J. Ocean Univ. China (Oceanic and Coastal Sea Research)
https://doi.org/10.1007/s11802-021-4768-2
ISSN 1672-5182, 2021 20 (6): 1505-1514
http://www.ouc.edu.cn/xbywb/
E-mail:xbywb@ouc.edu.cn

Polymorphism in the *Ras* and *β-Glucosidase* Genes and Their Association with Growth Traits in the Pacific Oyster *Crassostrea gigas*

HAN Ziqiang[1)], CONG Rihao[1)], and LI Qi[1), 2), *]

1) *Key Laboratory of Mariculture*, *Ministry of Education*, *Ocean University of China*, *Qingdao* 266003, *China*
2) *Laboratory for Marine Fisheries Science and Food Production Processes*, *Qingdao National Laboratory for Marine Science and Technology*, *Qingdao* 266237, *China*

(Received September 16, 2020; revised December 1, 2020; accepted March 2, 2021)

Abstract The *Ras* gene, a conserved member of the insulin pathway, and *β-glucosidase* gene, an important cellulase, are two important growth-related genes. However, there is no study on the association between mutations of these two genes and growth traits in bivalves. Here, the polymorphism of these two genes in *Crassostrea gigas* were revealed. Their association with growth traits was evaluated in 290 oysters from five families, and was further confirmed in another 186 oysters from three fast-growing strains. Seventeen and twelve SNPs were identified in the *Ras* gene and *β-glucosidase* gene, respectively. Among these SNPs, four SNPs in each gene (*Ras*: C.86C>A, C.90T>C, C.112A>G and C.118G>A; *β-glucosidase*: C.247G>A, C.284C>T, C.1260C>T and C.1293T>C) were significantly ($P<0.05$) associated with the growth of these oysters. Furthermore, eight and nine haplotypes were constructed in the *Ras* gene and *β-glucosidase* gene, respectively. Oysters with both haplotypes R-Hap5 (CCAA) and β-Hap7 (ACCT), or with both R-Hap 6 (ATGG) and β-Hap 6 (ACTC), or with both R-Hap 6 and β-Hap 9 (ACTT), or with both R-Hap 7 (ATAA) and β-Hap 7, showed the highest growth performances. These results provide candidate markers for selecting *C. gigas* with fast growth.

Key words *Crassostrea gigas*; growth; SNP; *Ras*; *β-glucosidase*

1 Introduction

The Pacific oyster (*Crassostrea gigas*), which was once endemic to China, Japan and Korean, is now one of the most widely farmed aquaculture species worldwide due to its strong adaptability (Miossec *et al*., 2009). However, the production and profitability of *C. gigas* has been limited, as world production is mainly based on un-improved populations or stocks (Gjedrem *et al*., 2012; Gjedrem and Rye, 2018). With the development of efficient hatchery technique, many breeding programs of *C. gigas* have been established in many countries (Ward *et al*., 2000; Langdon *et al*., 2003; Dégremont *et al*., 2007; Li *et al*., 2011). Fast growth, as one of the traits that directly affects the production and profitability, is the preferred target trait in almost all breeding programs (Ward *et al*., 2000; Langdon *et al*., 2003; Li *et al*., 2011). Compared with the selection only relying on phenotype, incorporating genome information into selective breeding has become an important aspect to enhance the efficiency of selection (Hollenbeck and Johnston, 2018).

Recently, a lot of candidate genes associated with growth traits have been revealed in scallop (Sun *et al*., 2020), mussel (Prieto *et al*., 2019) and clam (Saavedra *et al*., 2017) with the rapid development of genome resources. Many single nucleotide polymorphisms (SNPs) in these candidate genes, such as *alpha-amylase* gene (Huang *et al*., 2016), *myostatin* gene (Niu *et al*, 2015; Fan *et al*., 2017), long-chain fatty acid-CoA ligases gene (Dai *et al*., 2015), zinc finger transcription factor (Yang *et al*., 2020) and insulin-like growth factor (Feng *et al*., 2014; Ning *et al*., 2018) have been identified associated with growth traits. For *C. gigas*, however, only *amylase* gene (Huvet *et al*., 2008), *bHLH* gene (Chen *et al*., 2020) and a few genes from insulin family (Cong *et al*., 2013, 2014; Moon and Choi, 2019) have been reported to have growth-related mutations. Considering the complexity of the genetic mechanism underling growth traits and the growth performances are affected by a series of processes, including the food acquisition and absorption, energy conversion and distribution, *etc*., it is necessary to further clarify the relationship between more genes and growth and their role in growth, which will be helpful to select *C. gigas* with fast growth.

The insulin family is widely distributed and highly conserved in vertebrates and invertebrates, and plays critical roles in regulating development, reproduction, growth and carbohydrate metabolism (Gricourt *et al*., 2006; Schlueter *et al*., 2007; Zhang and He, 2020). In *C. gigas*, several genes from the insulin-like family, such as insulin recep-

* Corresponding author. E-mail: qili66@ouc.edu.cn

tor-related receptor (Cong *et al*., 2014), insulin-related peptide (Cong *et al*., 2013; Shi *et al*., 2013), insulin growth factor binding protein (Zhang *et al*., 2017; Choi *et al*., 2018), have been identified as being associated with growth traits. *Ras* gene, as a conserved member of the insulin family, plays an important role in regulating cell growth, differentiation and apoptosis (Ciocan *et al*., 2006). It has been cloned and identified to be involved in carcinogenesis in *Mytilus trossulus* (Ciocan *et al*., 2006) and *Mytilus galloprovincialis* (Lima *et al*., 2008). In *C. gigas*, *Ras* gene is mainly expressed in mantle edges and visceral ganglia, and is overexpressed during the tissue rebuilding stage in the storage tissue, which indicates that this gene may be related to the regulation of growth (Jouaux *et al*., 2012).

C. gigas is a filter-feeder whose natural diet is mainly phytoplankton. An important component of phytoplankton is cellulose, which is a macromolecular polysaccharide composed of glucose (Watanabe and Tokuda, 2001). Cellulose needs to be degraded into monosaccharide under the catalysis of cellulase, before it can be absorbed by organisms (Beguin and Aubert, 1994). Cellulase is not only widely distributed in protozoa, bacteria, fungi, and plants, but also in many aquatic invertebrates (Tanimura *et al*., 2013). The *β-glucosidase*, an important cellulase, plays a critical role in the cellulose degradation (Lynd *et al*., 2002). In bivalves, endogenous *β-glucosidase* has been identified in *Corbicula japonica* (Sakamoto *et al*., 2009) and *C. gigas* (Liu, 2012). It is expressed only in the digestive glands, suggesting that it might be related to the digestive and absorption functions (Liu, 2012).

In order to determine whether *Ras* and *β-glucosidase* genes can be used as indicators in breeding program for *C. gigas*, this study explored the polymorphisms of these two genes and analyzed their association with growth traits in different families and fast-growing strains of *C. gigas*.

2 Materials and Methods

2.1 Animals and Traits

In June 2009, 54 full-sib families were established with each male mating to three females, using 80 wild *C. gigas* collected from Rushan Bay, Shandong province, China as parents (Cong *et al*., 2013, 2014). In March 2011, five families (55 to 60 oysters per family) were randomly selected from these 54 families, and 290 oysters were randomly selected from these five families for preliminary association analysis.

Three selected strains of *C. gigas*, strain C, strain J and strain K, were initiated in 2007 in our breeding program targeting at fast growth. The oysters collected from three stocks in Rushan in Shandong province, China (36.4˚N, 121.3˚E), Onagawa Bay in Miyagi Prefecture, Japan (38.3˚N, 141.3˚E), and Pusan, South Korea (35.1˚N, 129.1˚E) as parents, respectively (Li *et al*., 2011). In 2008, the second-generation of these three strains were established with oysters collected from the first-generation of strain C (30 × 31 cross, selection intensity = 1.872), strain J (34 × 36 cross, selection intensity = 1.870) and strain K (30 × 35 cross, selection intensity = 1.728) as parents, respectively (Wang *et al*., 2012). In July 2009, ten females and ten males were selected from each strain as parents to generate the third generation of strain C, strain J and strain K. After fourteen months, 62 oysters were randomly selected from each strain for verifying analysis.

