Management and monitoring of microalgal & cyanobacterial master stock-cultures

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Additional Notes

This SOP is based on the experience of managing algal master stock-cultures in CCAP, which has been based at SAMS since 1986, and the author's experience working in the algal biotech sector. The author would like to acknowledge the invaluable contributions of current and former CCAP staff, affiliated researchers and students.

Apparatus: Incubators with controlled light/dark cycle; a class I biological safety cabinet; database, or hard-copy equivalent; capacity to monitor and document environmental data; facilities and equipment to assess culture "stability & functionality".

Additional information:

CCAP Knowledgebase: *www.ccap.ac.uk/knowledgebase*

World Federation for Culture Collections *(2010) Guidelines for the establishment and operation of collections of cultures of microorganisms. http://www.wfcc.info/guidelines/*

ISO 9001:2008 *Quality management systems. www.iso.org/iso/iso_9000*

Day JG and Stacey GN *(2008) Biobanking. Molecular Biotechnology 40, 202-213.* ***OECD*** *(2007) OECD Best Practice guide -lines for Biological Resource Centres. www.oecd.org/sti/biotech/38777417.pdf*

Lorenz M, Friedl T and Day JG *(2005) Perpetual maintenance of actively metabolizing microalgal cultures. In: Algal Culturing Techniques. Andersen R.A. (ed.) Academic Press, New York. pp 145-155.*

The most common approach used to conserve microalgal cultures *in vitro* is by their maintenance under controlled environmental conditions. Routine serial sub-culturing is performed using aseptic microbiological technique and involves transferring an inoculum generally from a late log/stationary-phase culture into fresh, pre-sterilised medium. This leads to metabolically active cultures that can be used at short notice. The objective is to retain a healthy, physiologically, morphologically, genetically and functionally representative culture. This SOP lists the key criteria that need to be managed, monitored and documented to ensure the long-term value of biotechnologically relevant microalgae and cyanobacteria.

1. Compliance with legislation. Operations must be carried out safely and compliant with the various legislation and regulations that control the management and exploitation of genetic resources (see Annex 1 for further detail). Additionally, Health and Safety responsibilities associated with the materials stored must be competently assessed, managed and documented to conform to local legislation.

2. Strain designation & documentation.

   As with any biological resource, base-line information needs to be held on the strain. All isolates/strains require a unique identifier, or strain number (whilst taxonomic classification may change as more data and taxonomic understanding accumulate the strain number remains constant). Other data that needs to be held include: hazard status, taxonomic identity (genus & species), geographical origin, environmental origin (terrestrial, ice, freshwater, brackish, marine, hypersaline etc.), date of isolation, name and affiliation of isolator, culture medium preference and cultivation regime. In addition, linkage to other relevant molecular, metabolomic, or functionality data add value to the conserved material. These data can most effectively be held in a data-base, but where small numbers of isolates are held other electronic, or hard-copy, formats may suffice.

3. Methodology used to maintain the material.

   Full details of the culture regime, culture age, density of the inoculum, records of when sub-culturing has been performed, methodology employed to maintain the culture and, if necessary, tests to check axenicity should be detailed in an SOP.

   It is worth noting that the maintenance approaches used to conserve Master stock-cultures are generally different from those to maintain Working stock-cultures. Master stock-cultures are maintained in medium, and under regimes, that maximise the sub-culture/ culture transfer interval. This may include the cultivation of normally planktonic taxa on solid medium, the use of high nutrient medium, lower than optimal temperature and lighting regimes. Master stock-cultures invariably have a significant lag-phase prior to growth being

reactivated. An approach employed in some of the major collections involves an initial period of incubation under optimal environmental light and temperature conditions to re-establish a healthy culture, followed by a prolonged period of the culture being incubated under a relatively low light and low temperature regime. Working stock-cultures need to be physiologically active and ideally have little or no lag-phase on transfer to fresh medium. Therefore these should be maintained separately and under appropriate environmental conditions. Both of these regimes have the potential to select for a sub-population of algae that are not identical to the initial sample.

