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Research Article

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Hyaluronic acid production of *Streptococcus zooepidermicus* in fish gill washing water

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ABSTRACT

Hyaluronic acid or hyaludronan (HA) was applied in pharmaceutical field. This study was conducted to produce HA by Streptococcus zooepidermicus ATCC 43079 in fish gill washing watered medium. Streptococcus zooepidermicus ATCC 43079 was cultured in fish gill washing water medium and LB medium in 72 h, 96 h and 120 h. HA evaluation in these media was performed based on uronic acid, one monomer of HA by colorimetric method. Molecular weight determination of HA in media was done by using gel permeable chromatography. In the fish gill washing water medium and LB, the maximum of molecular weight was 50.000 Da and 20.000 Da, respectively. The highest HA yield was produced in both media at 120 h. The ratios of HA yield in the fish gill washing water medium and LB are 9.5:15.2. The study showed that fish gill washing water medium could be used to produce HA.

Keywords: *Streptococcus zooepidermicus* ATCC 43079, hyaluronic acid, yield, fish gill washing water medium

INTRODUCTION

Hyaluronic acid or hyaluronan (HA) that isolated HA from cow's vitreous body was detected in 1934 [1]. HA composed of glucuronic acid and N-acetyl-D-glucosamine. Hyaluronic acid has long chain polymer with large size from 5,000 to 20,000.000 Da. Bioavailability of HA was evaluated based on molecular weight (size). HA with low molecular weight (<30000Da) was used in cosmetics and dermatology because it is easy to be absorbed to go deeper into the skin where it can promote collagen creation and aid in tissue repair [2]. The high molecular weight (HA) was applied in medicine such as viscous supplementation for knee [3]. HA can be detected in human's body (bone, cartilage, joint, ligament, skin and hair) and play many different functions in the human's body such as it lubricates the joints, prevents wrinkle. HA was applied in foods, cosmetics and pharmaceuticals. HA was found in cock's combs and human's body with low molecular weigh. However, HA was extracted from cock's comb will get a high risk of infection and high cost. Moreover, HA was not only found in animal and bacteria but also can be found in Japanese sweet potato (starchy roots and tubers). However, HA produced from *Streptococcus* was normal and easy to extract.

Streptococcus zooepidemicus is a gram-positive belonging to Lancefield's group A, grown at 37^{o}C. *Streptococcus zooepidermicus* was isolated from the different kinds of animals such as cow, rabbit, horse and human throat swab [4] that produced HA [5]. *Streptococcus zooepidermicus* has HA synthesizing genes (Has), leading to HA synthesis. Otherwise, this bacterium didn't produce hyaluronidase to destroy HA. Therefore, *Streptococcus zooepidermicus* was used to study for HA production.

HA production by *Streptococcus zooepidemicus* was studied in marine by-products such as media made from mussel processing wastewaters and tuna peptone viscera [6]. Cheese whey was also used to produce HA by *Streptococcus zooepidemicus*. Amino acid requirements were studied on HA-producing *Streptococcus zooepidemicus* [7]. Recently, hydrolyzed and non-hydrolyzed viscera were studied for HA production [8]. However, all those media didn't discuss HA properties.

In the study, HA production from *Streptococcus zooepidemicus* ATCC 43079 was studied in fish gill washing water and compared to Luria broth (LB).

EXPERIMENTAL SECTION

The fish gill washing water preparation

Pour 100 ml of distilled water into 200g of catfish's gill put in the beaker. Shake well and then filter to get the fine fluid. The fluid was collected and divided into test tubes. Each test tube contained 5 ml. All test tubes were auto-claved at 121^{o}C/30 min. Bacterium (10^5 CFU) was added in each tube and incubated for 24 h, 48 h, 96 h, 120 h.

Extraction of hyaluronic acid

Hyaluronic acid was precipitated by method of Rosa [9]. After removing the cell by centrifugation (1000 rpm at 4^{o}C and 5 min), protein was removed by chloroform. The water fraction was added with 1% cetyl pyridinium chloride (CPC) and left the sample for 24 h at 25^{o}C for HA precipitation. HA was collected after centrifuging at 15000 rpm/4^{o}C/15 min. The collected pellet was solubilized with 0.9M $CaCl_2$ before adding absolute ethanol mixture put the sample at 24h in 25^{o}C. Then, centrifugation was done with the speed of 15000 rpm in 5 min at 4^{o}C. Supernatant was discarded and the pellet was washed with 80% of ethanol. The pellet was collected after centrifugation (1000 rpm, 4^{o}C for 4 min) and left at 24h for drying the pellet. The pellet was solubilized in distilled water.

Method for quantitation of uronic acid by spectrophotometer

Glucuronic acid was determined by using carbazole calorimetric reagent [10, 11]. Take out (500µl) of sample and mix with 3ml of sulfuric acid containing borate and boiling the mixture at 100^{o}C for 10 min then put the tube into ice. Then adding 40µl of carbazole (0.1%) to the mixture. The tube was mixed well and heated at 100^{o}C in 10 min. The tube was cooled in ice and measured at 620 nm.

Gel permeable chromatography (GPC)

The HA was dried and resuspended in the distill water and ready for GPC. The column was use in this experiment ultrahydrogel 250µm of Waters and the solvent for running the gel was $NaNO_3$ (0.1N).

