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厦门大学

博 士 学 位 论 文

福建牡蛎(*Crassostrea angutala*)生长相关基因和群体选育研究

Studies on Growth Related Gene and Population Selective Breeding for the Fujian Oyster (*Crassostrea angutala*)

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中文摘要

福建牡蛎(*Crassostrea angulata*)旧称僧帽牡蛎，是福建的重要养殖贝类。《2011 年农业部渔业局》的数据显示，2010 年福建省牡蛎养殖产量达 1,456,106 吨，占全国的 40%，居全国首位，其中主要养殖品种为福建牡蛎。本论文对福建牡蛎生长相关基因和群体选育进行了研究，拟对福建牡蛎生长相关基因做深一步的了解，同时进行了福建牡蛎群体选育，有望为福建牡蛎良种选育提供理论支持。

本研究采用 SSH、454/Roche 转录组文库方法，建立牡蛎早期发育不同阶段 cDNA 文库。SSH 文库随机调取 672 个克隆，送交上海生工测序。获得序列 653 条，测序成功率 97.2%，将其数据进行拼接，得到 Unigenes(contigs+singlets) 553 条，重复序列占测序成功序列 15.3%。CAP3 除去重复和小于 100 的低质量 EST，得到有效 EST 553 条，平均长度 600bp，其中 54 个重叠组是由两条 EST 拼接而成，9 条是 3 条 EST 拼接而成，3 条是由 4 条或 4 条以上 EST 拼接而成的。非冗余序列占全部有效测序序列的 71.51%。

454/Roche 转录组文库共得到 566917 条 EST，平均长度 319bp。牡蛎发育的 8 个时期通过添加不同的物理标签用以区分，去除接头和小于 60bp 的短片段之后，剩余 555215 条序列，共 173Mb，平均长度 309bp。对所得数据进行拼接得到了 10462 条 contig,，112366 条 singleton。Contigs 平均长度 723bp，singleton 平均长度 275bp，6878 条 contig 长度超过 500bp，1907 条 contig 长度超过 1kb。所有基因最后可以 Mapping 到 262 个 Pathway 通路上，其中 Pathway 包含了生长、代谢、发育、信号转导和细胞膜离子泵等。基因波动最大的 30 条基因中有 10 条基因完全不能和 genebank 中的序列配对，说明双壳贝类可能在发育、生长、变态中具有独特的分子机制。

对 454 转录组中的 IGF-like 基因应用 RNAi、定量 PCR、免疫组化等方法进行了功能验证，其中 RNAi 干扰后的牡蛎生长速率显著比对照变慢，同时，干扰

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后的牡蛎其 IGF 在转录和翻译水平均下降。通过 T7 展示文库，淘选了一批和牡蛎 IGF 相结合的 EST，根据 EST 序列，通过 RACE 实验，克隆了和牡蛎 IGF 相关的基因 11 条，分别是：细胞凋亡蛋白，DAP (death-associated protein)；氯离子通路基因，CIC (Chloride intracelluar channel)；福建牡蛎细胞凋亡基因，Gadda (growth arrest and DNA-damage-inducible, alpha)；生长转录因子，RAS (RAS domain subfamily bZIP)；福建牡蛎同源基因，HOX (HOX12 protein isoform 3)；低密度脂肪受体，LDLtor (Low-density lipoprotein receptor)；类胰岛素生长因子受体基因，IGFR (Insulin-like growth factor receptor)。

针对牡蛎生长性状进行了群体选育，2009 年 6 月，本研究进行了漳浦北江养殖群体为亲本的群体选育，选育目标为生长快速性状。2010 年 9 月和 2011 年 11 月分别进行了以生长快速为目标的第二代和第三代选育，选育效果显著，在 90 天日龄时，第三代选育组总湿重比对照组增加 27.93%，差异显著($P < 0.01$)。对选育三代群体进行遗传分析学分析，结果表明尽管选育三代，但选育后代仍具有很高的基因多态性($PIC > 0.5$)，没有发生明显的近交衰退($P > 0.05$)。

在对 IGF 及其相关基因和福建牡蛎的性状关联研究中，IGF 的基因拷贝数和牡蛎湿重、软体重正相关，随着选育世代的进行，其 IGF 基因拷贝数增多，变异系数变小，*PIC* 指数下降，IGF 拷贝数可以作为一个候选功能分子标记，进而应用到牡蛎选择育种上。