The adductor muscles of 290 oysters from these five families and 186 oysters from these three strains were obtained and stored in −20℃ for extracting DNA. Shell height, shell length and shell width of 476 oysters were measured using an electronic vernier caliper (0.01 mm), and body weight and soft-tissue weight were weighed using an electronic balance (0.01 g).

2.2 DNA Extraction and Primer Design

Genomic DNA of each sample was extracted by using the phenol-chloroform method (Li *et al*., 2002). PCR primers were designed by Primer Premier 5.0 according to the cDNA sequence of *Ras* gene and *β-glucosidase* gene (Table 1). The cDNA sequence of *Ras* gene (1145 bp, see Fig.1) was amplified by primers 5’-CCCGTCCTCATGT ACTGGTC-3’ and 5’-ATCTTGGATACGGCAGGTCA-3’ reported by Jouaux *et al*. (2012), while the cDNA sequence of *β-glucosidase* gene has been reported by Liu (2012).

2.3 SNP Genotyping

A total of 476 oysters were genotyped using the single-strand conformation polymorphism (SSCP) technique and confirmed by random sequencing as described by Cong *et al*. (2014). Briefly, 5 μL of each PCR product was added to 10 μL denaturing buffer (98% formamide, 0.09% xylene cyanole FF, and 0.09% bromophenol blue). These samples were denatured at 94℃ for 5 min and then immediately placed on ice for 10 min. Electrophoresis of the denatured DNA was performed in 8%−12% nondenaturing polyacrylamide gel with 120 V for 12−14 h at 4℃. Finally, SSCP patterns on the gels were visualized by silver staining (Ou *et al*., 2005). In order to confirm these genotypes obtained by SSCP, the ABI 3730 sequencer (Applied Biosystems) was used to sequence more than three individual PCR products with the same SSCP pattern in both directions.

2.4 Association Analysis

The association analysis included the preliminary association analysis between SNPs and growth traits using oysters from families, and verification of growth-related SNPs using oysters from three strains. First, the polymorphisms of both *Ras* gene and *β-glucosidase* gene were analyzed with the parents of five oyster families. According to the polymorphisms of these two genes in the parents, the families that need to be genotyped were determined, and a preliminary association analysis was preformed to screen out potential SNPs associated with growth traits. Then the association between these SNPs and growth traits were verified in three fast-growing strains. Finally, haplotypes based on verified growth-related SNPs were constructed to further verify their correlation with growth traits of *C. gigas*.

Table 1 Primers for analysis of SNPs in the *Ras* and *β-glucosidase* genes in *C. gigas*

Gene	Name	Primer sequence (5' to 3')	Location	Length (bp)	Annealing temp. (℃)
Ras	R2	AACAGCTTCATCAATGTTACAG ATTTCAAGAGTTGCTTGGATA	9-205	197	53
	R4	CACCACCAATCCTATACATAG CTAACCAGGTTTGACTGAAC	557-658	102	56
	R6	TCAAACCTGGTTAGCCTGCTC CCCTCCTTATCTCCACCTCC	645-813	169	60
	R8	GAGGTGGAGATAAGGAGGG TTGGTGTAATTGATAATAAACG	817-1031	215	55
β-glucosidase	G1	GGACGGAACAACGAAATAC TCGGCATACTCTTGGAAAT	189-374	186	54
	G2	CCTTCAATGAGCCCTGGAT GATGTCATGCGACACGATG	419-525	107	57
	G3	TGACATCATCAAAGCCCACG GAGCGAACCAACCAAAGTCGA	519-700	182	51
	G4	GGGTCTGGTTCAGGTTGG TCCGTTACATACACTGGCATA	958-1058	101	58
	G5	ATTCGGCCTGCATTATGTC GCTCCGAGTTTATTAGCATTT	1233-1379	147	55

Fig.1 Distribution of 17 SNPs in the *Ras* gene of *Crassostrea gigas*.

2.5 Statistical Analysis

The association between SNPs and growth traits were analyzed using the general linear model (GLM) procedure of SAS v8.2 (SAS Institute Inc.). The significance of the differences between genotypes or between haplotypes was analyzed by Bonferroni's multiple comparison, and the significance of the differences was $P<0.05$.

The model of ANOVA for the genotype or haplotype of each growth trait is $y_{ij}=\mu+G_i$ or H_i+e_{ij}, where y_{ij} is the observed value of jth individual of genotype or haplotype i; μ is the mean of observed values; G_i is the fixed effects of the genotype i; H_i is the fixed effects of the haplotype i; and e_{ij} is the random residual effect corresponding to the

observed values. No other effects such as generation and site were taken in these analyses, because all the oysters of preliminary association analysis or verified analysis were cultured under the same condition and sampled at the same age.

The allelic frequency, heterozygosity and polymorphism information content (PIC) were calculated using an online software PowerMarker v3.25 (http://statgen.ncsu.edu/powermarker/).

3 Results

3.1 SNP Identification

For *Ras* gene, a total of seventeen SNPs, including C.63C > T, C.86C > A, C.87A > G, C.90T > C, C.92C > A, C.112A > G, C.118G > A, C.625T > A, C.642C > T, C.672G > A, C.675G > A, C.747T > A, C.775T > A, C.786G > A, C.918G > A, C.949G > A and C.953A > G, were detected in the 669 bp coding sequence amplified by these four pairs of primers (Table 1). In addition, twelve SNPs, including C.247G > A, C.270C > A, C.276C > T, C.284C > T, C.288C > T, C.301C > T, C.352G > C, C.1260C > T, C.1272G > A, C.1287G > A, C.1293T > C and C.1301C > T were identified in the 716 bp coding sequence of *β-glucosidase* gene amplified by these five pairs of primers (Table 1).

3.2 Preliminary Association Analysis Between SNPs and Growth Traits

Among the seventeen SNPs locating in *Ras* gene, four SNPs were determined to be significantly ($P<0.05$) related to at least one of the five growth traits by preliminary association analysis in five families (Table 2). At C.86C > A, oysters with genotypes AA or AC were significantly ($P<0.05$) larger than those with genotype CC in shell height, shell length, body weight and soft-tissue weight. Similarly, significant ($P<0.05$) differences in these four growth traits were also observed at C.112A > G and C.118G > A. At C.112A > G, oysters with genotypes AA were significant-

Table 2 Association between SNPs in *Ras* and *β-glucosidase* genes and growth traits in five families of *C. gigas*

