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Note

Cloning of phosphoglucomutase gene (*pgm*) in *Streptococcus parauberis*

Sung-Ho Woo, Hyun-Ja Han*, Do-Hyung Kim** and Soo Il Park†

Department of Aquatic Life Medicine, Pukyong National University, Busan 608-737, Korea
**Fish Health Center, Chonnam National University, San96-1, Yeosu 550-749, Korea*
***Department of Aqualife Medicine, Chonnam National University, San96-1, Yeosu 550-749, Korea*

Here, we have cloned and sequenced *Streptococcus parauberis pgm* gene, encoding the enzyme phosphoglucomutase (PGM), which is known to be in association with virulence in other streptococcal species. The PGM of *S. parauberis* is the most closely related to that of *S. iniae* based on their amino acid sequences.

Key words : *Streptococcus parauberis*, *pgm* gene, phosphoglucomutase, virulence

Streptococcicosis is one of major problems in wild and farmed populations of diverse freshwater and marine fishes worldwide (Austin and Austin, 2007). The main pathogenic species responsible for fish streptococcicosis, which have been reported in many different countries are *Streptococcus iniae, Streptococcus parauberis, Streptococcus difficilis, Vagococcus salmoninarum, Lactococcus piscium* and *Lactococcus garviae* (Domeénech *et al*., 1996; Eldar *et al*., 1994; Eldar *et al*., 1996; Zlotkin *et al*., 1998). In particular, *S. iniae* and *S. parauberis* were initially thought to be dominant causative agents in streptococcal diseases of olive flounder (*Paralichthys olivaceus*), a major aquacultured species in Korea. However, *S. parauberis* tends to be as often isolated as *S. iniae* from diseased olive flounder in recent years (Cho *et al*., 2007; Jeong *et al*., 2006). Indeed, initial disease outbreaks caused by *S. parauberis* occurred in Spanish turbot farms between 1993 and 1996 (Toranzo *et al*., 1994; Domeénech *et al*., 1996). Although a vaccine has been available for the disease, Streptococcicosis continues to be endemic in turbot farms (Curras *et al*., 2002). Similarly, streptococcal vaccines available in the Korean market did not seem to confer olive flounder protection against *S. parauberis* infection. Althougn infection route of *S. parauberis* is known to be horizontal through the water or direct contact with infected fish (Romalde *et al*., 1996), virulence factors of this pathogen are still largely unknown. Thus, understanding of pathogenicity of *S. parauberis* is an urgent necessity and it will provide important information for the development of an effective prophylaxis.

The enzyme phosphoglucomutase (PGM) is responsible for the conversion of D-glucose 1-phosphate into D-glucose 6-phosphate, and it participates in both the breakdown and synthesis of glucose. Also, it is known to play an important role in polysaccharide capsule production and pathogenicity in a variety of gram-negative

†Corresponding Author: Soo Il Park, TEL : 051-629-5939,
Fax: 051-629-5938, E-mail : parksi@pknu.ac.kr

and gram-positive bacterial pathogens (Buchanan *et al*., 2005). Phosphoglucomutase gene (*pgm*) of varying streptococci, including *S. iniae* (Buchanan *et al*., 2005), *S. gordonii* (Bizzini *et al*., 2007) and *S. pneumonia* (Hardy *et al*., 2001), has been identified and characterized, suggesting that the PGM plays a critical role in virulence. In particular, the PGM of *S. iniae* is associated with normal cell wall morphology, surface capsule expression, and resistance to innate immune clearance mechanism (Buchanan *et al*., 2005). Here, we describe the nucleotide and deduced amino acid sequence of the *pgm* of *S. parauberis*, and compare with those of other streptococci.

Materials and Methods

S. parauberis JJI51, isolated from the kidney of diseased olive flounder in 2005, was used for the *pgm* cloning. The bacterial strain, identified as *S. parauberis* based on its 16S rRNA gene sequence (GenBank accession number FJ009631), was routinely cultured on Todd-Heweitt agar or in broth at 27℃.

