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FULL LENGTH ARTICLE

One-step detection of matured females using immunochromatography measuring kit for aquaculture of the sea cucumber *Apostichopus japonicus* (Selenka)

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KEYWORDS

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Abstract The present study aimed to develop a gonad-stimulating substance-like peptide (GSSL) immunochromatography measuring kit (GIM-Kit) to detect females of the sea cucumber *Apostichopus japonicus* that are ready for the collection of matured eggs for artificial insemination in hatcheries. *A. japonicus* is the major species consumed in East Asian countries. With regard to the increasing demand for the species in the region, collecting the minimum number of females for hatching is beneficial for sustaining the livestock and increasing the efficiency of breeding. A prototype GIM-Kit was constructed based on the double-antibody sandwich immunosorbent assay technique using two polyclonal antibodies that were generated against a physiologically active AEIDDLAGNIDY amino acid sequence of GSSL and a 40-nm-diameter colloidal gold-tagged antibody. The GIM-Kit detected as little as 80 ng of GSSL in approximately 0.4 g mL^{-1} of ovaries that was diluted in distilled water. The detection time was approximately 20 min in the early (April) to mid (May) breeding season. The immunoreaction of the ovaries was high among the females

Abbreviations: Ab, antibody; APad, absorbent pad; CG-Ab, colloidal gold-tagged anti-GSSL Ab; GIM-Kit, gonad-stimulating substance-like peptide immunochromatography measuring kit; GSSL, gonad-stimulating substance-like peptide; L-Ab, anti-GSSL-L antibody; N-Ab, anti-GSSL-N antibody; SAP, sample absorbent pad.

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during the early to mid-breeding season and low at the end of the season. Thus, the present GIM-Kit is applicable for practical use in hatcheries.

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Introduction

Increasing demand for food production in the world necessitates the development of an efficient and sustainable technology to generate the primary products. Particularly in sea cucumber fisheries, acquiring the maximal amount of matured eggs from the minimal number of animals will contribute to sustainability of fishery resources. Selecting sexually matured females is laborious because of the extremely asynchronous development of the ovary not only seasonally but also ovarian regions in the same females (Smiley and Cloney, 1985; Katow, 2012). Furthermore, identifying matured eggs requires skills in Reproductive Biology and Immunochemistry and the laboratory facilities with a quality that is often unaffordable to the majority of seaside hatcheries. Since male animals are easily identified due to their stable production of sperm (Ahmed et al., 2011), the present study focused on females.

The present study proposes a prototype device for detecting the females that have matured ovaries using the one-step technology of immunochromatographic lateral flow strips. The technology has been applied to detect pathogens in medical sciences (Seto and Gillam, 1994; Depierreux et al., 2000; Watanabe et al., 2001; Thongprachum et al., 2010), agriculture (Lakner et al., 1998; Li et al., 2011; Hua et al., 2012), veterinary medicine (Oh et al., 2006; Waritani et al., 2007) and fisheries (Takahashi et al., 2003; Adams and Thompson, 2008; Gas et al., 2010; Kum and Sekkin, 2011). The technique has been also applied to detect hormones, such as human chorionic gonadotropin in urine, for early detection of pregnancy (May, 1991). Furthermore, the accuracy of the one-step immunochromatography is regarded to be comparable to that of the reverse transcription-polymerase chain reaction tests in detecting respiratory syncytial virus (Kuroiwa et al., 2004). However, no such technique has been applied to aquaculture to date, despite the potential benefits to efficient propagation of fishery resources at hatcheries. In sea cucumber hatcheries, a large number of adults are collected to obtain matured eggs, which are available only from a very small proportion of these animals. If a technique to detect the females that have matured eggs alone was available, the collection of such a large number of adults will not be required.

Gonad-stimulating substance-like peptide (GSSL) is expressed during the breeding season of *Apostichopus japonicus* (Selenka) (Katow et al., 2009). Although GSSL is present in various organs, such as the body wall, warts, radial nerves, testes and ovaries, the gonads alone express the peptide in a manner closely associated with that of the breeding season (from March to June), whereas the other organs express GSSL throughout the year (Ahmed et al., 2011). Thus, GSSL in the gonads is a molecular marker of the animals being in breeding season. The present study aimed at developing a GSSL-immunochromatography measuring kit (GIM-Kit). Two antibodies [anti-GSSL-L antibody (L-Ab) and -N Ab (N-Ab)] were generated, and their specificity in the detection of the gonads in the matured stage has been previously observed (Katow et al., 2009; Ahmed et al., 2011). In this study, we report the successful creation and practical application of a GIM-Kit.

The basic design of the GIM-Kit is similar to known immunochromatography measuring kits (Depierreux et al., 2000; Takahashi et al., 2003; Hua et al., 2012) and is based on the double-antibody sandwich immunosorbent assay technique (Riske et al., 1990; Fig. 1A). The GIM-Kit was constructed on a strip of nitrocellulose membrane that was combined with a sample absorbent pad (SAP) on one end. The sample being applied to the SAP flows laterally in a nitrocellulose membrane to the first colloidal gold-tagged anti-GSSL Ab (CG-Ab)-containing section (CG-Ab). When the sample contains GSSL, a CG-Ab-GSSL complex is formed at the CG-Ab-containing section and trapped at the first test line (T1 line) by the presence of another immobilised anti-GSSL Ab that also binds to the complex through the GSSL epitope by forming a sandwich of two Abs and antigen (Fig. 1B). Thus, the T1 line concentrates the CG-Ab, which is visualised as a red line. The extra T1-line-unbound CG-Abs that does not bind the GSSL is trapped by the next immobilised goat anti-mouse IgG (T2 line) via mouse IgG of the CG-Ab (Fig. 1C). Again, the T2 line was also visualised as a red line by the accumulated CG-Ab, which confirms that the red colour at the T1 line derives from the CG-Ab. Thus, the samples that contain GSSL will be detected by two red lines, whereas the absence of the GSSL is indicated by a single red line at the T2 line region. The two-step detection is necessary to ensure that T1 line is derived from mouse anti-GSSL Ab but not from some non-specific proteins in the sample that occasionally happens in immunoblotting.

Materials and methods

Animals

Adults of the sea cumber *A. japonicus* were used. Green and black body-coloured variants were collected around the Research Center for Marine Biology, Tohoku University, Aomori, Asamushi, Japan, and cultured at 16 °C in a temperature-controlled seawater tank. For the warts analysis, black body-coloured variants were also used. In an attempt to find easier access to detect GSSL in the animal, the body fluid was collected from the body cavity using syringes. The body fluid was stored at −80 °C, vacuum-dried with VD-800F Vacuum Freeze Dryer (TAITEC, Koshigaya, Japan), hydrated with sterilised double distilled water (DDW) to 1/3 of original volume. The sample was filtered through 0.2 μm cellulose acetate filters (MN Sterilizer CA, Macherey-Nagel GmbH & Co. KG, Düren, Germany), desalted with HiTrap Desalting columns (GE Healthcare UK Ltd., Buckinghamshire, UK), and analysed using mouse anti-GSSL antibodies with immunoblotting as described below in the present study.

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