	SNP	Genotype	Number	Shell height (mm)	Shell length (mm)	Shell width (mm)	Body weight (g)	Soft-tissue weight (g)
Ras	C.86C > A	AA	97	72.09 ± 10.60^{a}	45.04 ± 6.83^{a}	23.77 ± 4.84^{a}	37.56 ± 14.51^{a}	5.05 ± 2.18^{a}
		AC	70	74.16 ± 9.98^{a}	45.26 ± 5.63^{a}	23.78 ± 4.29^{a}	38.32 ± 12.84^{a}	5.30 ± 2.20^{a}
		CC	45	65.36 ± 8.18^{b}	42.43 ± 5.41^{b}	22.67 ± 4.05^{a}	31.87 ± 10.41^{b}	4.12 ± 1.39^{b}
		P-value		**0.000**	**0.020**	0.310	**0.035**	**0.007**
	C.90T > C	TT	142	70.10 ± 10.30^{b}	44.22 ± 6.51^{a}	23.42 ± 4.62^{a}	37.01 ± 14.14^{a}	4.76 ± 2.01^{a}
		TC	70	74.16 ± 9.98^{a}	45.26 ± 5.63^{a}	23.78 ± 4.29^{a}	38.32 ± 12.84^{a}	5.30 ± 2.20^{a}
		P-value		**0.007**	0.250	0.579	0.178	0.074
	C.112A > G	AA	149	73.30 ± 10.72^{a}	45.24 ± 6.51^{a}	23.86 ± 4.69^{a}	37.65 ± 15.14^{a}	5.22 ± 2.25^{a}
		GA	63	67.02 ± 7.88^{b}	42.94 ± 5.27^{b}	22.79 ± 3.98^{a}	31.80 ± 9.35^{b}	4.27 ± 1.45^{b}
		P-value		**0.000**	**0.014**	0.110	**0.005**	**0.002**
	C.118G > A	GG	97	72.09 ± 10.60^{a}	45.04 ± 6.83^{a}	23.77 ± 4.84^{a}	37.56 ± 14.51^{a}	5.05 ± 2.18^{a}
		GA	70	74.16 ± 9.98^{a}	45.26 ± 5.63^{a}	23.78 ± 4.29^{a}	38.32 ± 12.84^{a}	5.30 ± 2.20^{a}
		AA	45	65.36 ± 8.18^{b}	42.43 ± 5.41^{b}	22.67 ± 4.05^{a}	31.87 ± 10.41^{b}	4.12 ± 1.39^{b}
		P-value		**0.000**	**0.020**	0.310	**0.035**	**0.007**
β-glucosidase	C.247G > A	GG	109	73.40 ± 10.67^{a}	46.69 ± 6.49^{a}	24.33 ± 4.60^{a,b}	41.16 ± 15.10^{a}	5.59 ± 2.07^{a}
		GA	168	69.69 ± 10.01^{b}	43.16 ± 6.16^{b}	23.07 ± 4.05^{b}	32.51 ± 11.59^{b}	4.33 ± 1.74^{b}
		AA	13	66.56 ± 11.73^{b}	46.64 ± 7.77^{a,b}	25.81 ± 5.13^{a}	39.32 ± 24.99^{a,b}	4.85 ± 3.36^{a,b}
		P-value		**0.010**	**0.001**	**0.010**	**0.001**	**0.001**
	C.257A > T	AA	97	73.32 ± 10.34^{a}	47.12 ± 6.19^{a}	24.38 ± 4.64^{a,b}	41.34 ± 14.59^{a}	5.57 ± 1.93^{a}
		AT	167	70.00 ± 10.33^{b}	43.00 ± 6.29^{b}	23.01 ± 3.88^{b}	32.62 ± 12.16^{b}	4.36 ± 1.83^{b}
		TT	26	69.64 ± 11.28^{a,b}	46.01 ± 6.77^{a,b}	25.23 ± 5.50^{a}	38.56 ± 19.82^{a,b}	5.08 ± 2.88^{a,b}
		P-value		**0.040**	**0.001**	**0.007**	**0.001**	**0.001**
	C.270C > A	CC	240	71.92 ± 10.71^{a}	45.04 ± 6.74^{a}	23.84 ± 4.48^{a}	36.87 ± 15.14^{a}	4.97 ± 2.16^{a}
		CA	50	67.02 ± 8.47^{b}	42.87 ± 5.40^{b}	22.86 ± 3.71^{a}	32.21 ± 9.00^{b}	4.18 ± 1.26^{b}
		P-value		**0.003**	**0.040**	0.150	**0.040**	**0.010**
	C.284C > T	CC	262	71.42 ± 10.64^{a}	44.87 ± 6.60^{a}	23.75 ± 4.50^{a}	36.57 ± 12.77^{a}	4.92 ± 2.11^{a}
		CT	28	67.80 ± 8.58^{b}	42.56 ± 6.06^{a}	22.87 ± 2.74^{a}	31.39 ± 8.65^{b}	3.98 ± 1.04^{b}
		P-value		**0.010**	0.070	0.310	**0.030**	**0.020**
	C.1260C > T	CC	184	72.98 ± 9.88^{a}	46.04 ± 6.56^{a}	24.49 ± 4.82^{a}	39.99 ± 12.86^{a}	5.47 ± 2.02^{a}
		CT	28	69.24 ± 9.31^{a}	43.10 ± 6.13^{a}	23.42 ± 3.24^{a}	33.14 ± 9.79^{b}	4.25 ± 1.26^{b}
		TT	17	66.02 ± 8.45^{b}	44.65 ± 5.51^{a}	22.87 ± 4.96^{a}	33.28 ± 9.89^{b}	4.16 ± 1.41^{b}
		P-value		**0.006**	0.060	0.240	**0.020**	**0.005**
	C.1293T > C	TT	75	73.91 ± 10.12^{a}	46.20 ± 6.31^{a}	24.71 ± 5.20^{a}	42.89 ± 13.99^{a}	6.04 ± 2.35^{a}
		TC	90	70.53 ± 8.88^{a}	43.87 ± 6.03^{b}	23.42 ± 3.68^{a}	33.31 ± 9.75^{b}	4.52 ± 1.48^{c}
		CC	64	71.87 ± 10.78^{a}	47.26 ± 6.85^{a}	24.83 ± 5.27^{a}	41.46 ± 13.04^{a}	5.37 ± 1.98^{b}
		P-value		0.090	**0.003**	0.100	**0.001**	**0.001**

Notes: Means with different superscripts within the same column differ significantly at $P<0.05$. Bold values indicate *P*-value < 0.05.

ly ($P<0.05$) larger than those with genotype GA, and oysters with genotypes GG or GA at C.118G>A were significantly ($P<0.05$) larger than those with genotype AA. In addition, at C.90T>C, significant ($P<0.05$) difference was only observed in shell height, in which the oysters with genotype TC were significantly larger than those with genotype TT. As the result, all four growth-related SNPs belong to the same intron.

For *β-glucosidase* gene, six SNPs were determined to be significantly ($P<0.05$) related to at least one of the five growth traits (Table 2). At C.247G>A, oysters with genotype GG were significantly ($P<0.05$) larger than those with genotype GA in shell height, shell length, body weight and soft-tissue weight. Similarly, for these four growth traits, oysters with genotype AA at C.257A>T were significantly ($P<0.05$) larger than those with genotype AT, and oysters with genotype CC in C.270C>A were significantly ($P<0.05$) larger than those with genotype CA. In both C.284C>T and C.1260C>T, oysters with genotype CC showed significantly ($P<0.05$) higher performances in shell height, body weight and soft-tissue weight. In addition, in C.1293T>C, oysters with genotypes TT and CC were significantly ($P<0.05$) larger than those with genotype TC in shell length, total weight and soft-tissue weight. C.247G>A and C.284C>T were nonsynonymous mutations (p.Asp63Asn and Thr75Ile), while C.1260C>T and C.1293T>C were synonymous mutations.

3.3 Verification of Growth-Related SNPs

Similar trends as the abovementioned results were observed in these verified individuals, although there were slight differences in specific traits (Table 3). For *Ras* gene, at C.86C>A, oysters with genotype AA or AC were significantly ($P<0.05$) larger than those with genotype CC only in shell height and soft-tissue weight, and oysters with genotype AA were significantly ($P<0.05$) larger than those with genotype CC in shell weight and body weight, but no significant ($P>0.05$) differences were observed in shell length. At C.112A>G, oysters with genotype AA were significantly ($P<0.05$) larger than those with genotype GA not only in shell height, shell length, body weight and soft-tissue weight, but also in shell width. Similarly, at C.118G>A, oysters with genotypes GG and GA were significantly ($P<0.05$) larger than those with genotype AA in all five growth traits. Moreover, at C.90T>C, no significant ($P>0.05$) difference was observed in shell height, but the oysters with genotype TC were significantly ($P<0.05$) larger than those with genotype TT in soft-tissue weight.

