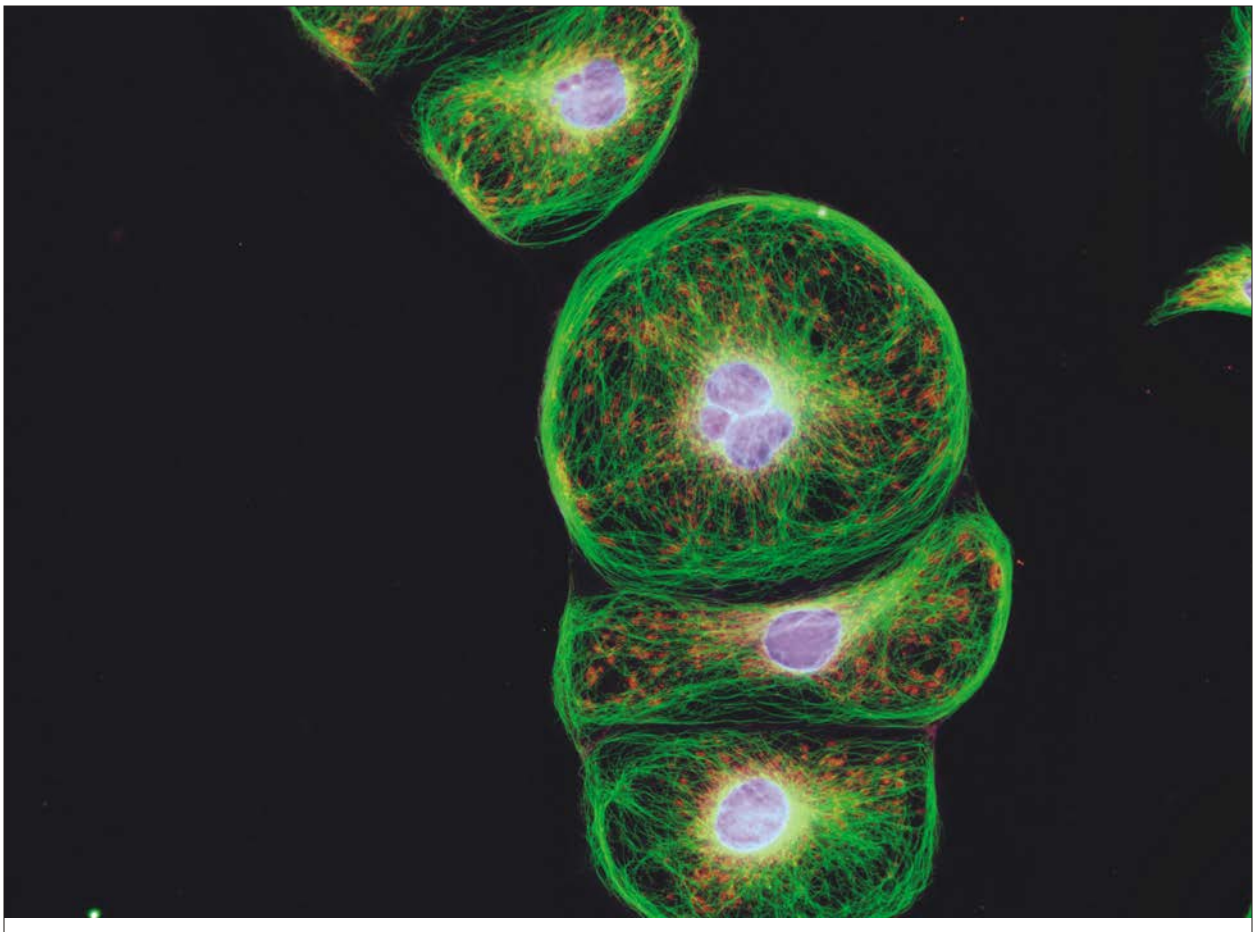

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Sebae Anemone (*Heteractis crispa*) Venom as an Alternative Cell Lysis Buffer Reagent

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ABSTRACT

Sea anemone venoms are rich reservoirs of biologically active proteins which include actinoporins, a large family of lethal pore-forming 20 kDa polypeptides. In this study, venom isolated from leathery sebae anemone *Heteractis crispa*, was examined for presence of actinoporins and its potential to be used as a substitute for commercial cell lysis buffers. Proteome profiling of the venom extract using SDS-PAGE and silver staining revealed proteins with molecular weights 20-215 kDa, indicating the presence of 20 kDa actinoporins among its active proteins. The venom extract was tested for functionality as a stand-alone cell lysis reagent or as a complement to established cell lysis solutions. *Heteractis crispa* venom when used with sodium dodecyl sulfate and proteinase K, demonstrated efficient isolation of highly pure DNA. The isolated nucleic acid samples were run in 1% (w/v) agarose gel and confirmed the absence of nuclease activity throughout all treatments. Taken together, venom isolated from *H. crispa* with detergent and protease additives, was found to be a viable alternative cell lysis reagent used in isolating undamaged genomic DNA with high purity.