关键词：福建牡蛎(*Crassostrea angulata*)；选育；基因；RNA 干扰；基因拷贝数；转录组

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Abstract

Fujian oyster (*Crassostrea angulata*), formerly known as mitral oysters is the Fujian important cultured shellfish. "2011 China Fishery Statistics Yearbook data, Fujian oyster aquaculture production in 2010 reached 1,456,106 tons, or 40% of the country's total, ranking first in the country. The thesis of Fujian oyster growth-related genes and groups of breeding the proposed Fujian Oyster deep step understanding of growth-related genes, while oyster populations breeding in Fujian, is expected to provide theoretical support for Fujian oyster breeding.

SSH and 454/Roche transcriptome library were adopted in this study to establish cDNA library in different phases of oyster early development. SSH library drew 672 clones randomly and sent them to Shanghai Sangon Company for sequencing. 653 sequences were obtained and the success rate was 97.2%. The data was spliced and got 553 Unigenes (contigs+singlets) in which the repetitive sequences accounted for 15.3% of the success sequences. Repetitive and low quality ESTs which were less than 100 were removed from the CAP3 and 553 effective ESTs was obtained whose average length was 600 bp. Among the ESTs, 54 overlapped groups were consisted of two splicing EST, 9 were consisted of 3 splicing ESTs, and 3 were consisted of 4 or more than 4 splicing EST. Non-redundant sequences accounted for 71.51% of the whole effective sequences.

454/Roche transcriptome library has obtained 566917 ESTs whose average length was 319bp. 8 phases of oyster growth were distinguished by adding different physical tags. After wiping out joints and short fragments which were less than 60bp, 555215 sequences were left which were of 173Mb and an average length of 309bp. The obtained data were spliced to get 10462 contigs and 112366 singletons. The average length of Contigs was 723bp, and average length of singleton was 275bp. The lengths of 6878 contigs exceeded 500bp and the lengths of 1907 contigs exceeded 1kb. Finally all genes could map to 262 pathways which contained growth, metabolism, development, signal transduction, cell membrane ionic mercury and so on. In the 30 genes of the greatest volatility, there were 10 genes which were totally unable to

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match to the sequences in the genebank. It indicated that bivalves have a unique molecular mechanism in development, growth and metamorphosis.

Methods of RNAi, realtime PCR, and immunohistochemistry were applied to verify the functionality for IGF-like genes in the 454 transcriptome. The growth of oyster after RNAi interference was obviously slower than that of the control. Meanwhile, IGF of the interfered oyster were reduced both in the transcription and translation level. A group of ESTs combining with oyster IGF was selected through T7 display library. According to the EST sequences, 11 genes related to oyster IGF were cloned by RACE experiment, which were cell apoptosis protein, DAP (death-associated protein), chloride ion channel gene, CIC (Chloride intracelluar channel), cell apoptosis gene of portugal oyster, Gadda (growth arrest and DNA-damage-inducible, alpha), growth and transcription factor, RAS (RAS domain subfamily bZIP), homologous gene of portugal oyster, HOX (HOX12 protein isoform 3), low density fat receptor, LDLtor (Low-density lipoprotein receptor), receptor gene of insulin-like growth factor, IGFR (Insulin-like growth factor receptor)..

Population selection and breeding were made for oyster growth trait. In June 2009, this study conducted population selection and breeding with cultured population of Beijiang in Zhangpu as parent. The breeding goals were tachyauxesis characters. In September 2010 and November 2011, selection and breeding of the second and third generation were conducted respectively with the goal of tachyauxesis whose effect was remarkable. At 90 days of age, the gross wet weight of the third breeding group has increased 27.93% than that of the control group and the difference was significant ($P > 0.01$). Genetic analysis was carried for the three generations of breeding groups, and the result indicated that though three generations were selected and bred, the breeding offspring had high genetic polymorphism ($PIC > 0.5$) and there weren't obvious inbreeding depression.

In the study on relationship between IGF and its related genes and characters of portugal oyster, there was a positive correlation between gene copy number of IGF and wet weight and soft weight of oyster. As the development of breeding generation, the IGF gene copy number increased while coefficient of variation decreased and PIC index declined. As a candidate functional molecular marker, IGF copy number could

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be applied to selective breeding of oyster.

