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Analysis of vp28 Gene Expression Efficiency and Photosynthetic Characteristics in Transgenic Synechococcus sp. PCC 7942 (Postprint)

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Abstract

【Background】White Spot Syndrome Virus (WSSV) is one of the most devastating viruses in shrimp aquaculture, with no effective chemotherapeutic agents currently available for large-scale application. However, significant advances have been made in WSSV immunoprophylaxis in recent years. Vp28 protein, a major structural protein of the WSSV envelope, has had its encoding gene successfully expressed in eight host species since 2004, demonstrating significant prophylactic and therapeutic efficacy against WSSV in laboratory trials, yet its application in the shrimp industry remains unreported.【Objective】To express Vp28 recombinant protein using Synechococcus, a natural feed organism for shrimp; this medicine-food integration approach can simplify operations, reduce costs, and facilitate industrial application.【Methods】The expression efficiency of the vp28 gene in vp28-transgenic Synechococcus sp. PCC7942 was quantified by fluorescence quantitative PCR. Photosynthetic activity variations of the vp28-transgenic Synechococcus under different temperatures, light intensities, pH values, and salinities were measured using an oxygen electrode method to determine its optimal growth conditions.【Results】The vp28 gene expression efficiency was determined to be 9.52%, threefold higher than that in Anabaena sp. PCC7120. The optimal harvest time was the late logarithmic growth phase (approximately 15 days). The optimal growth conditions for the transgenic cyanobacterium PCC7942 were: temperature 40 °C, salinity 0-0.1 mol NaCl/L, pH 7.5, and light intensity 450 mol $\cdot$ m^{-2} $\cdot$ s^{-1}.【Conclusion】This study determined the expression efficiency of the vp28 gene in Synechococcus and the optimal culture conditions for this transgenic cyanobacterium, providing a foundation for large-scale preparation of oral agents based on medicine-food integration using vp28-transgenic Synechococcus sp. PCC7942.

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Full Text

Expression Efficiency and Photosynthetic Characteristics Analysis of the vp28 Gene in Transgenic Synechococcus sp. PCC 7942

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Abstract

Background: White Spot Syndrome Virus (WSSV) is one of the most devastating pathogens in shrimp aquaculture, causing cumulative mortality rates of 100% within 2-10 days post-infection. Despite significant progress in immunological prevention, no effective therapeutic agents have been deployed at scale. Vp28, the major envelope protein of WSSV, has been successfully expressed in eight host species since 2004, demonstrating remarkable prophylactic efficacy in laboratory trials, yet industrial application remains unrealized.

Objective: This study employed Synechococcus sp. PCC 7942, a natural shrimp feed, as a host for recombinant Vp28 protein expression. This "medicine-food homology" approach simplifies operational procedures, reduces costs, and facilitates practical application in aquaculture production.

Methods: The expression efficiency of the vp28 gene in transgenic Synechococcus 7942 was quantified using real-time fluorescent quantitative PCR. Photosynthetic activity under varying temperature, illumination, pH, and salinity conditions was measured via oxygen electrode methodology to determine optimal growth parameters.

Results: The vp28 gene expression efficiency reached 9.52% in transgenic Synechococcus 7942, threefold higher than that achieved in Anabaena sp. PCC 7120. The optimal harvest time was identified as the late logarithmic growth phase (approximately 15 days). The optimal growth conditions for the transgenic cyanobacterium were: temperature 40 °C, salinity 0-0.1 mol NaCl/L, pH 7.5, and light intensity 450 $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Conclusion: These findings establish the expression efficiency of vp28 in Synechococcus and define optimal cultivation conditions, providing a foundation for large-scale preparation of oral agents using transgenic Synechococcus 7942 that integrate prophylactic and nutritional functions.

Keywords: Transgenic Synechococcus with vp28 gene; Photosynthetic activity; Real-time fluorescent quantitative PCR; vp28 gene expression efficiency

Introduction

White Spot Syndrome Virus (WSSV) is a highly lethal and contagious pathogen of penaeid shrimp that has become the principal disease impeding aquaculture development over the past two decades. Vp28, the major envelope structural protein of WSSV, plays a critical role in viral infection of host organisms. Van Hulten et al. successfully cloned the vp28 gene into a baculovirus expression vector and expressed it in insect cells, demonstrating that high-concentration animal serum prepared from this Vp28 protein could neutralize WSSV, suggesting its pivotal role in viral pathogenesis. Vaccination strategies, particularly those targeting recombinant Vp28 envelope protein, have attracted considerable research attention. Since 2004, the vp28 gene has been successfully expressed in eight microbial hosts, with laboratory trials showing significant therapeutic efficacy. However, these immunological approaches have not achieved large-scale application due to operational complexity and high costs.

