; modified according to McFadden, G.I. [19], Melkonian, M. (1986): Use of Hepes buffer for microalgal culture media and fixation for electron microscopy. Phycologia 25, 551–557. Trace Metals according to L1 culture medium [20] (Guillard & Hargraves 1993; Phycologia 32:234–236), but K_2CrO_4 omitted.

The rationale for the replacement of the trace element mixture of ASP12 (P-II and S-II; [18]) with the trace element mixture of L1 was that the L1 trace element mixture contains several essential trace elements that are not included in ASP12, namely Nickel, Vanadium, and Selenium. In particular, Selenium has long been known to be an essential element for many species of marine phytoplankton [21], particularly diatoms, and recent (phylo)genomic analyses identified a plethora of selenoproteins in algae [22]. In contrast, a biological role for Chromium (especially in its hexavalent form Cr(VI)) is not established for marine microalgae, and Chromium-binding proteins have not yet been identified (although it was recently reported that minor additions of Cr(VI) partially rescued growth of *Ostreococcus tauri* under Fe-limited conditions [23]). Hexavalent Chromium may become difficult to obtain in some countries (e.g., the EU) in the future due to its carcinogenic properties, and since its biological role in algae is uncertain, we decided to omit K_2CrO_4 from the trace element mix of ASP-MEL. Borate is routinely added to many synthetic seawater media, such as the ASP-media [18] and others [24], but is not present in the trace element mix of the L1 medium (because there is sufficient boron in natural seawater). Boron is known to leak from borosilicate glass over a longer time [25], e.g., from stock media flasks, and could thus be present as a contaminant trace element in ASP-MEL. We did not observe any adverse effect of omitting the addition of borate to ASP-MEL on the marine algae tested with this culture medium. A vitamin mix of four vitamins (Thiamine, Biotin, Vitamin B12, Niacinamide; Table 5) according to Kies [26] is a constituent of the ASP-MEL culture medium.

ASP-MEL (listed as ASP12 modified) has been adopted as the primary culture medium for marine algae by two major public algal culture collections (CCAC and BEA). Overall, more than 1000 strains are held in both collections in ASP-MEL (see Table 6 for an overview of the taxa cultivated).

2.2. SFM

SFM (Synthetic Freshwater Medium) has had a long and convoluted history associated with the authors' affiliations over the past 55 years. Zygnematophyceae were the central focus of research in the group of Horst Drawert during the 1960s–1973s at the University of Hamburg (Germany). The culture medium used at that time for Zygnematophyceae was based on Pringsheim [27] and consisted of five major salts

supplemented with soil extract [28]. In 1972, a trace element mix (derived from the P8 trace elements of the ASP6 artificial seawater medium [17]) was added to the major salts ($FeCl_3$ was replaced by $FeSO_4$, which was included in the trace element mix with EDTA). Soil extract was still used (100 mL/L of culture medium). Algae other than Zygnematophyceae were grown in a synthetic culture medium originally devised by Kessler et al. [29] for species of *Chlorella* and later complemented by the addition of EDTA [30]. The medium contained 11 components, but no vitamins, and the pH was adjusted to 5.5 to cope with the rising pH caused by the consumption of nitrate in the phosphate-buffered medium. This medium was used, e.g., to grow *Microthamnion* and *Fritschiella* [31,32].

In 1977 Kattner and colleagues [33] devised a new simple multi-purpose synthetic culture medium based on three stock solutions ($Ca(NO_3)_2 \cdot 4H_2O$, $MgSO_4 \cdot 7H_2O$, and K_2HPO_4; derived from the "L/10 Medium" of Korn [34] and including a simplified trace element mix from the P-II metals of Provasoli [17], which consisted of EDTA, $FeSO_4 \cdot 7H_2O$, H_3BO_3 and $MnCl_2 \cdot 4H_2O$. The culture medium contained the vitamin mix of Kies [26], although the concentrations of the four vitamins listed were 20-fold higher than those given by Kies. The medium pH was adjusted to 5.5. Kattner et al. [33] used their culture medium to study the chemical composition of the cell wall of *Netrium digitus* (Zygnematophyceae). This culture medium represents the nucleus of the SFM culture medium. It was first modified in 1997 by the addition of 1 mM HEPES as a pH buffer. In 2003, the medium was designated as SFM in the authors' laboratory when it was further modified by replacing the previous simple trace element mix with the trace element mix from L1 [20] and using the stock solution mix of $K_2HPO_4 \cdot 3H_2O$, Na_2CO_3, and $NaNO_3$ from the ASP2 medium [16]. This medium was recently refined by introducing NH_4 as a second source of combined nitrogen (in addition to nitrate), because it was found that many algae (e.g. Euglenophyceae, almost all species of *Closterium* and some other Zygnematophyceae, and other algae) are unable to use nitrate as a nitrogen source (the nitrate concentration was reduced to keep the total N-concentration stable). To approximate N/P ratios optimal for microalgal growth (N/P = 16 [35–37]), phosphate concentrations were recently raised. As in ASP-MEL, Chromium was omitted from the final trace element mix in SFM. The pH is adjusted to 6 (Table 2).

