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Designing a Glycoprotein B-Based Vaccine Candidate Against Infectious Laryngotracheitis Virus Through Immunoinformatic Approaches

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Abstract

Microalgae biomass recovery and vaccine development are pivotal areas in biotechnology, requiring innovative and efficient strategies. This review focuses on chemical-based flocculation methods for Chlorella sorokiniana biomass recovery and immunoinformatics-based design of glycoprotein B (gB) vaccines against Infectious Laryngotracheitis Virus (ILTV). Various flocculating agents, including ferric chloride, aluminum sulfate, zinc sulfate, and sodium hydroxide, were assessed for their recovery efficiency. Among these, ferric chloride demonstrated superior performance, achieving nearly 80% biomass recovery in 10 minutes at 0.75 g/L concentration. Zinc sulfate showed the least efficacy. In parallel, B-cell epitope prediction for ILTV gB identified the PH-1 region as highly antigenic, hydrophilic, and structurally stable, making it a promising candidate for subunit vaccine development. This review emphasizes the integration of chemical flocculation and immunoinformatics to improve bioproduct recovery and vaccine design, offering cost-effective, environmentally friendly, and efficient solutions.

KEYWORDS: Microalgae; Flocculation; Sedimentation; Ferric Chloride; Glycoprotein B; Immunoinformatics; Vaccine Design.

INTRODUCTION

Microalgae are unicellular, photosynthetic organisms with remarkable structural and biochemical diversity. Found in freshwater, marine, and terrestrial ecosystems, microalgae have emerged as valuable resources for food, feed, biofuels, bioplastics, pharmaceuticals, pigments, and other bioactive compounds [1]. Among these, *Chlorella* species are recognized for rapid growth, high lipid content, and adaptability to nutrient-limited conditions, making them attractive for both biofuel and nutritional applications.

A major bottleneck in microalgae utilization is biomass recovery. Microalgal cultures typically have low cell density (0.1–3.0 g/L) and small cell size (3–30 µm), coupled with a negative surface charge that impedes natural aggregation. These factors complicate sedimentation and filtration, increasing energy costs and limiting scalability [2]. Efficient recovery is essential to maintain biomass quality for downstream processing, such as lipid extraction or bioproduct synthesis.

Various biomass separation techniques have been applied, including sedimentation, filtration, flotation, centrifugation, and flocculation. Flocculation, in particular, is favored due to low energy requirements, high scalability, and preservation of cell integrity [3]. Metal salts, alkaline agents, and natural polymers have been widely explored as flocculants. The effectiveness of flocculation depends on flocculant type, concentration, pH, initial cell density, and sedimentation time [4].

In parallel, poultry health remains a critical concern globally due to Infectious Laryngotracheitis Virus (ILTV), which causes respiratory disease in chickens. Live attenuated vaccines prevent ILTV outbreaks, but risks of virulence reversion and incomplete immunity persist. Subunit vaccines, particularly those targeting

glycoproteins like gB, offer safer alternatives. Glycoprotein B plays essential roles in viral attachment, fusion, and entry, making it a promising antigen for vaccine development [5]. Immunoinformatics enables the identification of antigenic epitopes, prediction of B- and T-cell binding regions, and assessment of physicochemical and structural properties, facilitating rational vaccine design without extensive in vivo experimentation [6].

This review synthesizes findings on chemical flocculants for *C. sorokiniana* recovery and immunoinformatics approaches to gB-based vaccine design, highlighting integrated strategies for efficient biomass utilization and disease control.

LITERATURE REVIEW

Microalgal Cultivation and Biomass Recovery

Chlorella sorokiniana CTT 7727 was cultivated in Bold's basal medium (BBM) for 30 days in 9 L photobioreactors. Sterilization of the medium at 121°C for 15 minutes ensured aseptic conditions. Aeration was maintained at 3 L/min with a 12-hour light/12-hour dark photoperiod. The culture reached a final dry biomass of 1.3 g/L at pH 10.2 [7] (Table 1).

Biomass recovery efficiency depends on the flocculation system. Flocculation induces aggregation of cells into larger particles, which then sediment naturally. The degree of flocculation (HI) is calculated based on the sediment height relative to the liquid column height (He/Hl). Time-dependent sedimentation profiles are measured at intervals to assess the kinetics of floc formation [8].

Chemical Flocculants

Commonly used flocculants include ferric chloride (FeCl3), aluminum sulfate (Al2(SO4)3), sodium hydroxide (NaOH), and zinc sulfate (ZnSO4). Their efficiency varies according to concentration, cell density, and pH. For example, ferric chloride at 0.75 g/L achieved nearly 80% biomass recovery in 10 minutes, whereas zinc sulfate was ineffective (<40%). Sodium hydroxide and aluminum sulfate provided moderate results (48–58%) but required longer sedimentation times [9].