4. Number of replicate samples and inoculum choice.

The number of replicate samples depend on a number of factors, not least the stability of the individual strain being maintained. In general 2-4 replicate cultures are inoculated, but some workers may choose to generate larger batches.

It is recommended to hold at least three batches of Master stock-cultures; i.e. the most recently inoculated batch of cultures and representatives/ all of the two previous batches.

Historically at the CCAP collection based at SAMS and elsewhere, the decision on the duration of sub-culture interval has been dictated by the longevity/ stability of the strain in culture (generic guidance is available in the EnAlgae SOP on "Maintenance of microalgal master stock-cultures by routine serial transfer"). Normally one would utilise the oldest "healthy", Q/C confirmed (see 6. Quality control), culture as the inoculum. Two alternative strategies are routinely employed, firstly one culture is selected from the most recent batch as an inoculum source (Fig. 1a), or alternatively using material from the previous batch prior to its discard (Fig. 1b). In either case if the Q/C results are unsatisfactory (e.g. an axenic culture has become contaminated), then a culture from the last batch of material with satisfactory Q/C results should be employed as an inoculum. The latter option (Fig. 1b) has the advantage of providing a back-up stock should the 2nd oldest culture become contaminated or fail in some other way.

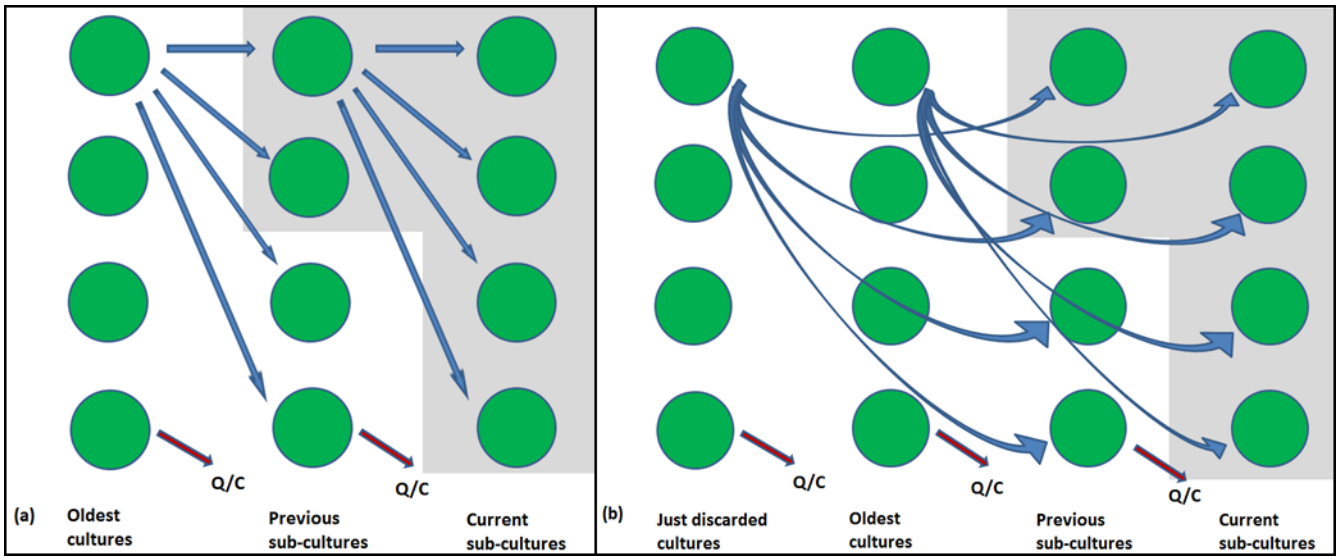

Figure 1 Transfer schedule based on employing a culture from the most recent batch (a); or previous Master stock-culture batch (b)

5. Storage facility.

It is crucial that Master stock-cultures of any individual algal strain are held in more than one incubator and in more than one physical location. For some organisations it may not be practicable to maintain separate sub-batches of the most recent inoculated cultures in separate laboratories; however, as outlined above (Fig. 1 grey boxes) one should maintain these, with exemplar cultures from the previous batch, within the main algal facilities and transfer the older cultures to a separate incubator, off site, or in a different building/ laboratory as an insurance against catastrophic incubator failure.