A degenerate primer set for *pgm* (pgmF and pgmR) (Table 1) was designed using conserved regions of the published nucleotide sequences of *S. pyogenes* (Accession number NC_002737), *S. mutans* (Accession number NC_004350) and *S. iniae* (Accession number AY846302). The nucleotide sequence of the complete *pgm* was obtained using the LA PCR in vitro cloning kit (TaKaRa Bio Inc., Otsu, Japan). Primers, pgm5I (inverse primer)/pgm5IN (inverse nested primer) and pgm3I/pgm3IN, were used to amplify the 5' and the 3' end of the *pgm*, respectively. Chromosomal DNA of *S. parauberis*, used as template DNA, was digested with restriction enzymes, EcoRI and PstI, and the DNA fragments were ligated into the corresponding cassettes of the LA PCR in vitro cloning kit. The ligated cassettes were used for the 1st PCR using C1 (provided by the manufacturer) and a pgm3I or a pgm5I primer. After 10 min of initial denaturation at 94℃, the following conditions were applied; 30 cycles of 30 s at 94℃, 30 s at 55℃ and 4 min at 72℃, and 7 min of final extension. The 1st PCR products were used for 2nd PCR using C2 (provided by the manufacturer) and pgm3IN or pgm5IN with the same PCR condition, except for an extension step that was for 2 min at 72℃. Amplification products were analysed by electrophoresis in 1% (w/v) agarose gel containing ethidium bromide (1 ng/㎖). The PCR products, purified with a QIAquick PCR purification kit (Qiagen, Germany) as described by the manufacturer's instructions, were cloned by using *Escherichia coli* DH5α and pGEM-T easy vector (Promega, Madison, WI, USA). Sequencing reactions were carried out using the BigDye terminator v.1 cycle sequencing kit (Applied Biosystems, Foster City, CA), and sequencing analysis was performed using a Prism 310 Genetic Analyzer (Applied Biosystems). DNA sequence identities and homology of amino acid sequence was analyzed by BioEdit Ver. 7.0.9 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The multiple alignment of protein sequences was produced by the Clustal W program (http://www.ddbj.nig.ac.jp/E-mail/clustalw-e.html).

Table 1. PCR primers used for the identification of the *pgm* gene of *Streptococcus parauberis*

Primer	Orientation of sequence	Base sequences (5'-3')
pgmF	Forward	ATGACTTATACAGAAAATTATC
pgmR	Reverse	AGAA(C/T)TTGATTTT(C/T)GG(C/T)TC(G/A/T)G
pgm5I	Reverse	TCAAAGGCAAATTCTGGTGA
pgm5IN	Reverse	TCCTGCACCAATGTAACCAC
pgm3I	Forward	TGCTGAAAGCTACAGTGCAAC
pgm3IN	Forward	GTGATAAAGATGCTATTCAAGCCAG

Results and Discussion

The nucleotide sequence for *S. parauberis pgm* identified in this study has been deposited in GenBank under accession number FJ004970. Multiple alignment of *pgm* (*Streptococcus parauberis*) deduced amino acid sequence with other *pgm* proteins is shown in Fig. 1. DNA sequence identities between *pgm* of *S. parauberis* and those of other streptococcal species are as follows; *S. iniae* (AY846302, 79.1%), *S. mutans* (NC_004350, 77.5%), and *S. pyogenes* (NC_002737, 77.3%). Homology of amino acid sequence of the PGM between four streptococcal species (*S. parauberis*, *S. iniae*, *S. pyogenes*, and *S. mutans*) is shown in Table 2. Of these, the PGM of *S. parauberis* is the most closely related to that of *S. iniae*. Primary structure of the putative *pgm* of *S. parauberis* was analyzed by a SMART architecture research computer program (http://smart.EMBL-heidelberg.de/) (Schultz *et al*., 2000). The gene of *S. parauberis* consists of four domains with c-terminal in its 4th one, which is different from the other streptococcal species composed of only three main domains (I, II and III). It is inferred that domain I, II, III and IV of *S. parauberis* contain the catalytic phosphoserine residue, a metal-binding loop, the sugar-binding loop, and a phosphate-binding site, respectively (Shackelford *et al.*, 2004).