Various modifications of SFM are currently being used in the authors' laboratory: To grow diatoms, SFM is supplemented with silicate (Table 2; SFM + Si). To enable growth of algae, which are sensitive to NH_4, ammonium is omitted, and nitrate is the only nitrogen source (Table 2; SFM – NH_4). The pH may also be raised to 7 (SFM pH 7 and SFM – NH_4 pH 7). Finally, dilution of SFM to half- or quarter strength (1/2 SFM 1/4 SFM) can be employed for algae isolated from oligotrophic environments (e.g., Zygnematophyceae from bogs). The electrical conductivity of SFM is lower (0.245 mS/cm, Table 7) than that of many other freshwater algal culture media.

Table 2. SFM (pH 6 or pH 7).

Component	Stock Solution	Addition per 1 L Culture Medium	Molar Concentration in Final Medium
HEPES	238.31 $g \cdot L^{-1}$ dH_2O	1.0 mL	1.00 mM
$Ca(NO_3)_2 \cdot 4H_2O$	100.00 $g \cdot L^{-1}$ dH_2O	0.5 mL	0.21 mM
$MgSO_4 \cdot 7H_2O$	20.00 $g \cdot L^{-1}$ dH_2O	2.5 mL	0.203 mM
$(NH_4)_2HPO_4$	20.00 $g \cdot L^{-1}$ dH_2O	0.58 mL	87.8 µM
$K_2HPO_4 \cdot 3H_2O$	5.00 $g \cdot L^{-1}$ dH_2O	2.1 mL	46.00 µM
Na_2CO_3	32.00 $g \cdot L^{-1}$ dH_2O	0.6 mL	0.19 mM
$NaNO_3$	50.00 $g \cdot L^{-1}$ dH_2O	0.3 mL	0.176 mM
H_3BO_3	1.00 $g \cdot L^{-1}$ dH_2O	1.0 mL	16.00 µM
Trace Elements	See Table 4	1.0 mL	See Table 4
Vitamins	See Table 5	1.0 mL	See Table 5

Modifications of SFM:

SFM + Si

Component	Stock Solution	Addition per 1 L Culture Medium	Molar Concentration in Final Medium
$Na_2SiO_3 \cdot 9H_2O$	28.42 g·L^{-1} dH_2O	5.0 mL	0.50 mM

SFM with lower phosphate content

Component	Stock Solution	Addition per 1 L Culture Medium	Molar Concentration in Final Medium
$K_2HPO_4 \cdot 3H_2O$	5.00 g·L^{-1} dH_2O	0.6 mL	13.20 µM

SFM with higher nitrate content (and omission of NH_4; SFM – NH_4)

Component	Stock Solution	Addition per 1 L Culture Medium	Molar Concentration in Final Medium
$NaNO_3$	50.00 g·L^{-1} dH_2O	0.6 mL	0.35 mM

SFM with lower phosphate and higher nitrate content (and omission of NH_4)

Component	Stock Solution	Addition per 1 L Culture Medium	Molar Concentration in Final Medium
$K_2HPO_4 \cdot 3H_2O$	5.00 g·L^{-1} dH_2O	0.6 mL	13.20 µM
$NaNO_3$	50.00 g·L^{-1} dH_2O	0.6 ml	0.35 mM

In the genesis of SFM, we see an initial shift from media containing soil extract to culture media, in which soil extract is replaced by a simple trace element mix (initially derived from the P-II metals of the marine ASP-media), and finally by a more complex trace element mix (from the enriched seawater medium L1). The transfer of a complex trace element mix developed for marine algal culture media to synthetic culture media for freshwater/terrestrial algae may be regarded as a major innovative step in the development of our synthetic culture media for all algae.

