

## PEPCY PRACTICAL GUIDELINES

**TITLE:** Extraction and crude fractionation of extracts from lyophilized cyanobacterial cells for bioassays

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**PPG ID:** PPG\_UnivKon002 | **DATE:** 21/06/05 | **PAGE:** 1 of 3 | **REVISION LEVEL:** 1

### 1. PURPOSE

Description of the extraction procedure for potentially bioactive peptides from lyophilised cyanobacterial cells by crude fractionation.

### 2. INTRODUCTION

Crude cyanobacterial cell extracts are frequently described as producing effects in toxicity testing in addition to those of the previously described cyanobacterial toxins. Determining the factors responsible for these effects from a crude cyanobacterial extract is time consuming and complicated. However, by producing multi-cyanopeptide fractions from cyanobacterial extracts for use in toxicity testing it may be possible to determine whether or not it is the cyanopeptide component which is responsible for the effects.

### 3. REQUIREMENTS

#### Materials

- Lyophilised cyanobacterial cells (stored at -20°C until use)
- SPE C18 columns (e.g. Chromabond, Macherey-Nagel, 3 ml/500 mg)
- Acetic acid
- Methanol
- H<sub>2</sub>O-MQ

#### Equipment

- Speed Vac or lyophilisator (or/ and rotation evaporator)
- Sonication bath
- Centrifuge

### 4. PROCEDURE

#### A. Extraction with acetic acid

1. Weight app. 400 mg lyophilised cyanobacterial material in a 15 ml plastic vial, note the mass as precise your balance allows.
2. Add 10 ml 5% acetic acid in H<sub>2</sub>O-MQ.
3. Sonicate the suspension for 15 minutes or for 2 min with a sonication rod fitting to the centrifuge tube. Keep the sample cool with ice in the sonication bath.
4. Centrifuge the sample with ~3000 g for 15 min.

5. Decant the supernatant, resuspend the pellet by vortexing or with a clean spatula and repeat the extraction procedure from step 2 to 5 one time.
6. Combine all supernatants to a final of 15-20 ml (the volume depends on the expansion of the material when moistened).
7. Keep the extracted pellet for methanolic extraction (see below).

### **B. Solid phase extraction**

1. Condition the solid phase columns (C18 ec) with 6 ml 100% MeOH.
2. Wash the column with 6 ml 100% H<sub>2</sub>O -MQ.
3. Apply the 15-20 ml sample and collect the flow-through as **fraction 1** (very hydrophilic).
4. Wash again with 6 ml H<sub>2</sub>O -MQ.
5. Finally elute moderately hydrophobic compounds (e.g. peptides) with 6 ml 100% MeOH: **fraction 2** (moderately hydrophobic =moderately lipophilic).

### **C. Methanolic extraction**

1. Take the pellet from acetic acid extraction A. When very voluminous a drying step (lyophilisation) may precede the following steps.
2. Add 10 mL of 100% MeOH and resuspend the pellet by vortexing or with a clean spatula.
3. Put the tubes on a shaker or shake manually several times during one hour. A sonification step is not necessary since cells should be already disrupted.
4. Centrifuge and collect the supernatant. Repeat from step C2.
5. Combine all supernatants as **fraction 3** (lipophilic)

### **D. Sample preparation and storage**

All fractions should be completely dry before sending them to partners.

**Fraction 1** best will be dried in a lyophilisator in large (50 mL) centrifuge tubes. Dried fractions 1 can be send in 50 mL centrifuge tubes, no transfer to smaller vials is required.

**Fraction 2** best will be dried in a Speed Vac in 2 mL reaction tubes. Add volumes of 1.5 mL of an individual extract successively to a single tube. Alternatively drying can be done under nitrogen flow at 30°C maximum.

**Fraction 3** best will be dried in a rotary evaporator, redissolved in a small volume of 100% MeOH, transferred (in multiple steps if necessary) to 2 mL reaction tubes, and dried in a Speed Vac. Alternatively drying can be done under nitrogen flow at 30°C maximum.

### **E. Labelling and data to be provided**

All tubes have to be labeled individually and unambiguously. This can be a simple number that should then be specified by some institutes acronym (e.g. TU-01) to avoid the confusion of multiple number for tubes.

Put all information relevant to the tubes in a spreadsheet and send a print-out together with the samples as well as an electronic version via email to Stefan Höger.

As information the following is necessary:

Strain: strain ID, taxon, growth conditions, dry weight; optional: any information available on peptide production, esp. microcystin production

Fraction: number and if necessary any comments on derivations from the protocol

Example table:

tube ID	strain	taxon	dry weight [g]	fraction	comment
Ukon01	PCC7654	Aphanizomenon	0,86	1	
Ukon02	PCC7654	Aphanizomenon	0,86	2	
Ukon03	PCC7654	Aphanizomenon	0,86	3	
Ukon04	TUB054	Nostoc	1,12	1	pellet very gelatinous
etc.					