RESULTS AND DISCUSSION

Optimization of conditions of *Streptococcus zooepidermicus* ATCC 43079

S. zooepidermicus biomass in each culture was collected after centrifugation and then the biomass was weighed to know whether medium was good or not for growth although biomass didn't relate to HA production. However, if the growth was too weak, HA production was also problematic. The biomass was weighed and recorded as in figure 1.

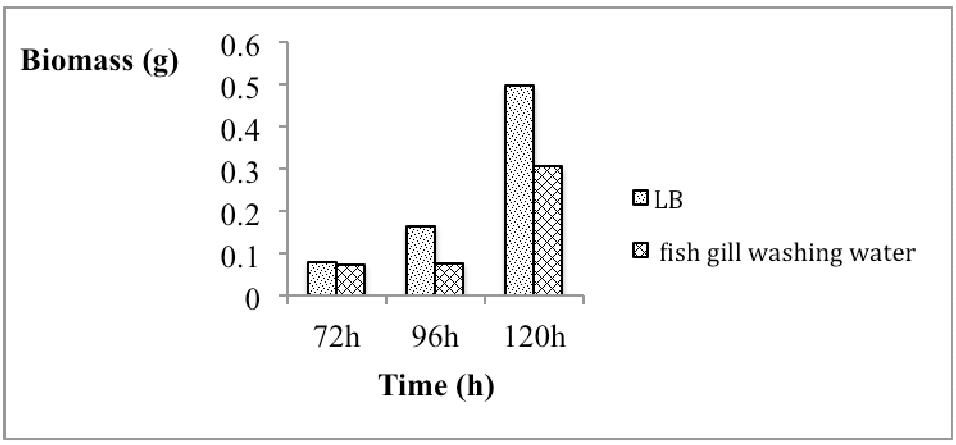

Figure 1: The biomass in media

Evaluation of hyaluronic acid yield

In order to compare the ability of HA yield in different media, uronic acid in HA produced in the media was quanti-fied so that the ratios of HA yield in all media were determined. In table 1, the highest HA was obtained at 120 h. Based on uronic acid measurement, uronic acid or HA produced in LB was higher than fish gill washing water was lower. Generally, the ratios of HA yield in the gill washing medium and LB was 9.5:15.2. Moreover, based on mo-lecular weight of HA produced in media, the HA produced in the fish gill medium had higher molecular weight than LB (Figure 2). In the fish gill washing medium, the molecular weight of the product was to 50.000 Da while the molecular weight of HA collected in LB was to 20.000 Da.

Consequently, medium components involves in not only the yield but also the molecular weight and the application. However, fish gill washing water was very cheap and the limitation of difference in molecular weight of HA in both media, HA produced in the fish gill medium will be used for cosmetics to improve aging.

Table 1: The uronic acid yield in different medium

Sample	Time (hours)	Uronic acid yield (g)
Fish gill washed water	72	4.98 ±0.000382
Fish gill washed water	96	7.96 ±0.000122
Fish gill washed water	120	9.50 ±0.00018
LB medium	72	7.10 ±0.000156
LB medium	96	8.30 ±0.000474
LB medium	120	15.2 ±0.000252

Mean ±Standard deviation. a is mean x10^{-3}

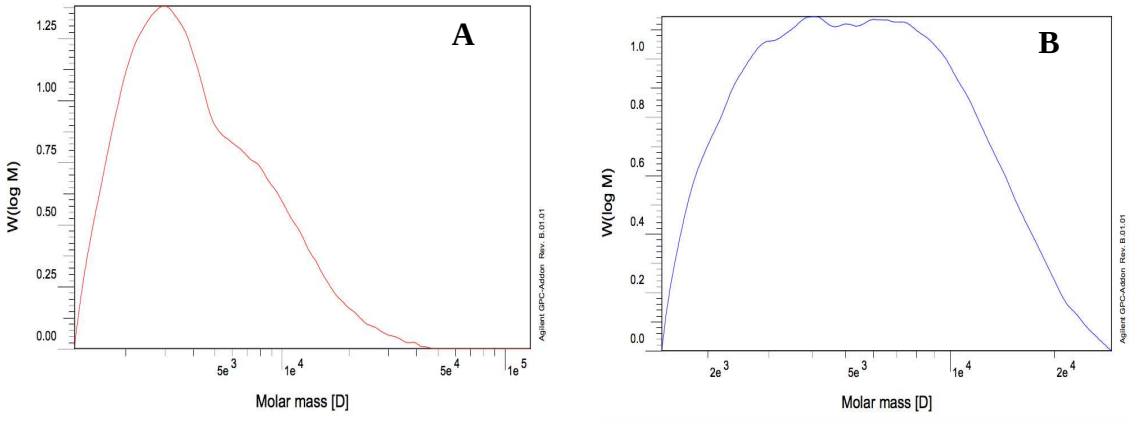

Figure 2: Molecular weight of HA in different media. (A): fish gill washing water; (B): LB

CONCLUSION

Streptococcus zoopeidermicus ATCC 43079 could produce HA in fish gill washing water, leading HA with low cost and acceptable molecular weight for cosmetics and wound recovery.

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