In the pre-SFM era, the culture medium of Kattner et al. [33], with the addition of HEPES, was used in 2001 for only 17 algal strains in the CCAC (Culture Collection of Algae at the University of Cologne), among them *Tetraselmis cordiformis*, *Limnothrix redekei*, *Cryptomonas* spp., some Zygnematophyceae, and *Pediastrum* spp. In the subsequent 10 years, this number increased only minimally (39 strains in 2012, including some Volvocales, filamentous Zygnematophyceae [*Zygnema*, *Spirogyra*], some dinoflagellates, *Chromulina* and *Monomastix*). Today, the CCAC (Central Collection of Algal Cultures at the University of Duisburg-Essen) holds 52% (3874 strains) of its 7395 algal strains in SFM (with or without NH_4). In the authors' laboratory at the MPIPZ, ~5500 algal strains are currently grown in SFM (pH 6), see Table 6 for an overview of the taxa cultivated.

2.3. W-MEL

The W-MEL culture medium derives from a synthetic culture medium developed originally for Zygnematophyceae by Waris [38,39]. Waris [39] introduced EDTA (to replace citric acid) as a chelator for iron (as $FeSO_4 \cdot 7H_2O$) in algal culture media (it had previously been used as an iron chelator for diverse plants by Jacobson [40]). Waris [39] proposed two culture media containing Fe-EDTA: The MS medium consisted of four major salts (1 mM KNO_3, 0.15 mM $(NH_4)_2HPO_4$, 81.1 µM $MgSO_4 \cdot 7H_2O$, and 0.29 mM $CaSO_4 \cdot 2H_2O$) and 179 µM of the Fe-EDTA complex. The second medium (MXS), in addition, contained a simple trace element mix of six components ($ZnSO_4 \cdot 7H_2O$, $CuCl_2 \cdot 2H_2O$, $(NH_4)_6Mo_7O_{24}$, $MnCl_2$, H_3BO_3,

and $CoCl_2$), very similar to the P8 trace element mix of the ASP6 medium [17]. The MXS medium contained 89.5 µM of the Fe-EDTA complex. The pH for both media was adjusted to 6. Waris [39] reported excellent growth of 13 species of Zygnematophyceae (and of *Eremosphaera viridis*). The MXS culture medium was later modified by Kies [26] by reducing the number of components in the trace element mix to four ($ZnSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, H_3BO_3, and $CoCl_2 \cdot 6H_2O$) and adding 100 mL of soil extract/L of medium (10 g unfertilised garden soil was boiled in 125 mL distilled water for 5 min, centrifuged and extensively filtered until clear). Kies [26] used anhydrous, saturated $CaSO_4$ (instead of $CaSO_4 \cdot 2H_2O$ used by Waris [39]), and the concentration of the Fe-EDTA complex was reduced to 17.9 µM. The pH was adjusted to 5.4. Kies [26] named the medium WEES (in German: Waris Erdextrakt Sauer, translated: Waris soil extract acidic). In 1986, McFadden and Melkonian [19] modified WEES by including HEPES as a pH buffer (at 3 mM) and adjusting the pH of the medium to 7. They renamed this culture medium Waris-H. Waris-H underwent further modifications (without name change) in the authors' laboratory: $CaSO_4 \cdot 2H_2O$ was replaced by $Ca(NO_3)_2 \cdot 4H_2O$ (0.42 mM), the concentration of HEPES was reduced to 1 mM, and the volume of soil extract was reduced to 10 mL/L medium. This medium remained unchanged until 2024, when we omitted soil extract and the trace element mix of Waris-H and added the trace element mix of the L1 culture medium of Guillard and Hargraves [20] instead, but without Chromium. In consequence, the modified Waris-H medium has been renamed W-MEL (Tables 3–5), now a multi-purpose, synthetic culture medium for freshwater and terrestrial algae.

Waris-H is extensively used by two large public algal culture collections (CCAC and BEA [Banco Español de Algas]), but also some other public collections (sometimes listed as WEES). Today, the CCAC grows 36% of its strains in Waris-H (with soil extract), and in the BEA, almost all of their freshwater/terrestrial strains are grown in Waris-H. We anticipate that in the coming years soil extract will be phased out from most, if not all, algal culture media (including Waris-H) in public algal culture collections and replaced by well-designed trace element mixtures (such as the trace element mix of the L1 culture medium). Experiments in the authors' laboratory, performed during the past two years, have shown that many algae currently grown in Waris-H can be equally well grown in W-MEL (including, among others, many euglenophytes and dinoflagellates, but also the cercozoan photosynthetic amoeba *Paulinella chromatophora*). For a list of taxa currently grown in W-MEL (or Waris-H), see Table 6.