Key words: Sea anemone, actinoporin, cell lysis, DNA extraction

INTRODUCTION

Sea anemones are sedentary invertebrates that rely on their venom for capture and digestion of prey, as well as defense against other predators (Sher *et al*., 2005). Their venoms are abundant sources of biologically active polypeptides such as neurotoxins, enzymes and cytolysins (Linder and Bernheimer, 1978). These cytolysins can be classified into four polypeptide groups based on molecular weight that vary per genera. Group I range from 5-8 kDa and reacts with phosphatidylcholine. Group III comprises of lethal cytolytic phospholipases that range from 30-40 kDa while group IV comprises of thiol-activated cytolysins weighing 80 kDa. Group II, comprised of 20 kDa actinoporins, are the highlight of this research due to its pore-forming activity (Anderluh and Macek, 2002).

Actinoporins have been characterized in *H. crispa* in previous studies and are noted for their hemolytic activity (Leichenko *et al*., 2014). Fedorov *et al*. (2010) have reported cytotoxic properties in human tumor lines through the p53 apoptotic pathway. The pore-forming mechanism of actinoporins, through spontaneous insertion into membranes containing sphingomyelin, underlies most of these properties (Bakrac and Anderluh, 2010). The DNA extraction entails the use of reagent buffers with similar cell lysis properties to disrupt the cell membrane and isolate the nucleic acids of interest. However, most of these lysis buffers are health hazards or expensive.

This study investigates the potential of venom isolated from *Heteractis crispa* as an alternative cost-efficient component in DNA extraction protocols. The cytolytic activity of the crude venom extract and its effect on DNA purity in conjunction with proteases and different detergents is examined. The present study examines the prospect of using pore-forming toxins and other similar biological compounds in inducing cell lysis in DNA extraction protocols, as most cell lysis buffers are mediated by the solubilization of the cell membrane and proteins.

MATERIALS AND METHODS

***Heteractis crispa* venom extraction:** Venom extraction followed a modified protocol adopted from Kem *et al.* (1989). The tentacles of the specimen were severed using a sterile blade. Sixty milliliters of cold water were added to 15 g of tentacles placed in a beaker. The solution was homogenized using Heidolph SilentCrusher M Homogenizer for 15 min at room temperature. The homogenate was then centrifuged at 3000 rpm for 20 min at room temperature. The supernatant was collected and stored at 4°C for subsequent use.

Proteomic profiling using SDS-PAGE and silver staining: Crude samples were resolved with (30% T, 2.7% C) SDS-PAGE gel. An equal volume of sample buffer was added to 20 μL of each sample. The samples were boiled at 90°C for 5 min. The gel was loaded 2 μL Sigma-S8320 high range marker and 7.5 μL samples then run for 20 min at 150 V. After electrophoresis, the gel was visualized using silver staining.

Assessment of purity of extracted DNA: The Phenol-Chloroform Method by Sambrook and Russel (2001) was used in isolating DNA from chicken liver tissues. The cells were treated with 340 μL of different cell lysis buffer. The following set-ups were used as positive control: The SDS-based lysis reagent, 340 μL Triton X based lysis reagent, 16:1 (v/v) solution of SDS and proteinase K. The Triton X-100-based lysis reagent was composed of 20 mM EDTA, 10 mM Tris pH 8.0, 200 mM NaCl, 0.2% Triton X-100 and 100 μg mL^{-1} proteinase K. The SDS-based lysis reagent was comprised of 10 mM EDTA, 10 mM Tris pH 8.0, 100 mM NaCl, 0.5% SDS and 100 μg mL^{-1} proteinase K. The following set-ups were used for treatment: *Heteractis crispa* crude venom extract, 16:1 (v/v) solution of *H. crispa* venom and proteinase K, 5:11:1 (v/v) solution of *H. crispa* venom, 10% SDS and proteinase K and a 319:1:20 (v/v) solution of venom, Triton X-100 and proteinase K. These set-ups were used as treatments in order to determine whether sea anemone venom would be a viable substitute or component in commercial cell lysis solutions. The DNA extraction was performed in triplicate for all seven set-ups. Using NanoDrop™ 2000, absorbance values of samples at wavelengths of 260 and 280 nm were obtained. The DNA purity and concentration were calculated based on the absorbance values.