For *β-glucosidase* gene, only four of the six growth-related SNPs were observed significantly related to growth traits in verified individuals (Table 3). At C.247G>A, oysters with genotype AA were significantly ($P<0.05$) larger than those with genotype GA in all five growth traits, while

Table 3 Association between SNPs in *Ras* and *β-glucosidase* genes and growth traits in three strains of *C. gigas*

SNP		Genotype	Number	Shell height (mm)	Shell length (mm)	Shell width (mm)	Body weight (g)	Soft-tissue weight (g)
Ras	C.86C>A	AA	79	58.36±11.91^{a}	35.85±8.62^{a}	20.77±5.95^{a}	22.54±13.49^{a}	2.31±1.19^{a}
		AC	78	55.40±12.00^{a}	33.65±9.09^{a}	19.58±5.36^{a,b}	20.56±13.22^{a,b}	2.18±1.27^{a}
		CC	29	49.59±9.01^{b}	32.63±6.76^{a}	17.43±5.13^{b}	15.72±8.74^{b}	1.64±0.86^{b}
		P-value		**0.001**	0.077	**0.025**	**0.019**	**0.018**
	C.90T>C	TT	157	54.91±12.88^{a}	33.56±7.71^{a}	19.57±5.70^{a}	18.98±11.98^{a}	1.95±1.16^{b}
		TC	29	57.61±12.23^{a}	35.36±8.00^{a}	20.06±5.77^{a}	21.99±12.96^{a}	2.39±1.23^{a}
		P-value		0.147	0.110	0.573	0.114	**0.017**
	C.112A>G	AA	147	57.76±11.48^{a}	36.58±7.39^{a}	20.60±5.53^{a}	22.44±13.27^{a}	2.32±1.23^{a}
		GA	39	48.21±10.29^{b}	32.61±7.23^{b}	16.56±5.08^{b}	13.89±8.59^{b}	1.52±0.81^{b}
		P-value		**0.000**	**0.011**	**0.000**	**0.000**	**0.000**
	C.118G>A	GG	67	55.31±8.92^{b}	33.83±7.09^{a}	19.51±5.19^{a}	18.70±10.53^{b}	1.97±1.00^{b}
		GA	86	60.71±11.37^{a}	36.30±8.16^{a}	20.98±5.54^{a}	24.19±13.37^{a}	2.54±1.29^{a}
		AA	19	39.25±10.57^{c}	27.96±5.69^{b}	15.83±6.68^{b}	12.49±6.11^{c}	1.10±0.56^{c}
		P-value		**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
	C.247G>A	GG	64	55.22±11.03^{b}	34.75±7.56^{a,b}	19.51±5.83^{a,b}	20.57±11.34^{a,b}	2.26±1.21^{a}
		GA	14	45.81±10.16^{c}	30.41±4.94^{b}	16.89±3.33^{b}	12.99±6.36^{b}	1.19±0.61^{b}
		AA	65	61.13±11.13^{a}	36.63±8.04^{a}	21.01±4.24^{a}	24.56±13.82^{a}	2.52±1.23^{a}
		P-value		**0.001**	**0.020**	**0.010**	**0.004**	**0.001**
β-glucosidase	C.284C>T	CC	128	57.46±12.12^{a}	35.66±7.96^{a}	20.17±5.19^{a}	22.45±12.91^{a}	2.36±1.25^{a}
		CT	15	52.91±8.71^{a}	31.08±3.94^{b}	17.88±3.36^{a}	14.74±6.46^{b}	1.52±0.58^{b}
		P-value		0.160	**0.030**	0.090	**0.020**	**0.010**
	C.1260C>T	CC	8	59.86±6.30^{a,b}	36.81±4.01^{a}	21.25±3.59^{ab}	21.43±4.14^{a,b}	2.13±0.63^{a,b}
		CT	85	54.40±11.53^{b}	34.12±7.74^{a}	18.99±4.86^{b}	18.61±11.49^{b}	2.05±1.24^{b}
		TT	40	62.54±8.81^{a}	36.89±6.28^{a}	21.21±4.66^{a}	25.40±13.85^{a}	2.64±1.13^{a}
		P-value		**0.001**	0.110	**0.030**	**0.020**	**0.030**
	C.1293T>C	TT	23	58.43±12.97^{a,b}	38.24±8.33^{a}	21.31±5.15^{a}	24.03±13.94^{a}	2.61±1.27^{a}
		TC	49	53.06±9.56^{b}	31.87±5.38^{b}	17.32±3.75^{b}	14.97±6.94^{b}	1.51±0.67^{b}
		CC	61	60.02±10.73^{a}	38.54±7.21^{a}	25.21±4.71^{a}	24.31±13.35^{a}	2.77±1.24^{a}
		P-value		**0.004**	**0.001**	**0.001**	**0.001**	**0.001**

Notes: Means with different superscripts within the same column differ significantly at $P<0.05$. Bold values indicate *P*-value<0.05.

the performances of oysters with genotype GG were between AA and GA. At C.284C>T, oysters with genotype CC were significantly ($P<0.05$) larger than those with genotype CT in shell length, body weight and soft-tissue weight, while no significant ($P>0.05$) difference was observed in shell height. Notably, at C.1260C>T, the greatest performances were observed in oysters with genotype TT not in those with genotype CC. In addition, at C.1293T>C, oysters with genotypes TT and CC were significantly ($P<0.05$) larger than those with genotype TC not only in shell length, total weight and soft-tissue weight, but also in shell width.

3.4 Construction of Haplotypes and Their Association with Growth Traits

Eight haplotypes were constructed based on four growth-related SNPs (C.86C>A, C.90T>C, C.112A>G and C.118G>A) of *Ras* gene, and these eight haplotypes were significantly ($P<0.05$) associated with all five growth traits (Table 4). For all five growth traits, oysters with the haplotype R-Hap8 (ACAA) had the highest performances, and were significantly ($P<0.05$) larger than those with haplotype R-Hap1 (CTAA) and R-Hap2 (CCAG).

Table 4 Associations between haplotypes in *Ras* gene and growth traits in three strains of *C. gigas*

Haplotypes	Number	86	90	112	118	Shell height (mm)	Shell length (mm)	Shell width (mm)	Body weight (g)	Soft-tissue weight (g)
R-Hap1	43	C	T	A	A	45.86 ± 9.17^{a}	30.09 ± 6.29^{a}	17.53 ± 5.42^{a}	12.71 ± 7.36^{a}	1.34 ± 0.77^{a}
R-Hap2	10	C	C	A	G	$49.25\pm9.52^{a,b}$	30.87 ± 4.64^{a}	16.05 ± 3.57^{a}	12.14 ± 3.29^{a}	$1.50\pm0.34^{a,b}$
R-Hap3	68	A	T	A	G	$54.72\pm10.42^{a,b}$	$33.99\pm6.96^{a,b}$	19.31 ± 5.02^{a}	$18.43\pm10.79^{a,b}$	$1.93\pm1.03^{a,b}$
R-Hap4	6	A	T	G	A	$54.77\pm10.04^{a,b}$	$34.55\pm7.66^{a,b}$	19.09 ± 5.47^{a}	$20.35\pm8.56^{a,b}$	$2.03\pm0.79^{a,b,c}$
R-Hap5	71	C	C	A	A	58.05 ± 11.04^{b}	$35.77\pm8.01^{a,b}$	19.97 ± 5.30^{a}	$23.07\pm12.84^{a,b}$	$2.44\pm1.22^{a,b,c}$
R-Hap6	140	A	T	G	G	58.55 ± 10.23^{b}	$35.36\pm7.94^{a,b}$	$20.54\pm5.34^{a,b}$	$22.40\pm12.64^{a,b}$	$2.35\pm1.23^{a,b,c}$
R-Hap7	16	A	T	A	A	65.16 ± 18.09^{c}	$36.27\pm9.50^{a,b}$	$20.72\pm5.74^{a,b}$	27.57 ± 14.91^{b}	$2.71\pm1.46^{b,c}$
R-Hap8	5	A	C	A	A	66.46 ± 8.51^{c}	39.74 ± 7.36^{b}	25.88 ± 9.79^{b}	29.14 ± 6.86^{b}	3.22 ± 1.15^{c}

For *β-glucosidase gene*, nine haplotypes constructed based on four growth-related SNPs (C.247G>A, C.284C>T, C.1260C>T and C.1293T>C) were significantly ($P<0.05$) associated with all five growth traits (Table 5). Furthermore, oysters with the haplotype β-Hap9 (ACTT) had the highest performances, and were significantly ($P<0.05$) larger than those with other haplotypes.