Cyanobacteria (also known as blue-green algae) represent the only prokaryotic algal group, characterized by simple structure, rapid growth, strong adaptability, and ease of genetic manipulation, making them valuable model organisms for molecular biology and genetic engineering research. Capitalizing on the natural feeding relationship between cyanobacteria and shrimp larvae, Zhang et al. first achieved successful expression of the WSSV envelope protein vp28 gene in Anabaena sp. PCC 7120, demonstrating that oral administration through feed represents a more practical approach for industrial application. However, the expression efficiency in Anabaena was too low to meet production demands. Effective enhancement of vp28 expression rates would further reduce costs and enable true commercialization of oral agents.

Although the vp28 gene was successfully introduced into Synechococcus sp. PCC 7942 years ago, comprehensive analysis of its expression efficiency and optimal growth conditions was not pursued due to harvesting difficulties. The recent establishment of a simplified harvesting method for unicellular algae now enables large-scale cultivation of transgenic Synechococcus 7942. This study investigates photosynthetic and respiratory rates of transgenic Synechococcus in response to environmental factors using oxygen electrode analysis to determine optimal cultivation conditions, while employing real-time fluorescent quantitative PCR to analyze vp28 expression efficiency across growth stages and identify optimal harvest timing. As a natural feed for shrimp larvae, transgenic Synechococcus 7942 expressing Vp28 can provide both nutrition and disease protection through this medicine-food homology approach, facilitating its adoption in shrimp aquaculture.

1.1 Materials

The cyanobacterial strains used in this study are listed in Table 1.

1.2 Instruments and Reagents

Key instruments and reagents included: FTC-3000 real-time fluorescent quantitative PCR system (Funglyn Biotech Inc., Canada), liquid-phase oxygen electrode (Hansatech, UK), total RNA extraction kit (Tiangen Biotech), RNA reverse transcription kit (Tiangen Biotech), KAPA Universal SYBR Fast quantitative reagent kit (Jieyi Biotechnology), gel extraction kit (Tiangen Biotech), TAE electrophoresis buffer, kanamycin sulfate, BG-11 medium, and spectrophotometer.

1.3 Methods

1.3.1 Western Blot Detection of Target Protein Transgenic vp28-type, empty vector-type, and wild-type Synechococcus 7942 samples were collected during logarithmic growth phase ($OD_{750} = 0.5$). After repeated freeze-thaw cycles and centrifugation, supernatants were removed. Protein concentrations were determined using the Bradford method to calculate loading volumes, with approximately 10 g protein loaded per lane. Following electrophoresis, proteins were transferred to membranes, blocked, and incubated with monoclonal antibodies.

1.3.2 Establishment of Real-Time Fluorescent Quantitative PCR Method a) Primer Design: Based on WSSV nucleotide sequences deposited in GenBank, a pair of RT-PCR primers was designed (Table 2) and used to amplify the vp28 gene from WSSV viral DNA. The amplified product was electrophoretically separated, identified, and sequenced to verify the selected conserved sequences.

b) Standard Preparation: The vp28 gene was amplified from WSSV DNA, and the purified fragment was used as standard DNA after gel extraction. Following 10-fold serial dilution, DNA concentration was calculated using the formula:

$$DNA\ concentration = 50 \times OD_{260} \times dilution\ factor = ng/\mu l \quad (1)$$

Copy number was determined using:

$$Number\ copies = (mass \times 6.022 \times 10^{23})/(length \times 10^{9} \times 650) \quad (2)$$

c) Reaction System: The 20 l reaction system contained: SYBR Mix (2×) 10 l, Forward Primer 0.4 l, Reverse Primer 0.4 l, DNA template 2 l, and PCR-grade water 7.2 l. Reaction conditions are shown in Table 3 .

1.3.3 Analysis of vp28 Gene Expression Efficiency in Transgenic Synechococcus at Different Growth Stages Samples of transgenic Synechococcus at different growth stages were collected for total RNA extraction and reverse transcription to cDNA for RT-qPCR amplification. Ct values were converted to log copy numbers via standard curve, and sample copy numbers and DNA concentrations were calculated using formula (2). Percentage content and protein concentration were then calculated based on Synechococcus dry weight.

1.3.4 Synechococcus Cultivation Cultures were grown in BG-11 (+N/-N) medium at 35 °C under continuous illumination (50 mol photons $m^{-2}s^{-1}$) with shaking at 135 r/min.