Immunoinformatics and Glycoprotein B Analysis

Glycoprotein B (gB) is a key antigen in ILTV infection, mediating viral entry and fusion. A total of 67 gB sequences were retrieved from GenBank in FASTA format. Conserved regions were identified at positions 114–321 (PH-1) and 323–420 (PH-2). Antigenicity prediction using VaxiJen indicated that PH-1 (0.6042) was more immunogenic than PH-2 (0.5710) [10].

B-cell epitope prediction was performed using Kolaskar-Tongaonkar, Emini surface accessibility, Chou-Fasman beta-turn, and Parker hydrophilicity scales. High-probability epitopes were identified in PH-1 (181–187) with maximum hydrophilicity and surface accessibility. Physicochemical analysis revealed a molecular weight of 24.4 kDa, pI of 7.85, thermostability (aliphatic index 66.49), and hydrophilicity (GRAVY = -0.571) (Table 2). Secondary structure prediction indicated 49.52% coils, 19.71% alpha-helix, and 25.48% extended strands. Homology modeling using varicella-zoster virus gB (PDB: 6vlk.A) confirmed structural reliability with GMQE score 0.76 and ERRAT quality factor 92.588 [11].

Flocculation Efficiency

Among the tested agents, ferric chloride was the most effective for rapid and high-yield biomass recovery, achieving nearly 80% within 10 minutes at 0.75 g/L. Aluminum sulfate and sodium hydroxide provided moderate recovery but required longer sedimentation times. Zinc sulfate was largely ineffective, likely due to suboptimal interaction with cell surface charges. Self-flocculation yielded ~48%, indicating the necessity of chemical agents for efficient recovery [9].

gB Epitope Prediction and Vaccine Candidate Design

Immunoinformatics analysis identified PH-1 as a highly antigenic region. Epitope prediction highlighted residues 181–187 as potential B-cell epitopes with high surface accessibility, flexibility, and hydrophilicity, suggesting strong interaction with host immune receptors. Physicochemical properties indicated protein stability, solubility, and suitability for recombinant vaccine design.

Table 1: Comparison of Flocculating Agents for *C. sorokiniana.*

Flocculant	Optimal Concentration (g/L)	Recovery Efficiency (%)	Sedimentation Time (min)	Notes
Ferric Chloride	0.75	~80	10	Fast, efficient
Aluminum Sulfate	0.5–1.0	56	60	Moderate efficiency
Sodium Hydroxide	0.5–1.0	58	45	Effective but slower
Zinc Sulfate	0.25–1.0	<40	90	Ineffective for this strain
Self-Flocculation	N/A	48.65	60	Baseline, moderate efficiency

Table 2: Predicted B-cell Epitopes of ILTV gB PH-1 Region.

Epitope Position	Amino Acid Sequence	Antigenicity Score	Surface Accessibility	Hydrophilicity	Flexibility
181–187	[Sequence]	1.024	High	6.312	High
147–153	[Sequence]	1.010	Moderate	5.2	Moderate
30–38	[Sequence]	1.008	Moderate	4.9	Moderate

Homology modeling and structural validation confirmed the reliability of the predicted 3D structure [11].

DISCUSSION

Efficient biomass recovery is critical for the economic viability of microalgal bioproducts. Flocculation, particularly using ferric chloride, offers a cost-effective, rapid, and environmentally friendly alternative to centrifugation or filtration. The recovery efficiency depends on flocculant type, concentration, pH, and cell density, with higher biomass concentrations favoring floc formation [12].

For ILTV vaccine design, gB protein represents an ideal antigen due to its central role in viral entry and its immunogenic potential. The integration of immunoinformatics approaches allows identification of high-probability epitopes, structural validation, and physicochemical characterization, expediting the development of recombinant vaccines while reducing reliance on animal models [13]. The PH-1 region, with its high antigenicity and structural stability, provides a promising target for subunit vaccine formulations.

Combining chemical biomass recovery and computational vaccine design illustrates a multi-disciplinary approach to biotechnology, optimizing both production efficiency and disease control. Future studies should explore experimental validation of predicted epitopes, large-scale flocculation, and integration of microalgal bioproducts with vaccine production platforms.

CONCLUSION

This review highlights ferric chloride as the most efficient flocculant for *C. sorokiniana* biomass recovery, achieving high yields in minimal time. Zinc sulfate is unsuitable for this strain. Immunoinformatics analysis of ILTV gB identified the PH-1 region as highly antigenic, structurally stable, and suitable for vaccine development. The combination of optimized flocculation and computational epitope mapping provides a cost-effective, scalable, and environmentally sustainable framework for microalgal biomass utilization and ILTV vaccine design. Further experimental studies are warranted to validate these findings in vitro and in vivo.

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