1. As previously mentioned in Section 2.4.1, three fish in each group were anesthetized in an MS-222 (ethyl 3-aminobenzoate methanesulfonate, Sigma) anesthetic bath on each of the sampling dates. The organ tissues (liver, spleen, kidney, and stomach) were then cut and fixed in 10% formalin solution (Formaldehyde solution, 35.0%, SAMCHUN, Korea) for 48 h. An automated tissue processing system (Leica, TP 1020, Germany) was subsequently used to conduct tissue trimming, followed by dehydration from 70% to 100% via serial dilution with ethanol, cleaning using xylene (SAMCHUN, Korea), and tissue embedding with paraffin. Following embedding, the tissues were serially sectioned to a thickness of approximately 5–6 µm using a microtome (Leica, RM 2135, Germany), stained using Hematoxylin and Eosin (H&E), and analyzed using an optical microscope (Leica, DM500, Germany).
2. This study analyzed effect of varying CH doses on the *S. parauberis* 2589 strain, which causes streptococcosis in olive flounder. Our results were obtained through conducting *in vitro* and *in vivo* experiments to determine the optimal dose regimen of CH in *S. parauberis*-infected olive flounder, and they showed that the optimum oral CH dose for treating *S. parauberis* infection was 400 mg/kg fish body weight for seven days. The results suggest that the oral administration of CH in olive flounder affects gram-positive bacteria-induced diseases in clinical trials. We therefore studied the therapeutic efficacy, determined residual profiles, and analyzed sensitivity relating to the oral administration of CH for treating streptococcosis in cultured olive flounder.
3. Of these, β-1,3-1,4-glucanase plays an important role in the degradation of glucan, widely used in brewing and poultry feed industries (Bamforth 2017, Raveendran et al. 2018). Applying biological enzymes in the pulping field is currently a strong focus of scientific investigation, and cellulase, hemicellulase, pectinase, laccase, and lipase are the most commonly used (Tsatsis et al. 2017).

β-1,3-1,4-glucanase sources include bacteria, fungi, and plant endosperm cell walls, and enzymes used in the industrial field are prepared by the submerged fermentation of Bacillus licheniformis. The Carbohydrate-Active Enzymes Database (<http://www.cazy.org/>) divides β-1,3-1,4-glucanase into 9 families, Of these, the β-1,3-1,4-glucanase structure of the GH16, GH17, and GH26 families has been analyzed (Rieder et al. 2015), and the enzymes of the other families are mostly cellulase and bifunctional glucanase. In this study, we selected the wild-type β-1,3-1,4-glucanase (*Plica*) (Cheng et al. 2014), which belongs to the GH16 family; its third-order structure comprises two groups of antiparallel β-lamellae, and its conserved sequence is EIDIGF. However, compared with the conserved sequence EIDIEF of the β-1,3-1,4-glucanase of other GH16 members (Liu et al. 2016), it is missing a glutamic acid site (Cheng et al. 2014). Most of the commercial enzymes currently on the market are acid-resistant and normal temperature enzymes, and these cannot meet the temperature requirements of feed pelleting (65 ℃–90 ℃) and malting (50 ℃–70 ℃). (Tang et al. 2012). Therefore, it would be of great significance to develop stable and heat-resistant glucanase.