Test for nuclease activity: To evaluate the nuclease activity of the cell lysis buffer reagents, nucleic acid isolates were run on 1% (w/v) Agarose Gel Electrophoresis (AGE).

RESULTS

Proteome profile of *Heteractis crispa* venom: The venom extracted from *H. crispa* yielded nine distinct bands occurring at 21.2, 48.4, 56.6, 66.3, 83.9, 115, 157, 184 and 215 kDa (Fig. 1). The second lane corresponds to the crude venom extract. Proteins associated with each band were compared with proteins isolate from sea anemone venom in related literature.

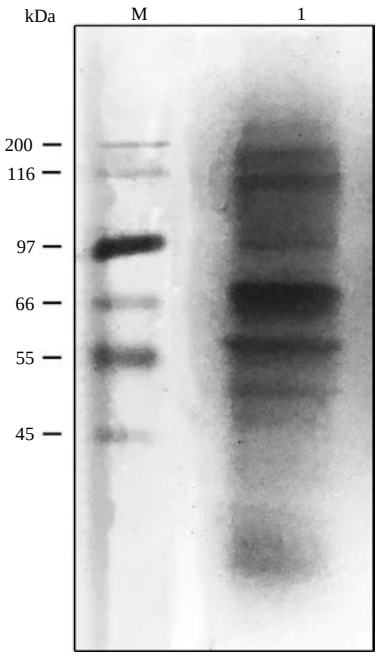

Fig. 1: SDS-PAGE gel profile of *H. crispa* venom. Proteins isolated from *Heteractis crispa* venom were resolved on 12% SDS-PAGE gel and the bands were visualized using silver staining. Lane M: Sigma S8320 Wide Range Marker, Lane 1: *Heteractis crispa* crude venom

Table 1: Average purity and concentrations of DNA samples extracted using different cell lysis agents

Cell lysis agent	DNA purity	DNA conc. (ng μL^{-1})
SDS-based lysis buffer[1]	1.77	2041
Triton X-100-based lysis buffer[2]	1.59	142
10% SDS, proteinase K (16:1)	1.69	1116
H. crispa venom, proteinase K (16:1)	1.69	322
H. crispa venom, 10% SDS, proteinase K (5:11:1)	1.79	2280
H. crispa venom, Triton X-100, proteinase K (319:1:20)	1.27	117
H. crispa venom	1.63	282

[1]10 mM EDTA, 10 mM Tris pH 8.0, 100 mM NaCl, 0.5% SDS, 100 μg mL^{-1} proteinase K, [2]20 mM EDTA, 10 mM Tris pH 8.0, 200 mM NaCl, 0.2% triton X-100, 100 μg mL^{-1} proteinase K

DNA extraction and quantitation: The concentration of the genomic DNA extracted varied considerably based on the cell lysis buffer used. Table 1 shows the average purity and concentrations of the DNA samples isolated with the seven cell lysis reagents. The DNA extracted with a cell lysis buffer composed of 100 μL venom crude extract, 220 μL 10% SDS and 20 μL proteinase K produced the DNA extract with the highest purity and concentration. The DNA extracted with high salt lysis buffer yielded the lowest purity. No significant difference was observed between set-ups except for *H. crispa* venom, Triton X-100, proteinase K (319:1:20) ($p>0.05$).

Test for nuclease activity: The DNA isolates were run in 1% (w/v) agarose gel to check for nuclease activity of the cell lysis buffer treatments. Figure 2 shows the gel profile of the DNA isolates. Undamaged genomic DNA was observed as a distinct band throughout all set-ups and no other banding pattern was observed.

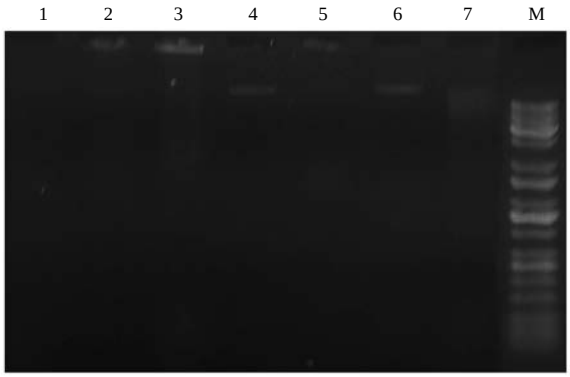

Fig. 2: Electrophoresis of genomic DNA isolated using different cell lysis buffers. Lane 1: SDS-based lysis buffer, Lane 2: Triton X-100-based lysis buffer, Lane 3: 10% SDS, proteinase K (16:1), Lane 4: *H. crispa* Venom, proteinase K (16:1) and Lane 5: *H. crispa* Venom

DISCUSSION

Sea anemone venom contains a plethora of biologically active compounds. Most of the proteins characterized in the venom extracted from *H. crispa* are toxins for immobilizing prey and for warding off predators. Sea anemones produce two types of protein toxins: Neurotoxins which act mainly on ion channels and cytolysins which exhibit lytic activity on a variety of cells through pore formation in the cell membrane (Anderluh and Macek, 2002; Kristan *et al.*, 2004).