Table 3. W-MEL (Waris-H modified: without soil-extract, + L1-trace elements, + H_3BO_3).

Component	Stock Solution	Addition per 1 L Culture Medium	Molar Concentration in Final Medium
HEPES	238.31 g·L^{-1} dH_2O	1 mL	1.00 mM
KNO_3	100.00 g·L^{-1} dH_2O	1 mL	1.00 mM
$MgSO_4 \cdot 7H_2O$	20.00 g·L^{-1} dH_2O	1 mL	81.14 µM
$(NH_4)_2HPO_4$	20.00 g·L^{-1} dH_2O	1 mL	0.151 mM
$Ca(NO_3)_2 \cdot 4H_2O$	100.00 g·L^{-1} dH_2O	1 mL	0.423 mM
H_3BO_3	1.00 g·L^{-1} dH_2O	1 mL	16.22 µM
Trace Elements	See Table 4	1 mL	See Table 4
Vitamins	See Table 5	1 mL	See Table 5

Table 4. Trace Element Stock Solution.

Components	Pre-Stock Solutions	Quantity Added per 1 L of dH_2O	Molar Concentration in Final Culture Medium
$Na_2EDTA \cdot 2H_2O$		4.36 g	11.7 µM
$FeCl_3 \cdot 6H_2O$		3.15 g	11.7 µM
$MnCl_2 \cdot 4H_2O$	180.0 g·L^{-1} dH_2O	1 mL	0.900 µM
$ZnSO_4 \cdot 7H_2O$	22.0 g·L^{-1} dH_2O	1 mL	0.085 µM

$CoCl_2 \cdot 6H_2O$	10.0 g·L^{-1} dH_2O	1 mL	0.042 µM
$CuSO_4 \cdot 5H_2O$	2.45 g·L^{-1} dH_2O	1 mL	0.0098 µM
$Na_2MoO_4 \cdot 2H_2O$	19.9 g·L^{-1} dH_2O	1 mL	0.0822 µM
H_2SeO_3	1.3 g·L^{-1} dH_2O	1 mL	0.100 µM
$NiSO_4 \cdot 6H_2O$	2.7 g·L^{-1} dH_2O	1 mL	0.103 µM
Na_3VO_4	1.84 g·L^{-1} dH_2O	1 mL	0.100 µM

The following components are added one after the other to ca. 950 mL dH_2O, starting with $Na_2EDTA \cdot 2H_2O$. After $Na_2EDTA \cdot 2H_2O$ is completely dissolved, $FeCl_3 \cdot 6H_2O$ is added next. After $FeCl_3 \cdot 6H_2O$ is also fully dissolved, the remaining components (Pre-Stock Solutions) are added. The final volume is brought to 1 L with dH_2O. The Trace Element Stock Solution is aliquoted and autoclaved. Pre-Stock Solutions can be aliquoted and stored frozen at −20 °C.

Table 5. Vitamin Stock Solution.

Components	Pre-Stock Solution: Amount Weighed per 1 L of dH_2O	Molar Concentration in Final Culture Medium
Thiamine-HCl	10 g	0.30 µM
Biotin	100 mg	4.10 nM
Vitamin B_{12}	20 mg	0.15 nM
Niacinamide	10 mg	0.80 nM

At first, a Pre-Stock Solution is prepared. Thiamine-HCl, Biotin, Vitamin B12, and Niacinamide are weighed out, added to ca. 950 mL of dH_2O, and brought to the final volume of 1 L. The pH of this solution is approx. pH 3.2. If there are undissolved particles left, the pH is slightly increased by the addition of e.g., 1N NaOH, dropwise. 10 mL aliquots of the Pre-Stock Solution can be stored at −20 °C and can be used to prepare 1 liter of the final Vitamin Stock solution.

Table 6. List of Algae cultivated in the three media ASP-MEL, SFM, and Waris-H/W-MEL.