From the above eight growth-related SNPs from *Ras* and *β-glucosidase* genes, 27 haplotypes significantly ($P<0.05$) associated with four growth traits were constructed (Table 6). Oysters with haplotypes R5_β7, R6_β6, R6_β9 and R7_β7 were significantly ($P<0.05$) higher than those of haplotype R1_β2 in shell height, body weight and soft-tissue weight.

Table 5 Associations between haplotypes in *β-glucosidase* gene and growth traits in three strains of *C. gigas*

Haplotypes	Number	247	284	1260	1293	Shell height (mm)	Shell length (mm)	Shell width (mm)	Body weight (g)	Soft-tissue weight (g)
β-Hap 1	14	A	T	T	C	$51.87\pm6.61^{a,b}$	30.74 ± 4.01^{a}	17.41 ± 3.42^{a}	13.42 ± 5.31^{a}	1.36 ± 0.50^{a}
β-Hap 2	64	G	C	C	T	50.95 ± 10.88^{a}	32.04 ± 6.62^{a}	17.49 ± 4.84^{a}	$15.79\pm8.30^{a,b}$	$1.60\pm0.84^{a,b}$
β-Hap 3	13	G	C	T	T	$53.84\pm11.43^{a,b,c}$	$36.04\pm7.20^{a,b}$	$20.84\pm6.15^{a,b}$	$19.80\pm9.11^{a,b,c}$	$2.29\pm0.91^{b,c}$
β-Hap 4	89	G	C	T	C	$55.89\pm9.84^{a,b,c}$	$33.82\pm7.05^{a,b}$	$19.32\pm5.26^{a,b}$	$20.24\pm11.30^{a,b,c}$	$2.11\pm1.19^{a,b,c}$
β-Hap 5	16	G	C	C	C	$57.90\pm7.67^{a,b,c}$	$36.07\pm7.41^{a,b}$	$19.58\pm5.33^{a,b}$	$20.86\pm10.91^{a,b,c}$	2.56 ± 1.25^{c}
β-Hap 6	83	A	C	T	C	$59.51\pm11.91^{b,c,d}$	$35.54\pm6.89^{a,b}$	$20.15\pm4.15^{a,b}$	$23.02\pm13.58^{b,c}$	$2.37\pm1.14^{b,c}$
β-Hap 7	40	A	C	C	T	$61.64\pm11.25^{c,d}$	$38.16\pm8.83^{b,c}$	$22.12\pm3.70^{b,c}$	25.59 ± 13.11^{c}	2.58 ± 1.21^{c}
β-Hap 8	9	A	C	C	C	$61.72\pm7.64^{c,d}$	$38.22\pm4.74^{b,c}$	23.33 ± 2.78^{c}	28.06 ± 6.69^{c}	2.93 ± 1.34^{c}
β-Hap 9	4	A	C	T	T	67.27 ± 9.80^{d}	42.93 ± 10.36^{c}	23.66 ± 2.62^{c}	36.60 ± 20.23^{d}	3.78 ± 1.36^{d}

Table 6 Associations between haplotypes in *Ras* and *β-glucosidase* genes and growth traits in three strains of *C. gigas*

Haplotypes	Number	Shell height (mm)	Shell length (mm)	Shell width (mm)	Body weight (g)	Soft-tissue weight (g)
R1_β2	11	41.25 ± 8.69^{a}	28.60 ± 6.70^{a}	15.95 ± 6.11^{a}	8.55 ± 4.40^{a}	0.89 ± 0.46^{a}
R1_β4	7	$51.16\pm10.54^{a,b}$	$36.79\pm5.62^{a,b}$	16.11 ± 4.97^{a}	$17.33\pm10.14^{a,b}$	$1.74\pm0.86^{a,b}$
R1_β6	8	$54.50\pm7.22^{a,b}$	$34.45\pm2.67^{a,b}$	19.43 ± 4.90^{a}	$17.84\pm5.82^{a,b}$	$2.36\pm0.40^{a,b}$
R1_β7	3	$44.82\pm1.18^{a,b}$	$28.32\pm2.44^{a,b}$	23.48 ± 5.87^{a}	$13.53\pm5.97^{a,b}$	$1.43\pm0.71^{a,b}$
R2_β2	6	$48.83\pm7.79^{a,b}$	$29.07\pm4.72^{a,b}$	15.76 ± 2.81^{a}	$12.98\pm3.43^{a,b}$	$1.32\pm0.31^{a,b}$
R3_β1	5	$57.19\pm8.79^{a,b}$	$33.11\pm7.36^{a,b}$	17.12 ± 2.68^{a}	$16.30\pm6.51^{a,b}$	$1.48\pm0.58^{a,b}$
R3_β2	16	$52.70\pm8.82^{a,b}$	$32.12\pm6.64^{a,b}$	19.14 ± 5.02^{a}	$15.31\pm5.99^{a,b}$	$1.83\pm0.86^{a,b}$
R3_β4	17	$52.84\pm11.20^{a,b}$	$32.94\pm6.37^{a,b}$	18.94 ± 5.26^{a}	$17.36\pm11.40^{a,b}$	$1.74\pm1.00^{a,b}$
R3_β5	5	$56.74\pm9.39^{a,b}$	$34.49\pm4.42^{a,b}$	19.13 ± 2.71^{a}	$16.18\pm5.46^{a,b}$	$2.32\pm0.54^{a,b}$
R3_β6	8	$55.57\pm11.93^{a,b}$	$35.79\pm6.06^{a,b}$	21.02 ± 6.30^{a}	$23.33\pm18.86^{a,b}$	$2.33\pm1.46^{a,b}$
R3_β7	8	$60.15\pm8.25^{a,b}$	$40.57\pm7.90^{a,b}$	22.75 ± 2.88^{a}	$28.03\pm8.96^{a,b}$	$2.60\pm1.11^{a,b}$
R4_β4	3	$53.08\pm13.77^{a,b}$	$33.23\pm6.09^{a,b}$	15.52 ± 4.74^{a}	$17.67\pm10.56^{a,b}$	$2.13\pm1.16^{a,b}$
R5_β1	4	$55.48\pm7.24^{a,b}$	$31.24\pm4.58^{a,b}$	19.75 ± 1.88^{a}	$16.75\pm4.85^{a,b}$	$1.45\pm0.19^{a,b}$
R5_β2	22	$52.09\pm12.25^{a,b}$	$34.24\pm6.71^{a,b}$	17.59 ± 5.98^{a}	$18.84\pm10.30^{a,b}$	$1.92\pm0.92^{a,b}$
R5_β4	11	$58.37\pm7.18^{a,b}$	$32.39\pm6.24^{a,b}$	21.39 ± 5.63^{a}	$20.70\pm8.02^{a,b}$	$2.15\pm0.81^{a,b}$

(*to be continued*)

(*continued*)