The *pgm* gene of several streptococcal species has been sequenced, some of which show that the gene is in association with virulence. Indeed, a PGM mutant strain of *S. iniae* may have value as a live vaccine as the mutant was able to stimulate an immune response (Buchanan *et al*., 2005). In line with this, *pgm* of *S. parauberis* may have an important role in virulence mechanism. Further study will be required to determine the functional role of the gene.

Table 2. Degree of homology of *Streptococcus parauberis* PGM to other streptococcal PGMs (amino acid sequences)

Bacterial species	vs. *S. parauberis* PGM	
	% identity	% similarity
S. iniae (AY846302)	88.5	92.8
S. pyogenes (NP_664668)	85.5	92.8
S. agalactiae (ZP_00785392)	85.1	92.1
S. mutans (NP_721469)	84.1	90.4

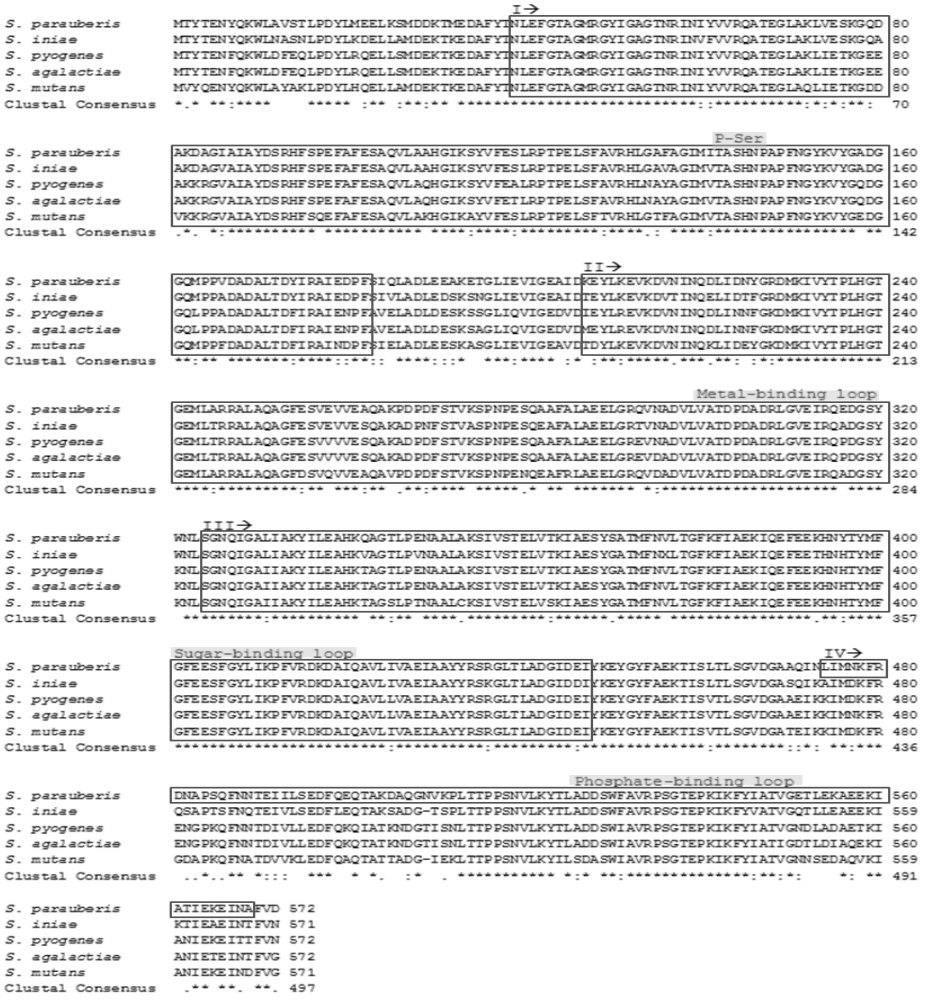

Fig. 1. Multiple alignment of *pgm* (*Streptococcus parauberis*) amino acid sequence with other *pgm* proteins. The putative conserved functional domains of *pgm* of *S. parauberis* are boxed in gray, which are indicated by I, II, III and IV. The residues identical in all sequences are shown with asterisks (*), whereas those with strong homologies and weak similarities are marked by colons (:) and dots (.), respectively. The accession numbers of *pgm* sequences are listed in Table 2.

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