Culture Collection	Culture Medium	Class	Number of Genera	Number of Strains
CCAC	ASP-MEL		110	482
		Bacillariophyceae	32	115
		Chlorodendrophyceae	1	84
		Chlorophyceae	5	10
		Chrysophyceae	2	6
		Compsopogonophyceae	1	3
		Cryptophyceae	3	44
		Cyanobacteria	18	47
		Dinophyceae	13	53
		Euglenophyceae	1	5
		Eustigmatophyceae	1	1
		Haptophyceae	4	12
		Mamiellophyceae	2	2
		Nephroselmidophyceae	1	10
		Pelagophyceae	4	10
		Phaeophyceae	4	6
		Prasinophytes-Chlorophyta	5	30
		Rhodophyceae	3	7
		Stylonematophyceae	2	4
		Trebouxiophyceae	2	3
		Ulvophyceae	6	30
CCAC	SFM		187	3874
		Bacillariophyceae	29	121
		Chlorodendrophyceae	1	3
		Chlorokybophyceae	1	5
		Chlorophyceae	28	102
		Chrysophyceae	15	93

		Cryptophyceae	3	53
		Cyanobacteria	25	302
		Dinophyceae	10	31
		Euglenophyceae	8	84
		Eustigmatophyceae	3	4
		Florideophyceae	2	5
		Glaucocystophyceae	1	5
		Haptophyceae	2	4
		Mamiellophyceae	1	4
		Mesostigmatophyceae	1	1
		Phaeophyceae	1	1
		Phaeothamniophyceae	1	2
		Rhodophyceae	1	4
		Trebouxiophyceae	10	53
		Ulvophyceae	2	3
		Xanthophyceae	8	15
		Zygnematophyceae	34	2979
CCAC	Waris-H/W-MEL		245	2646
		Bacillariophyceae	6	9
		Cercozoa	5	14
		Chlorodendrophyceae	2	11
		Chlorokybophyceae	1	3
		Chlorophyceae	86	767
		Chrysophyceae	12	28
		Coleochaetophyceae	2	5
		Cryptophyceae	5	315
		Cyanobacteria	5	8
		Dinophyceae	17	81
		Euglenophyceae	23	278
		Eustigmatophyceae	1	1
		Glaucocystophyceae	3	21
		Haptophyceae	3	5
		Klebsormidiophyceae	1	2
		Mamiellophyceae	1	7
		Mesostigmatophyceae	2	9
		Nephroselmidophyceae	1	8
		Pedinophyceae	1	2
		Phaeosacciophyceae	1	1
		Prasinophytes - Chlorophyta	2	6
		Raphidophyceae	2	3
		Synchromophyceae	1	1
		Trebouxiophyceae	19	192
		Ulvophyceae	3	4
		Xanthophyceae	8	32
		Zygnematophyceae	32	833
MEL-Collection	SFM		107	1580
		Bacillariophyceae	3	6
		Chlorophyceae	33	169
		Chrysophyceae	1	1
		Chlorokybophyceae	1	2
		Coleochaetophyceae	1	3
		Cryptophyceae	1	4

		Cyanobacteria	18	53
		Dinophyceae	2	2
		Euglenophyceae	2	14
		Klebsormidiophyceae	1	15
		Mesostigmatophyceae	1	1
		Trebouxiophyceae	12	95
		Ulvophyceae	2	2
		Xanthophyceae	1	4
		Zygnematophyceae	28	1209
CCAH	ASP-MEL		9	16
		Bacillariophyceae	2	5
		Chlorophyceae	1	1
		Cyanobacteria	3	3
		Heterokontophyta	1	4
		Trebouxiophyceae	1	2
		Ulvophyceae	1	1
CCAH	SFM		3	9
		Chlorophyceae	2	6
		Chrysophyceae	1	3
CCAH	W-MEL		26	47
		Bacillariophyceae	1	1
		Chlorophyceae	15	30
		Cyanobacteria	8	13
		Trebouxiophyceae	1	1
		Ulvophyceae	1	2

Algal classes, number of genera, and number of strains cultivated in ASP-MEL, SFM, and Waris-H/W-MEL in the CCAC, the authors' research collection (MEL collection), and the CCAH (Culture Collection of Algae at Ho Chi Minh City, Vietnam). Strains grown in the three media from other culture collections (BEA, AACC [the Armenian Algal Culture Collection]) and strains that have not yet been added to the MEL collection have not been included in the list.