In the present study, proteins with molecular weights between 20 and 215 kDa were separated and characterized (Fig. 1). Previously documented proteins from sea anemone have molecular weights similar or close to the characterized proteins of *H. crispa*. Neurotoxic proteins weighing 216, 193 and 103 kDa have been isolated by Veeruraj *et al.* (2008) from *S. mertensii* and *S. haddoni*. Anderluh and Macek (2002) have characterized proteins belonging to a ~55 kDa Sea Anemone MACPF toxin family and ~80 kDa cytolysins from *M. senile* which constitute the type IV toxins. Neurotoxins LdNt1 and LdNt2 weighing 62.50 and 58 kDa, respectively, have been isolated by Sanchez-Rodriguez and Cruz-Vazquez (2006) from *L. danae*. Anderluh *et al.* (2011) have characterized 30-40 kDa cytolytic phospholipases, representing type II cytolysins. Actinoporins which have been previously characterized in *H. crispa* by Tkacheva *et al.* (2011) and Leichenko *et al.* (2014), are the highlights of this present study because of their special cell lysis mechanism.

The concentration and purity of the DNA samples (Table 1) provide criteria for choosing the optimal cell lysis agent for animal tissue. The mechanism of the different cell lysis agents used in this study is reflected in the concentration and purity of the extracted DNA.

Actinoporins are pore-forming toxins profuse in sea anemones. A pocket in the protein recognizes sphingomyelin on the cell membrane. Electrons on the phenol groups of the tyrosine residues in actinoporin interact with the positively charged choline in the phosphocholine molecule of sphingomyelin in actinoporins. The negatively phosphate groups in sphingomyelin stabilize the arginine groups in this actinoporin pocket. After membrane recognition, oligomerization of four monomers of actinoporin and conformational changes in the N-terminal part and the loops occur. Protein-protein contacts are concerned in one segment while the other segment is involved in anchorage. A functional pore is subsequently produced (Bakrac and Anderluh, 2010). Pore-forming

toxins assemble transmembrane pores that induce cell lysis. Cellular regulation of ions and molecules is disrupted upon pore formation. Osmotic regulation is also disturbed which can cause membrane blebbing and lysis (Guerra *et al*., 2011; Vaidyanathan *et al*., 2014).

Sodium dodecyl sulfate and Triton X-100 were used as a detergent additive to solubilize protein and lipid components in the cell membrane which facilitates cell lysis. Also, it denatures contaminating proteins present once the subcellular components have been released. The SDS alone is a strong anionic detergent that can efficiently disrupt the cell membrane resulting in high DNA yield. No significant difference between the purities of the DNA isolated with crude venom extract and the SDS-based lysis reagent was observed ($p>0.05$). Hence, *H. crispa* venom alone can sufficiently isolate DNA from viable cells. The presence of the 20 kDa actinoporin, as confirmed by proteome profiling (Fig. 1), induced pore formation of the cell membranes and subsequently cell lysis. Using crude venom extract without SDS and proteinase K yielded marginally lower DNA purity, since there were no detergents such as SDS to solubilize the cell debris and protein components. However, *H. crispa* venom when added to 10% SDS, proteinase K (5:11:1) lysis buffer, afforded an increase in extraction efficiency yielding DNA with the highest purity (Table 1). The results affirm the lytic activity of actinoporins through pore formation and augments the report of Leichenko *et al.* (2014) describing the hemolytic effects of actinoporins in *H. crispa*.

Degradation or fragmentation of DNA by venom isolated by *H. crispa* was not observed after running the isolates in 1% (w/v) agarose gel (Fig. 2). The results clearly indicate lack of nuclease activity and the high quality of DNA isolated with the prepared lysis buffers.

Heteractis crispa venom was found to be capable of lysis and can be used a viable component in cell lysis buffers used in DNA extraction. Addition of strong anionic detergents like SDS complements its role in cell lysis and aids in increasing DNA purity of the isolates. These results foster the development of novel cell lysis buffer reagents mediated by pore-forming mechanisms.

Purification via gel filtration chromatography is recommended in order to isolate cytolysins. The identity could be confirmed via alignment of the determined amino acid sequence. Further characterization through 2D PAGE and further protein characterization for *H. crispa* venom is suggested to complement the current literature on the species and venom proteome.

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