Where practicable the environmental regime of the incubator(s) should be continuously monitored and recorded. Appropriate alarms and 'failsafe's should be automated (e.g. automatic cut-out of light/ heating if temperature rises >2°C above the pre-set regime). These should be supplemented by routine checks by competent personnel. If automation/ continuous monitoring are not available, then routine checking and documenting the temperature and light regime should be performed.

6. Quality control.

The choice of quality control system needs to be commensurate with the application of the material and for some applications/ processes the maintenance of the organisms will form a component of organisational adherence to Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), ISO 9001, or OECD Best Practice guide-lines for Biological Resource Centres. www.oecd.org/sti/biotech/38777417.pdf). For many practitioners such formal systems may be excessively complex and expensive to administer. However, security of the biological resource, i.e. the algal cultures, is of paramount importance. It is recommended that as a minimum a simple documented system is in place that assesses and records the points listed in Table 1.

Table 1 Q/C issues to be assessed1 and recorded for the maintenance of biotechnologically relevant microalgal & cyanobacterial master stock-cultures

Factor to be assessed	Confirmation procedure	Acceptable level
Strain number	Check labels of both inoculum and receiving culture vessels at actual time of transfer	Checked and confirmed
Strain Identity	Phenotypic characters confirmed by microscopy, or molecular bar code	Checked and confirmed
Medium, dates	Check labels of both inoculum and receiving culture vessels at actual time of transfer	Checked and confirmed
Culture status/ axenicity	Microscopy (phase contrast and/or DAPI staining) and axenicity testing using appropriate selective media	Bacteria and fungus-free for axenic strains and for non-axenic samples a pre-agreed acceptable level of commensal organisms
Functionality	Cell density measurements to track growth/metabolite production Confirmation that the trait of biotechnological relevance, e.g. metabolite production, is retained	Confirmation of a documented performance standard e.g. product yield, growth rate, enzymatic specific rate or gene function
Genotypic stability	Whole genome assessment by sequencing or AFLP. Alternatively, presence and function of key gene(s) of biotechnological relevance	Confirmation of a documented performance standard

[1]It may not be practicable to perform all assessments at each transfer; therefore, an acceptable standard must be agreed for each strain.

7. Labelling minimum standards.

It is essential that the culture vessels are appropriately labelled; they should as a minimum have: a strain number, the taxonomic name (if available), the culture medium name and the date of inoculation. Clarity of hand-writing is critical, as is the use of indelible marker pens; the use of pre-printed labels is advised. Modern methods such as the use of Barcoding allow automated and human-error-free management of the samples, but have the disadvantage of not having the possibility to instantly check details without access to a mobile scanner. Irrespective of the labelling approach employed the connection of the culture to a database that allows retention of all relevant data associated with the sample is critical for Q/C and management of the conserved material.

8. Sample man Management and monitoring of microalgal & cyanobacterial master stock-cultures agement and stock control.

   The management and monitoring of samples is critical to ensuring best practice. As samples are added, transferred or removed/discarded they should be simultaneously logged into the database or a hard-copy equivalent. It is also extremely important to ensure that Master stock-cultures experience the minimum possible level of disruption i.e. do not co-locate them with experimental materials or field samples. Where possible have dedicated incubator(s), or incubator space, for the stock-cultures.

   Note that discarded cultures should always be destroyed by sterilisation, such as autoclaving, before disposal.