3. Discussion

Culture media for algae serve different purposes. The primary purpose is to grow the alga in question reliably in an experimental setting outside its natural environment. Many culture media have been devised to grow a specific alga for a specific purpose: (1) to achieve the highest cell densities possible, (2) to obtain the most cost-efficient growth for applications in biotechnology or bioremediation, or (3) to mimic most closely the natural environment in which the alga thrives to study its life history and biology. General-purpose culture media aim to cultivate the broadest taxon sampling possible in a single culture medium. They are often employed in large research collections and public repositories of algae. Because of these different goals, the number of published recipes for algal culture media is enormous, yet their composition is often strikingly similar, reflecting both their genesis from the modification of empirically derived formulations and the generalities of the basic metabolism of photosynthetic organisms, including their requirements for macro- and micronutrients, including vitamins. In fact, vitamin mixes of almost all culture media are basically the same, consisting of 3–4 different vitamins in similar concentrations.

In the book 'Algal Culturing Techniques' [1] recipes for 55 different algal culture media are presented in five categories: synthetic culture media for freshwater/terrestrial algae (26 media), soil water enriched media for freshwater/terrestrial algae (6 media), synthetic seawater media for marine/brackish algae (7 media), enriched natural seawater media for marine/brackish algae (14 media), and soil water enriched natural seawater media (2 media). The list is by no means exhaustive; the medium Waris-H, which is listed in several large public culture collections (UTEX, CCAP, SAG, CCAC, BEA), e.g., has not been included. In fact, the NIES culture collection lists a total number of 113 different culture media for algae (including

modifications). As early as 1973, McLachlan [18] concluded that "Numerous enriched and synthetic media have been formulated, which together with trivial modifications, almost equal the number of investigators". In Andersen's book [1], enriched natural seawater media outnumbered the synthetic seawater media 2:1, whereas for freshwater/terrestrial algae, the opposite is true, synthetic media outnumbered soil water enriched media 4:1. The reason is probably that many trace elements needed by algae, and which are present in natural seawater, became limiting in synthetic media when the purity of water (ultrapure water) as well as that of sodium chloride (ACS grade) improved significantly in recent decades. This required trace element mixes in synthetic culture media to become more complex with new additions. Furthermore, for many laboratories located in coastal areas, it is much more cost-efficient to use natural seawater instead of synthetic seawater.

For freshwater/terrestrial algae, traditionally soil extract (as introduced by Pringsheim [41]) served the same function, providing a source of trace elements, as natural seawater did for marine algae. In consequence, soil extract is still widely used in the preparation of culture media for freshwater/terrestrial algae in public culture collections. Since the types of soil used for the preparation of soil extract vary widely in texture and composition, customers may find it difficult to grow some algae reliably using such soil extract-enriched culture media. Furthermore, for many physiological, biochemical, or molecular analyses, the use of soil extract to grow algae is essentially precluded. This may explain the predominance of synthetic culture media for research on algal model systems such as *Chlamydomonas reinhardtii*, and species of *Chlorella* and *Desmodesmus/Tetradesmus*. These chlorophyte algae apparently have simpler requirements for trace elements than many other freshwater/terrestrial algae. Complex trace element mixes have therefore been introduced into culture media for freshwater/terrestrial algae only relatively recently. As an example, we note that selenium is included in the trace element mix of culture media (as selenite, SeO_3) of only two of the 26 synthetic culture media for freshwater algae listed in [1], namely DY-V [42] and COMBO [43]. Selenite is associated with humic substances in many soil types [44]. The function of soil extract in culture media is still basically unknown, but it has been suggested to be linked to the chelation of trace elements by humic substances. Berges et al. [24] found it necessary to add selenite to the trace elements of their synthetic seawater when the manufacturer of sodium chloride provided a new (and possibly less contaminated) brand.

In our laboratory, we have taken a bold approach and replaced the soil extract in Waris-H with the trace element mix of the enriched natural seawater medium L1 (in W-MEL). Now all three culture media used in our laboratory have the same trace element mix, simplifying preparation of the different media. We hypothesize that Selenium is the element that renders soil extract beneficial to algal growth, and the addition of Selenium to the trace element mix makes soil extract largely superfluous.

Although the trace element mix in synthetic algal culture media now converges to a rather uniform composition, the choice of macronutrients and particularly the amount used in various synthetic culture media for freshwater/terrestrial algae still differs considerably, as exemplified by the electrical conductivity of selected media (Table 7).

Table 7. Electrical conductivity of selected marine and freshwater algal culture media.