Haplotypes	Number	Shell height (mm)	Shell length (mm)	Shell width (mm)	Body weight (g)	Soft-tissue weight (g)
R5_β5	10	$58.15\pm6.92^{a,b}$	$39.91\pm8.60^{a,b}$	20.78 ± 6.38^{a}	$26.83\pm13.06^{a,b}$	3.27 ± 1.55^{b}
R5_β6	5	63.6 ± 11.20^{b}	$37.93\pm10.12^{a,b}$	22.19 ± 2.57^{a}	$30.68\pm20.83^{a,b}$	$3.12\pm1.27^{a,b}$
R5_β7	10	64.55 ± 13.18^{b}	41.08 ± 9.61^{b}	21.86 ± 3.82^{a}	29.62 ± 16.82^{b}	3.11 ± 1.43^{b}
R6_β2	3	$47.03\pm22.23^{a,b}$	$30.89\pm9.92^{a,b}$	15.57 ± 7.65^{a}	$13.93\pm10.82^{a,b}$	$1.60\pm1.45^{a,b}$
R6_β3	12	$57.05\pm9.58^{a,b}$	$36.6\pm6.58^{a,b}$	22.32 ± 5.77^{a}	$19.82\pm8.28^{a,b}$	$2.44\pm0.78^{a,b}$
R6_β4	44	55.79 ± 8.69^{b}	$34.27\pm7.76^{a,b}$	19.86 ± 5.53^{a}	$21.42\pm12.02^{a,b}$	$2.28\pm1.31^{a,b}$
R6_β6	52	60.29 ± 12.31^{b}	$35.74\pm7.77^{a,b}$	20.82 ± 5.31^{a}	23.52 ± 13.10^{b}	2.38 ± 1.17^{b}
R6_β7	5	$59.92\pm9.30^{a,b}$	$40.09\pm7.51^{a,b}$	23.22 ± 5.18^{a}	$23.72\pm13.03^{a,b}$	$2.48\pm1.26^{a,b}$
R6_β9	5	67.29 ± 17.73^{b}	$43.39\pm13.28^{a,b}$	23.58 ± 2.92^{a}	37.28 ± 22.55^{b}	3.68 ± 2.05^{b}
R7_β2	3	$50.36\pm22.72^{a,b}$	$31.21\pm9.80^{a,b}$	19.16 ± 5.11^{a}	$17.17\pm16.35^{a,b}$	$1.47\pm1.07^{a,b}$
R7_β4	3	72.83 ± 2.65^{b}	$39.35\pm7.08^{a,b}$	21.61 ± 6.69^{a}	$33.33\pm14.77^{a,b}$	$3.57\pm1.84^{a,b}$
R7_β7	4	73.61 ± 8.78^{b}	$42.75\pm7.84^{a,b}$	24.73 ± 3.81^{a}	37.95 ± 5.88^{b}	3.58 ± 0.56^{b}

Note: Haplotype R1_β2 means haplotype R-Hap1 in Table 4 is grouped with β-Hap2 in Table 5, and so on.

3.5 Allelic Frequencies of Eight Growth-Related SNPs in Three Strains

Allelic frequencies of these eight growth-related SNPs were shown in Table 7. The PIC of C.284C>T were from 0.02 to 0.11, while the rest seven SNPs were from 0.26 to 0.37. For C.86C>A and C.284C>T, Hardy-Weinberg equilibrium was observed in all three strains of China, Japan and Korea, while C.90T>C, C.112A>G and C.247G>A did not meet Hardy-Weinberg equilibrium in any of these three strains. In addition, for C.118G>A, Hardy-Weinberg equilibrium was observed in China and Korea strains, but not in Japan strain. For C.1260C>T and C.1293T>C, Hardy-Weinberg equilibrium was observed in Japan strain, but not in China and Korea strains.

4 Discussion

The SNP detection of the candidate genes to obtain potential markers for specific traits can be used to improve the efficiency of selective breeding (Yang *et al*., 2020). The *Ras* gene and *β-glucosidase* gene are two important growth-related genes (Lynd *et al*., 2002; Jouaux *et al*., 2012), but there is no study on the association between mutations of these two genes and growth traits in bivalves so far. In this study, seventeen SNPs were revealed in 669 bp of the coding region of the *Ras* gene, and twelve SNPs were revealed in 716 bp of the coding region of the *β-glucosidase* gene. The SNP densities of these two genes were 2.54% and 1.68 respectively, which is consistent with the average polymorphism rate 2.3% in *C. gigas* (Zhang *et al*., 2012).

For *Ras* gene, four SNPs significantly ($P<0.05$) associated with growth traits have been identified through preliminary and validation analysis. Considering *Ras* gene identified by this study, as well as the insulin receptor-related receptor (Cong *et al*., 2014), insulin-related peptide (Cong *et al*., 2013; Shi *et al*., 2013), insulin growth factor binding protein (Zhang *et al*., 2017; Choi *et al*., 2018) reported in previous studies, now a total of four genes from the insulin pathway have been identified to be associated with growth traits in *C. gigas*. The regulatory effect of insulin pathway on growth traits has also been well documented in other animals (Gricourt *et al*., 2006; Schlueter *et al*., 2007; Zhang and He, 2020). Moreover, the *Ras* gene is abundantly expressed in the mantle that plays an important role in the shell formation and soft body growth during the rapid growth stage (Jouaux *et al*., 2012). However, the molecular mechanism under the association between these four SNPs and growth of *C. gigas* remain unclear. These four SNPs may affect the efficiency of transcription, because introns can increase transcript levels and increase the efficiency of mRNA translation by affecting the rate of transcription, nuclear export, and transcript stability (Shaul, 2017). In addition, the haplotype R-Hap8 was found to show the best growth performance. Haplotype, as a combination of alleles of multiple loci inherited on the same chromosome, can provide more information than SNP (Daly *et al*., 2001).

Similarly, for *β-glucosidase* gene, four SNPs significantly ($P<0.05$) associated with growth traits have been identified through preliminary and validation analysis. The *β-glucosidase*, as an important cellulase, plays a critical role in the cellulose degradation (Lynd *et al*., 2002). *β-glucosidase* that is expressed in the mammalian liver, kidney, intestine and spleen might be involved in the degradation and absorption of flavonoid glycosides (Berrin *et al*., 2003). In *C. gigas*, *β-glucosidase* gene is expressed only in the digestive gland, indicating that it might be involved in the digestion and absorption (Liu, 2012). Among the four growth-related SNPs of *β-glucosidase* gene, C.247G>A and C.284C>T were nonsynonymous mutations (p.Asp63Asn and Thr75Ile), which may cause changes in the spatial structure and function of the encoded protein, thereby affecting its physiological functions. C.1260C>T and C.1293T>C were synonymous mutations, which may affect transcription efficiency as a positive regulator, or may closely relate to the causal mutation (Beuzen *et al*., 2000). The haplotype β-Hap9 showed the highest performances. However, if it is used as a selective marker, some smaller oysters might be selected as a big variance of body weight was observed in this haplotype.

The accuracy of selection can be improved by considering both the haplotypes of *Ras* and *β-glucosidase* genes. Haplotypes R-Hap5 and β-Hap7, R-Hap 6 and β-Hap 6, R-Hap 6 and β-Hap 9, as well as R-Hap 7 and β-Hap 7 can be used to assist the selection of fast-growing individuals. In addition, the effect of both haplotypes R-Hap 8 and β-Hap 9 is not included here, because the oysters with this combination were too few to be analyzed.

Table 7 Allelic frequencies of growth-related SNPs in three *C. gigas* strains

Locus				China	Japan	Korea
Ras	C.86C>A	Allele	A	0.72	0.6	0.66
			C	0.28	0.4	0.34
		Heterozygosity		0.5	0.59	0.55
		PIC		0.32	0.37	0.35
		Equilibrium X2 test		$P=0.12$	$P=0.12$	$P=0.22$
	C.90T>C	Allele	T	0.77	0.78	0.76
			C	0.23	0.22	0.24
		Heterozygosity		0.47	0.43	0.48
		PIC		0.29	0.28	0.30
		Equilibrium X2 test		$P=0.03$	$P=0.05$	$P=0.05$
	C.112A>G	Allele	A	0.58	0.61	0.6
			G	0.42	0.39	0.4
		Heterozygosity		0.84	0.78	0.81
		PIC		0.37	0.37	0.37
		Equilibrium X2 test		$P<0.001$	$P<0.001$	$P<0.001$
	C.118G>A	Allele	A	0.34	0.41	0.28
			G	0.66	0.59	0.72
		Heterozygosity		0.47	0.6	0.38
		PIC		0.34	0.34	0.32
		Equilibrium X2 test		$P=1.00$	$P=0.03$	$P=0.71$
	C.247G>A	Allele	A	0.25	0.67	0.43
			G	0.75	0.33	0.57
		Heterozygosity		0.07	0.15	0.22
		PIC		0.30	0.34	0.37
		Equilibrium X2 test		$P<0.001$	$P<0.001$	$P<0.001$
β-glucosidase	C.284C>T	Allele	C	0.99	0.94	0.94
			T	0.01	0.06	0.06
		Heterozygosity		0.02	0.12	0.12
		PIC		0.02	0.11	0.11
		Equilibrium X2 test		$P=1.00$	$P=1.00$	$P=1.00$
	C.1260C>T	Allele	C	0.39	0.38	0.4
			T	0.61	0.62	0.6
		Heterozygosity		0.71	0.59	0.68
		PIC		0.36	0.36	0.36
		Equilibrium X2 test		$P<0.001$	$P=0.09$	$P=0.03$
	C.1293T>C	Allele	C	0.62	0.66	0.62
			T	0.38	0.34	0.38
		Heterozygosity		0.25	0.51	0.68
		PIC		0.26	0.35	0.36
		Equilibrium X2 test		$P<0.001$	$P=0.24$	$P<0.001$

Note: PIC, polymorphism information content.