9. Audit trail.

   It is important to ensure that all movements and manipulations of the cultures are monitored and logged. This includes who undertook the manipulation, what was done and where any individual culture vessel has been located over the duration of its storage. Also, for all microscopic examinations notes, and if practicable photomicrographs, should be recorded describing the appearance of the cultures.

10. Management responsibilities and training.

    Clear management responsibilities are a key pre-requisite to the maintenance of high standards. Only those who have been trained should have access rights to the cultures themselves and the management database/hard-copy documents. Wherever practicable all key changes and manipulations should be verified by a second, competent individual.

    Each individual should have the responsibility of keeping a record of their training records.

11. Emergency plan.

    It is important to ensure that there is a clear emergency plan in place to deal with any catastrophic failure in the incubators/ constant temperature rooms housing the Master stock-cultures. Routine visual inspection, in addition to any remote monitoring and alarm systems, is a pre-requisite. Clear lines of responsibility and communication are needed as is coverage over weekends and public holidays. An alternative lab/incubator(s) where samples can be relocated if there should be catastrophic equipment failure is also a pre-requisite.

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Annex 1: Additional information on regulations relevant to the commercial exploitation of microalgae & cyanobacteria

Action	Requirement	Law, Regulation, Convention	Further information
Collecting in the field	Prior Informed consent from a recognised authority	Convention on Biological Diversity (CBD)	http://www.cbd.int
	Mutually agreed terms	Convention on Biological Diversity (CBD)	http://www.cbd.int http://www.cbd.int/abs/instruments/
	Consent from the land owner	Property law	
Import	Non-indigenous plant pathogens require licenses from country authority	Quarantine regulations	
	Human, animal and plant pathogens can often only be imported to specified laboratories	Health and Safety	
Handling: Manipulation; Growth	Containment dependent on hazard	Control of Biological Agents - Health and Safety EC Directive 2000/54/EEC on Biological Agents	http://eur-op.eu.int/opnews/395/en/r3633.html
Genetic manipulation	Containment of manipulated organisms	EEC Directives 90/219/EEC. Contained use of genetically modified microorganisms (GMO's), *L117 Volume 33, 8 May 1990. EEC Directives 90/220/EEC. Release of GMO's, *L117 Volume 33, 8 May 1990. Cartagena Protocol on Biosafety	http://www.biodiv.org/biosafety/protocol.asp http://biosafety.ihe.be/Menu/BiosEur1.html http://biosafety.ihe.be/Menu/BiosEur1.html
Deposit as part of a patent process	Long-term storage and compliance with the Budapest Treaty	Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for the Purposes of Patent Procedure	http://www.wipo.int/treaties/en/registration/budapest/
Storage	Appropriate containment	Health and Safety Licence to hold pathogens Security	
Export to another country	Some plant and animal pathogens require export licences	Quarantine regulations	
	Dangerous organisms with potential for dual use	Export Licences for dangerous organisms, Biological and Toxin Weapons Convention (BTWC)	http://binas.unido.org/binas/regs.php3 http://www.opcw.nl/fact/rel_conv.htm http://www.dfat.gov.au/isecurity/pd/pd_4_96/pd9.html
Distribution	Packaging and transport considerations	IATA Dangerous Goods Regulations (DGR), Universal Postal Union Convention (UPU) United Nations Sub-Committee of Experts on the Transport of Dangerous Goods (UNSCETDG)	http://www.iata.org/cargo/dg/dgr.htm http://www.upu.int/ http://www.unece.org/trans/danger/danger.htm
	Sovereign rights over the strains	Convention on Biological Diversity	http://www.cbd.int
	Access and benefit sharing	Bonn Guidelines	http://www.cbd.int
	Intellectual Property Right ownership Customer licensed to receive organism?	Copyright	http://www.wipo.org
	Dangerous organisms export	EU Council Regulation 3381/94/EEC on the Control of Exports of Dual-Use Goods from the Community	http://eur-op.eu.int/opnews/395/en/r3633.html See national Export Offices