1.3.5 Physiological Activity Detection **a) Growth Curve Determination:** Samples were collected every 24 h over a 16-20 day period for cell counting using a hemocytometer and OD_{750} measurement.

b) Photosynthetic Oxygen Evolution Measurement: Logarithmic-phase cells were adjusted to 10 g/ml chlorophyll concentration. Net photosynthetic oxygen evolution of transgenic, empty vector, and wild-type Synechococcus 7942 was measured under different light, temperature, salinity, and pH conditions using an oxygen electrode. Oxygen evolution activity was calculated as:

$$V = S \times K \times 60 \times 1000/P \quad (3)$$

where V is oxygen evolution activity (mol O_2/mg Chl • hr), S is slope (min^{-1}), K is the constant representing dissolved oxygen in water at a given temperature (mol O_2/ml), and P is chlorophyll concentration (mg Chl/ml).

For chlorophyll extraction, samples were centrifuged at 4000 r/min for 10 min, supernatant was removed, and 90% methanol was added and mixed thoroughly. After 2 h extraction at room temperature and 8 h at 4 °C, samples were centrifuged again at 4000 r/min for 10 min. The supernatant was collected and OD_{665} was measured. Chlorophyll content was calculated as:

$$Chl\ (\mu g/ml) = 13.9 \times OD_{665} \quad (4)$$

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2 Results

2.1 Vp28 Protein Detection in Synechococcus 7942

The transgenic vp28-type Synechococcus 7942 was constructed in 2006 and maintained through successive liquid and solid cultures. To verify stable retention of the vp28 gene, Western blot analysis was performed. Soluble proteins were extracted from wild-type, empty vector-type, and transgenic vp28-type

Synechococcus 7942 at logarithmic phase, and protein concentrations were determined using the Bradford method to establish loading volumes. Western blot results showed no Vp28 protein expression in wild-type or empty vector-type cells, while a distinct Vp28 band was detected in transgenic cells (Figure 1 [Figure 1: see original paper]), matching the position of the standard protein band. This confirms stable Vp28 protein expression in transgenic Synechococcus.

2.2 Real-Time Fluorescent Quantitative PCR Standard Curve

Following qualitative confirmation of vp28 expression products, RT-qPCR was employed for quantitative analysis of vp28 expression efficiency during the growth period.

2.2.1 Primer Amplification and Melting Curve Using WSSV viral DNA as template and the primers listed in Table 2, amplification yielded a 164 bp DNA-specific band detected by 3% agarose gel electrophoresis (Figure 2 [Figure 2: see original paper]). Melting curve analysis of the amplified product from standard DNA showed a single specific peak at 81 °C, confirming excellent primer specificity for RT-PCR.

2.2.2 Standard Template and Standard Curve Construction During real-time fluorescent quantitative PCR detection, the amplified vp28 gene template was recovered via gel electrophoresis. The purified vp28 gene gel fragment was serially diluted 10-fold and amplified by PCR to generate the standard curve shown in Figure 3 [Figure 3: see original paper]. The initial copy number of the standard was 7.98×10^{13} copies/ l.

2.3 vp28 Gene Expression Efficiency in Transgenic Synechococcus 7942 and Anabaena 7120

Transgenic Synechococcus samples collected at 3, 6, 9, 12, 15, and 18 days post-inoculation were analyzed for vp28 expression efficiency across different growth stages. The resulting Ct values and copy number data are presented in Table 4 , with absolute quantitative expression efficiency shown in Figure 4 [Figure 4: see original paper]. Expression efficiency was also compared between transgenic Synechococcus 7942 and transgenic Anabaena 7120 (Table 5 and Figure 5 [Figure 5: see original paper]).

Growth curve analysis revealed the growth pattern of transgenic Synechococcus: 1-3 days lag phase, 4-14 days logarithmic phase, and entry into stationary phase at day 15. As shown in Figure 4, Vp28 protein yield reached 318 mg/L during late logarithmic phase, with maximum vp28 gene expression efficiency of 9.52% (based on dry weight) in transgenic Synechococcus. In contrast, transgenic Anabaena 7120 showed maximum expression efficiency of 3.23% (dry weight) during mid-logarithmic phase, with Vp28 protein yield of 108 mg/L. Thus, vp28

gene expression efficiency in transgenic Synechococcus 7942 is threefold higher than in transgenic Anabaena 7120.

2.4 Photosynthesis and Growth of Transgenic Synechococcus 7942

The high expression efficiency of vp28 in Synechococcus 7942 suggests potential for cost-effective oral agent development. To enable large-scale cultivation, optimal growth conditions were investigated.