The polymorphism level can be classified into three types according to the PIC value, including low polymorphism (PIC value<0.25), intermediate polymorphism (0.25<PIC value<0.5) and high polymorphism (PIC value>0.5) (Ma *et al*., 2011). In this study, C.284C>T belongs to the low polymorphism, while all other SNPs belong to intermediate polymorphism. In addition, not all SNPs in these three strains can be analyzed by Hardy-Weinberg equilibrium. Similar results have also been observed in the polymorphism analysis of insulin receptor-related receptor (Cong *et al*., 2014) and insulin-related peptide (Cong *et al*., 2013; Shi *et al*., 2013) in *C. gigas*. These results might be caused by the genotype of these three strains in three consecutive generations of artificial selection.

5 Conclusions

This study explored the polymorphisms of *Ras* and *β-glucosidase* genes and analyzed their association with growth traits in *C. gigas*. Four SNPs within *Ras* gene and four SNPs within *β-glucosidase* gene were significantly associated with growth traits. Grouping different *Ras* and *β-glucosidase* genes together, haplotypes R-Hap5 and β-Hap7, R-Hap 6 and β-Hap 6, R-Hap 6 and β-Hap 9, and R-Hap 7 and β-Hap 7 can be used to assist the selection of fast-growing individuals. These results provide candidate markers for the selective breeding of *C. gigas* with fast growth.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 31972789), the Industrial Development Project of Qingdao City (No. 20-3-4-16-nsh), and the Science and Technology Development Project of Weihai City (No. 2018NS01).

References

Beguin, P., and Aubert, J. P., 1994. The biological degradation of cellulose. *FEMS Microbiology Reviews*, **13**: 25-58, DOI: 10.1016/0168-6445(94)90099-x.

Berrin, J. G., Czjzek, M., Kroon, P. A., McLauchlan, W. R., Puigserver, A., Williamson, G., *et al*., 2003. Substrate (aglycone) specificity of human cytosolic beta-glucosidase. *Biochemical Journal*, **373**: 41-48, DOI: 10.1042/bj20021876.

Beuzen, N. D., Stear, M. J., and Chang, K. C., 2000. Molecular markers and their use in animal breeding. *Veterinary Journal*, **160**: 42-52, DOI: 10.1053/tvjl.2000.0468.

Chen, N., Li, L., Li, C. H., Lin, Z. H., Meng, J., Liu, S., *et al*., 2020. bHLH genes polymorphisms and their association with growth traits in the Pacific oyster *Crassostrea gigas*. *Journal of Oceanology and Limnology*, **38**: 862-868, DOI: 10.1007/s00343-019-9070-4.

Choi, Y. H., Kim, E. Y., and Nam, T. J., 2018. Involvement of insulin-like growth factor in intraspecific variation in growth of Pacific oyster *Crassostrea gigas* during winter. *Fisheries Science*, **84**: 1017-1024, DOI: 10.1007/s12562-018-1232-3.

Ciocan, C. M., Moore, J. D., and Rotchell, J. M., 2006. The role of Ras gene in the development of haemic neoplasia in *Mytilus trossulus*. *Marine Environmental Research*, **62**: S147-S150, DOI: 10.1016/j.marenvres.2006.04.020.

Cong, R. H., Kong, L. F., Yu, H., and Li, Q., 2014. Association between polymorphism in the insulin receptor-related receptor gene and growth traits in the Pacific oyster *Crassostrea gigas*. *Biochemical Systematics and Ecology*, **54**: 144-149, DOI: 10.1016/j.bse.2014.02.003.

Cong, R. H., Li, Q., and Kong, L. F., 2013. Polymorphism in the insulin-related peptide gene and its association with growth traits in the Pacific oyster *Crassostrea gigas*. *Biochemical Systematics and Ecology*, **46**: 36-43, DOI: 10.1016/j.bse.2012.09.008.

Dai, P., Huan, P., Wang, H. X., Lu, X., and Liu, B. Z., 2015. Characterization of a long-chain fatty acid-CoA ligase 1 gene and association between its SNPs and growth traits in the clam *Meretrix meretrix*. *Gene*, **566**: 194-200, DOI: 10.1016/j.gene.2015.04.047.

Daly, M. J., Rioux, J. D., Schaffner, S. E., Hudson, T. J., and Lander, E. S., 2001. High-resolution haplotype structure in the human genome. *Nature Genetics*, **29**: 229-232, DOI: 10.1038/ng1001-229.

Dégremont, L., Ernande, B., Bedier, E., and Boudry, P., 2007. Summer mortality of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). I. Estimation of genetic parameters for survival and growth. *Aquaculture*, **262**: 41-53, DOI: 10.1016/j.aquaculture.2006.10.025.

Fan, S. G., Xu, Y. H., Liu, B. S., He, W. Y., Zhang, B., Su, J. Q., *et al*., 2017. Molecular characterization and expression analysis of the myostatin gene and its association with growth traits in Noble scallop (*Chlamys nobilis*). *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*, **212**: 24-31, DOI: 10.1016/j.cbpb.2017.07.004.

Feng, L. Y., Li, X., Yu, Q., Ning, X. H., Dou, J. Z., Zou, J. J., *et al*., 2014. A scallop IGF binding protein gene: Molecular characterization and association of variants with growth traits. *PLoS One*, **9**: 7, DOI: 10.1371/journal.pone.0089039.

Gjedrem, T., 2012. Genetic improvement for the development of efficient global aquaculture: A personal opinion review. *Aquaculture*, **344**: 12-22, DOI: 10.1016/j.aquaculture.2012.03.003.

Gjedrem, T., and Rye, M., 2018. Selection response in fish and shellfish: A review. *Reviews in Aquaculture*, **10**: 168-179, DOI: 10.1111/raq.12154.

Gricourt, L., Mathieu, M., and Kellner, K., 2006. An insulin-like system involved in the control of Pacific oyster *Crassostrea gigas* reproduction: hrIGF-1 effect on germinal cell proliferation and maturation associated with expression of an homologous insulin receptor-related receptor. *Aquaculture*, **251**: 85-98, DOI: 10.1016/j.aquaculture.2005.05.015.

Hollenbeck, C. M., and Johnston, I. A., 2018. Genomic tools and selective breeding in molluscs. *Frontiers in Genetics*, **9**: 253, DOI: 10.3389/fgene.2018.00253.

Huang, G. J., Guo, Y. H., Li, L., Fan, S. G., Yu, Z. N., and Yu, D. H., 2016. Genomic structure of the alpha-amylase gene in the pearl oyster *Pinctada fucata* and its expression in response to salinity and food concentration. *Gene*, **587**: 98-105, DOI: 10.1016/j.gene.2016.04.044.

Huvet, A., Jeffroy, F., Fabioux, C., Daniel, J. Y., Quillien, V., Van Wormhoudt, A., *et al*., 2008. Association among growth, food consumption-related traits and amylase gene polymorphism in the Pacific oyster *Crassostrea gigas*. *Animal Genetics*, **39**: 662-665, DOI: 10.1111/j.1365-2052.2008.01776.x.