Keywords: *Crassostrea angulate*; Selective breeding; Gene; RNA interference; Gene copy number; Transcriptome

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第一章 绪论

1.1 我国牡蛎养殖业现状与趋势

牡蛎在我国已有 2000 多年的养殖历史，是我国四大养殖贝类之一。根据宋代梅尧臣的记载，宋时人们不仅知道食用牡蛎肉，而且沿海渔民已经从事“插竹养蚝”，在海滩上养殖牡蛎了，人工养殖历史可谓久矣。传统的养殖方法如投石养殖、桥式养殖、立桩养殖和插竹养殖等。这些养成方式的共同特点就是采苗器兼作养成器(董双林，2011)。

世界临海的国家几乎都有牡蛎生产，世界养殖牡蛎产量占贝类总产量的90％以上，20 世纪 90 年代初期，全世界的贝类养殖品种一共仅有 44 种，其中光养殖的牡蛎品种就占 11 种。牡蛎养殖比较发达的国家有中国、美国、日本、朝鲜、法国、墨西哥、新西兰、澳大利亚等(图 1-1-1)。各国对牡蛎相关研究较多，养殖方法在各种贝类中也最完善。根据联合国粮农组织 2007 年的统计，世界海产贝类中以牡蛎的产量最高(FAO, 2009)。

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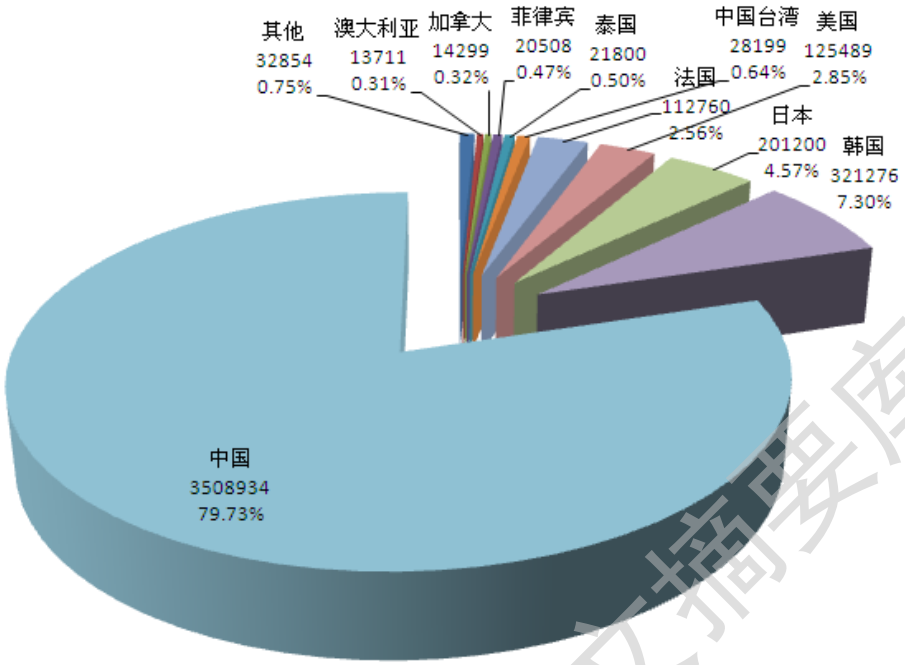

图 1-1-1 2007 年世界十大牡蛎生产国或地区产量(吨)及所占比例

Fig. 1-1-1 Yield of Oyster (Ton) in 10 Major Global Oyster Producing Countries or Areas and Proportions in 2007

通过几十年的快速发展，中国的牡蛎养殖规模和产量位居世界各国首位，2007年以后，中国每年牡蛎的产量都占世界总量的80%左右(中国渔业年鉴，2011)，但牡蛎养殖业的产值和效益却始终在低水平上徘徊。国际牡蛎市场巨大，价格高，但我国牡蛎出口量小，在国际市场所占份额很小(图1-1-2)。2007年中国牡蛎出口量1346吨，出口金额186万美元，仅占全世界牡蛎出口量和出口金额的3.33%和0.98% (FAO, 2009)，这与世界第一牡蛎生产大国的地位极不相称。2006年世界其他国家牡蛎平均产值达1360美元/吨，而中国为531.8美元/吨，仅为其他国家产值的39.1%。这种巨大的反差，很大程度上是由于我国牡蛎产品规格质量低，难以满足国际市场要求。

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