2.4.1 Effects of Light Intensity, pH, Temperature, and Salinity on Photosynthesis and Optimal Growth Conditions Figure 6a [Figure 6: see original paper] shows photosynthesis-light response curves at 35 °C for transgenic vp28-type, empty vector-type, and wild-type Synechococcus 7942. The wild-type light compensation point was 15 mol $\cdot\, m^{-2} \cdot s^{-1}$ with a saturation point at 300 mol $\cdot\, m^{-2} \cdot s^{-1}$ and net photosynthetic rate of 56.3 mol photons $m^{-2}s^{-1}$. Both empty vector-type and transgenic vp28-type showed light saturation at approximately 600 mol $\cdot\, m^{-2} \cdot s^{-1}$, with net photosynthetic rates of 60.8 and 64.3 mol photons $m^{-2}s^{-1}$, respectively. All three cell types exhibited photoinhibition at 700-900 mol $\cdot\, m^{-2} \cdot s^{-1}$. Transgenic vp28-type and empty vector-type displayed elevated light saturation points and 14.2% higher net photosynthesis compared to wild-type.

Figure 6b reveals optimal pH values of 8.0 for wild-type and 7.5 for both empty vector-type and transgenic cells. Transgenic photosynthetic rates increased steadily up to pH 7.5, then declined, though measurable activity persisted at pH 9.0. Empty vector-type showed similar pH responses but with more pronounced net photosynthesis decline above optimal pH.

Temperature responses were largely consistent across all strains (Figure 6c), with maximum growth at 40 °C. Temperatures of 45 °C inhibited growth in all types and could cause cell inactivation, though wild-type photosynthetic rates declined more rapidly at high temperatures compared to empty vector-type and transgenic cells. Overall, transgenic temperature responses aligned with wild-type patterns, establishing 40 °C as the optimal cultivation temperature.

Salinity tolerance analysis (Figure 6d) demonstrated that transgenic Synechococcus 7942 exhibited highest resistance to elevated salinity above 0.1 mol NaCl/L, followed by wild-type, while empty vector-type showed slightly poorer high-salinity tolerance. Wild-type and transgenic strains grew best at 0 mol/L NaCl, whereas empty vector-type tolerated 0-0.1 mol/L NaCl. All strains showed more pronounced photosynthetic rate changes in response to low salinity variations than high salinity changes. Based on these results, salinity for transgenic Synechococcus 7942 cultivation should be maintained at 0-0.1 mol/L NaCl.

2.4.2 Growth of Synechococcus 7942 at Different Growth Stages Figures 7 and 8 [Figure 8: see original paper] show that logarithmic growth for

transgenic vp28-type, empty vector-type, and wild-type Synechococcus 7942 occurred between days 4-14. Empty vector transformation slightly enhanced growth, while vp28 gene insertion provided an even greater growth-promoting effect, increasing growth rate by up to 15.4%, consistent with the light intensity curve results.

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3 Discussion

This study builds upon previous successful expression of the WSSV envelope protein gene vp28 in Synechococcus. Despite nine years of subculturing, the vp28 gene remained stably integrated and expressed in Synechococcus 7942. During late logarithmic phase, Vp28 protein yield reached 318 mg/L, with expression efficiency of 9.52% (dry weight)—tenfold higher than the 1% efficiency previously reported in Anabaena. No prior studies have examined vp28 expression efficiency variations across growth stages in Synechococcus 7942.

Physiological activity analysis under different environmental conditions (light, temperature, salinity, and pH) established optimal growth parameters for transgenic Synechococcus: temperature 40 °C (with transgenic cells showing enhanced thermotolerance), salinity 0-0.1 mol NaCl/L (with low-salinity variations exerting greater impact than high-salinity changes), pH 7.5 (though transgenic cells showed reduced pH adaptability compared to wild-type), and light intensity 600 $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (with transgenic cells demonstrating higher light tolerance and 14.2% greater net photosynthesis than wild-type at optimal intensity).

Quantitative detection of vp28 expression across growth stages provides guidance for optimal harvest timing and establishes a foundation for determining dosage in challenge experiments and quantitative analysis. Large-scale cultivation requires regulation of light, temperature, pH, and salinity to promote rapid growth of transgenic Synechococcus 7942, with physiological characterization providing the necessary parameters.

As a natural feed for shrimp larvae, transgenic Synechococcus expressing Vp28 leverages medicine-food homology advantages to develop easy-to-use, low-cost anti-WSSV oral agents for true industrialization.

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