Culture Medium	Conductivity after Autoclaving (mS/cm)
ASP-H	37.60
ASP-MEL	54.60
ASP18-MEL	41.40
ESM	50.40
f/2 – Si	50.20
L1	55.50
SFM	0.245
SFM – NH_4 *	0.194

SFM + NH_4 *	0.222
Waris-H	0.323
W-MEL	0.404
BG11-H	2.27
3N-BBM + 3V	1.55
BBM + 3V	0.80
DY-V	0.326
M7	0.255

* Phosphate-content: $K_2HPO_4 \cdot 3H_2O$ (13.2 µM).

BG11-H (a BG11 culture medium to which HEPES has been added as a pH buffer) and 3N BBM + 3V are high electrical conductivity media (2.27 and 1.55 mS/cm, respectively) that are optimized to achieve very high cell densities (in those algae that can tolerate these high salt concentrations), whereas in the general-purpose synthetic algal culture media the electrical conductivity ranges from 0.194 (SFM – NH_4 and 13.2 µM phosphate) to 0.404 mS/cm (W-MEL). Electrical conductivity in many oligotrophic freshwater environments (especially bogs [10–50 µS/cm]) is considerably lower than in the general-purpose synthetic culture media, and the electrical conductivity of the media represents a compromise between adjustment to natural conditions and the necessity to avoid too frequent transfers when nutrients become limiting. If necessary, appropriate dilutions of the culture media (1/2 or 1/4 strength) can be made to foster isolation of algae from low-conductivity environments or to preserve the species-specific morphology of the respective strain.

Does one need two general-purpose synthetic culture media for freshwater/terrestrial algae (SFM and W-MEL)? Probably not. Since W-MEL was only introduced in 2024, the comparison between the two media with respect to the diversity of algae that can be grown in them has only started. We have grown 500 strains of algae in both media in microplates, and after 4 months, evaluated growth macroscopically.

After 4 months, in general, the growth of algal strains was very similar in both media. In W-MEL, cell density was often somewhat higher, not surprisingly, given that the nitrogen content in W-MEL is about twice that of SFM (the phosphorus content is very similar in both media). We found a few algae that would grow well in W-MEL but not in SFM (and vice versa). This could be related to further differences between the two media: the Calcium content in W-MEL is twice that of SFM, as is the K+Na concentration (W-MEL has much more potassium compared to sodium, while in SFM it is the reverse). On the contrary, in SFM, the contents of sulfur and magnesium are twice those of W-MEL. At this interim stage of the development of general-purpose culture media for freshwater/terrestrial algae, we recommend growing algae with a higher Calcium requirement (such as flagellates) in W-MEL, while using SFM for algae thriving in low nutrient conditions in the environment.

Can we grow all algae in just two or three culture media (with modifications)? Probably not. We have already referred to *Limnospira platensis*. Some algae traditionally kept in soil water tubes, such as the large-celled species of *Euglena* (e.g., *E. ehrenbergii*), have defied transfer to synthetic culture media until now (although this does not necessarily relate to the chemical composition of the culture media). Other algae that require special environmental conditions, such as very low pH, can still be grown at moderately acidic or even pH neutral conditions, albeit sub-optimally (e.g., *Euglena mutabilis*). A different situation exists for algae that are mixotrophic (osmotrophic or phagotrophic). Here, appropriate organic substances or food particles (such as bacteria or eukaryotic organisms) must be provided (the chemical composition of the culture medium is then often of less importance). We also stress that the growth of marine macroalgae has not yet been extensively tested in ASP-MEL, and the trace element composition of this medium may have to be further modified to accommodate them.

4. Conclusions

In conclusion, this communication presents three new/modified synthetic algal culture media that have been used to grow ~12,000 diverse strains of algae in several public culture collections and the authors' research collection. In our estimation, there is no need anymore to use soil extract in algal culture media.

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Author Contributions

Both authors (B.M. and M.M.) contributed equally to this manuscript and the research described therein. Conceptualization, B.M. and M.M.; Methodology, B.M. and M.M.; Software, B.M. and M.M.; Validation, B.M. and M.M.; Formal Analysis, B.M. and M.M.; Investigation, B.M. and M.M.; Resources, B.M. and M.M.; Data Curation, B.M. and M.M.; Writing, B.M. and M.M.; Project Administration, B.M. and M.M.; Funding Acquisition, M.M.

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All research data linked to this manuscript are included in the manuscript. For further information contact the corresponding author.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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