Jouaux, A., Franco, A., Heude-Berthelin, C., Sourdaine, P., Blin, J. L., Mathieu, M., *et al*., 2012. Identification of Ras, Pten and p70S6K homologs in the Pacific oyster *Crassostrea gigas* and diet control of insulin pathway. *General and Comparative Endocrinology*, **176**: 28-38, DOI: 10.1016/j.ygcen.2011.12.008.

Langdon, C., Evans, F., Jacobson, D., and Blouin, M., 2003. Yields of cultured Pacific oysters *Crassostrea gigas* Thunberg improved after one generation of selection. *Aquaculture*, **220**: 227-244, DOI: 10.1016/s0044-8486(02)00621-x.

Li, Q., Wang, Q. Z., Liu, S. K., and Kong, L. F., 2011. Selection response and realized heritability for growth in three stocks of the Pacific oyster *Crassostrea gigas*. *Fisheries Science*, **77**: 643-648, DOI: 10.1007/s12562-011-0369-0.

Lima, I., Peck, M. R., Rendon-Von Osten, J., Soares, A. M. M., Guilhermino, L., and Rotchell, J. M., 2008. Ras gene in marine mussels: A molecular level response to petrochemical exposure. *Marine Pollution Bulletin*, **56**: 633-640, DOI: 10.1016/j.marpolbul.2008.01.018.

Liu, C. H., 2012. Cloning, characterization and expression analysis of cellulose gene in *Crassostrea gigas*. Master thesis. Ocean University of China (in Chinese with English abstract).

Lynd, L. R., Weimer, P. J., van Zyl, W. H., and Pretorius, I. S., 2002. Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, **66**: 506-577, DOI: 10.1128/mmbr.66.3.506-577.2002.

Ma, L., Qu, Y. J., Huai, Y. T., Li, Z. J., Wang, J., Lan, X. Y., *et al*., 2011. Polymorphisms identification and associations of *KLF7* gene with cattle growth traits. *Livestock Science*, **135**: 1-7, DOI: 10.1016/j.livsci.2010.04.014.

Miossec, L., Le Deuff, R. M., and Goulletquer, P., 2009. Alien species alert: *Crassostrea gigas* (Pacific oyster). *ICES Cooperative Research Report, No. 299*. 42pp.

Moon, J. S., and Choi, Y. H., 2019. Multiplex PCR for the rapid detection of insulin-like growth factor in the Pacific oyster, *Crassostrea gigas*: A useful indicator for growth assessment. *Molecular Biology Reports*, **46**: 1023-1031, DOI: 10.1007/s11033-018-4559-z.

Ning, X. H., Feng, L. Y., Li, X., Wang, S. Y., Zhang, M. R., Wang, S., *et al*., 2018. The scallop IGF2 mRNA-binding protein gene *PyIMP* and association of a synonymous mutation with growth traits. *Genes & Genetic Systems*, **93**: 91-100, DOI: 10.1266/ggs.17-00028.

Niu, D. H., Wang, L., Bai, Z. Y., Xie, S. M., Zhao, H. G., and Li,

J. L., 2015. Identification and expression characterization of the myostatin (*MSTN*) gene and association analysis with growth traits in the razor clam *Sinonovacula constricta*. *Gene*, **555**: 297-304, DOI: 10.1016/j.gene.2014.11.020.

Ou, L. J., Li, X. Y., Wu, G. Q., and Yang, N., 2005. Efficient and sensitive method of DNA silver staining in polyacrylamide gels. *Electrophoresis*, **26**: 99-101, DOI: 10.1002/elps.200406177.

Prieto, D., Markaide, P., Urrutxurtu, I., Navarro, E., Artigaud, S., Fleury, E., *et al*., 2019. Gill transcriptomic analysis in fast- and slow-growing individuals of *Mytilus galloprovincialis*. *Aquaculture*, **511**: 734242, DOI: 10.1016/j.aquaculture.2019.734242.

Saavedra, C., Milan, M., Leite, R. B., Cordero, D., Patarnello, T., Cancela, M. L., *et al*., 2017. A microarray study of carpet-shell clam (*Ruditapes decussatus*) shows common and organ-specific growth-related gene expression differences in gills and digestive gland. *Frontiers in Physiology*, **8**: 943, DOI: 10.3389/fphys.2017.00943.

Sakamoto, K., Uji, S., Kurokawa, T., and Toyohara, H., 2009. Molecular cloning of endogenous beta-glucosidase from common Japanese brackish water clam *Corbicula japonica*. *Gene*, **435**: 72-79, DOI: 10.1016/j.gene.2009.01.011.

Schlueter, P. J., Peng, G., Westerfield, M., and Duan, C., 2007. Insulin-like growth factor signaling regulates zebrafish embryonic growth and development by promoting cell survival and cell cycle progression. *Cell Death and Differentiation*, **14**: 1095-1105, DOI: 10.1038/sj.cdd.4402109.

Shaul, O., 2017. How introns enhance gene expression. *The International Journal of Biochemistry & Cell Biology*, **91**: 145-155, DOI: 10.1016/j.biocel.2017.06.016.

Shi, Y., Guan, Y. Y., and He, M. X., 2013. Molecular identification of insulin-related peptide receptor and its potential role in regulating development in *Pinctada fucata*. *Aquaculture*, **408**: 118-127, DOI: 10.1016/j.aquaculture.2013.05.038.

Sun, X. J., Li, L., Liu, Z. H., Zhao, D., Yang, A. G., Zhou, L. Q., *et al*., 2020. Molecular characterization of the myostatin gene and its regulation on muscle growth in Yesso scallop *Patinopecten yessoensis*. *Aquaculture*, **520**: 734982, DOI: 10.1016/j.aquaculture.2020.734982.

Tanimura, A., Liu, W., Yamada, K., Kishida, T., and Toyohara, H., 2013. Animal cellulases with a focus on aquatic invertebrates. *Fisheries Science*, **79**: 1-13, DOI: 10.1007/s12562-012-0559-4.

Wang, Q. Z., Li, Q., Kong, L. F., and Yu, R. H., 2012. Response to selection for fast growth in the second generation of Pacific oyster (*Crassostrea gigas*). *Journal of Ocean University of China*, **11**: 413-418, DOI: 10.1007/s11802-012-1909-7.

Ward, R. D., English, L. J., McGoldrick, D. J., Maguire, G. B., Nell, J. A., and Thompson, P. A., 2000. Genetic improvement of the Pacific oyster *Crassostrea gigas* (Thunberg) in Australia. *Aquaculture Research*, **31**: 35-44, DOI: 10.1046/j.1365-2109.2000.00388.x.

Watanabe, H., and Tokuda, G., 2001. Animal cellulases. *Cellular and Molecular Life Sciences*, **58**: 1167-1178, DOI: 10.1007/pl00000931.

Yang, C. Y., Yang, J. M., Hao, R. J., Du, X. D., and Deng, Y. W., 2020. Molecular characterization of *OSR1* in *Pinctada fucata martensii* and association of allelic variants with growth traits. *Aquaculture*, **516**: 734617, DOI: 10.1016/j.aquaculture.2019.734617.

Zhang, G. F., Fang, X. D., Guo, X. M., Li, L., Luo, R. B., Xu, F., *et al*., 2012. The oyster genome reveals stress adaptation and complexity of shell formation. *Nature*, **490**: 49-54, DOI: 10.1038/nature11413.

Zhang, H., and He, M. X., 2020. The role of a new insulin-like peptide in the pearl oyster *Pinctada fucata martensii*. *Scientific Reports*, **10**: 433, DOI: 10.1038/s41598-019-57329-3.

Zhang, H., Shi, Y., and He, M. X., 2017. Molecular identification of an insulin growth factor binding protein (IGFBP) and its potential role in an insulin-like peptide system of the pearl oyster, *Pinctada fucata*. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*, **214**: 27-35, DOI: 10.1016/j.cbpb.2017.09.003.

(Edited by Qiu Yantao)