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# A Review of Discovery Strategies and Production Technologies for Natural Bioactive Peptides

Natural bioactive peptides refer to short amino acid sequences derived from biological sources such as animals, plants, and microorganisms that possess specific biological activities. They play crucial roles in regulating physiological functions, defending against pathogens, and facilitating cell communication, making them vital resources for new drug development, functional food, and cosmetic formulations. This paper provides a systematic review of the primary strategies for discovering and production technologies for natural bioactive peptides.

## I. Strategies for Discovering Natural Bioactive Peptides

The discovery of novel natural bioactive peptides primarily relies on three complementary strategies: traditional isolation and identification, omics-based mining, and computer-aided design.

### 1. Traditional Separation and Bioactivity-Directed Screening

This is the most classical approach. First, crude extracts of proteins or peptides are isolated from biological materials, followed by stepwise separation and purification using chromatographic techniques. The entire

process is guided by bioactivity assays. For example, components are separated from animal venoms, plant extracts, or fermentation broths using ion exchange, gel filtration, and reverse-phase high-performance liquid chromatography (HPLC). Concurrently, their antioxidant, antibacterial, or enzyme-inhibitory activities are measured, tracking the bioactivity until a pure compound is obtained. Finally, mass spectrometry and sequencing determine its amino acid sequence. This method is direct and reliable but involves cumbersome procedures, is time-consuming, and may overlook low-abundance peptides.

2. Genome- and Proteome-Based Mining

With advances in bioinformatics, this approach has become the mainstream method for high-throughput discovery of novel peptides.

Genome Mining: Analyzes an organism's genomic sequence using bioinformatics tools to predict gene sequences potentially encoding bioactive peptides. Particularly suitable for microorganisms, where analysis of gene clusters can predict novel antimicrobial peptides or other peptides involved in ribosomal synthesis and post-translational modifications.

Transcriptome and Proteome Mining: Direct analysis of all mRNAs or proteins in tissues or cells under specific conditions. By comparing expression differences across various physiological or pathological states, potential functional peptides and their precursor proteins can be identified.

This method systematically reveals the composition and dynamics of endogenous peptides within organisms.

3. Computer-Aided Design and Virtual Screening

This represents a “top-down” rational design strategy. First, establish a database of sequence-activity relationships for known active peptides or develop pharmacophore models. Then, train models using machine learning or deep learning algorithms to predict the potential activity of newly designed peptides or those in virtual libraries. Researchers can perform molecular docking simulations based on the 3D structure of specific protein targets to design peptide sequences capable of binding to them. This approach significantly reduces the blindness and workload associated with experimental screening.

II. Production Technology for Natural Bioactive Peptides

After obtaining the sequence information of the target peptide, efficient and economical large-scale production is required. The primary techniques include chemical synthesis, microbial fermentation, and enzymatic conversion.

1. Chemical Synthesis Method

Solid-phase peptide synthesis: This is a common method for laboratory synthesis of custom peptides. The carboxyl group of the first amino acid is immobilized on a solid-phase carrier. Subsequently, the next amino acid, with its amino group protected, is sequentially linked

according to the predetermined sequence. Through cycles of deprotection, coupling, and washing, the complete peptide chain is ultimately cleaved from the carrier and purified. This method offers flexibility, including the incorporation of non-natural amino acids. However, costs increase significantly with peptide chain length, and it may produce racemic byproducts, making it unsuitable for large-scale production of long peptides.

Liquid-Phase Peptide Synthesis: Suitable for industrial production of specific medium-length peptides. Fragment coupling occurs in solution. Although step management is more complex, it may offer cost advantages over SPPS for large-scale production.

2. Microbial Fermentation (Recombinant DNA Technology)

This represents the most promising industrial method for producing long-chain or complex peptides. The fundamental workflow involves inserting the gene sequence encoding the target peptide into an expression vector, which is then transferred into host cells such as engineered bacteria or yeast. Through fermentation, the host cells synthesize the target peptide using their own translational machinery. Finally, the cells are lysed, and the peptide is isolated and purified. E. coli and Pichia pastoris are commonly used hosts. This method offers high yields and relatively low costs, making it suitable for large-scale production. However, for peptides containing multiple disulfide bonds or complex post-translational modifications,

selecting an appropriate host system is crucial to ensure proper folding and activity of the final product.

3. Enzymatic Conversion (Bio-Enzymatic Hydrolysis)

This is the primary method for producing bioactive peptides from natural protein sources, particularly suited for functional food ingredient production. Food-grade proteases hydrolyze animal or plant proteins under mild conditions, cleaving macromolecular proteins into bioactive small peptide fragments. By controlling parameters such as protease type and degree of hydrolysis, mixtures of peptides with different activities can be selectively released. This method is safe, mild, and cost-effective, but the products are typically mixtures of multiple peptides, making isolation of a single pure compound challenging.

III. Comparison and Selection of Different Production Technologies

Chemical Synthesis: Advantages include high precision and flexibility, enabling synthesis of any sequence. It is suitable for producing short peptides, non-natural amino acid-containing peptides, or modified peptide drugs. Disadvantages include significant environmental impact and extremely high costs for long peptides.

Microbial Fermentation: Advantages include suitability for large-scale production of long-chain peptides with favorable cost-effectiveness. Disadvantages involve high technical barriers, lengthy process development cycles, and potential difficulties in expressing structurally

complex peptides.

Enzymatic Conversion: Advantages include a simple, green, and safe process yielding natural products, making it highly suitable for large-scale preparation of food and cosmetic ingredients. Disadvantages include the production of mixtures, typically with low active peptide content and purity.

The choice of technical route depends on the target peptide's sequence length, structural complexity, application requirements, and production cost considerations.

IV. Challenges and Future Outlook

The field currently faces several core challenges: First, efficiently screening and identifying true key active peptides from complex mixtures is time-consuming and labor-intensive. Second, many peptides exhibit poor in vivo stability and short half-lives, limiting their drug potential. Third, balancing cost, yield, and product activity during large-scale production remains a significant hurdle.

Future development trends will focus on:

Technology Convergence: Integrating genomic mining, AI prediction, and high-throughput experimental validation to form an intelligent discovery closed-loop.

Rational Design: Developing next-generation peptides with enhanced stability, potency, and reduced toxicity based on deep understanding of peptide structure-activity relationships.

Green Manufacturing: Optimizing fermentation and enzymatic hydrolysis processes to develop more efficient, environmentally friendly large-scale production technologies.

Delivery System Innovation: Developing novel delivery technologies to enhance oral bioavailability and targeting of peptide therapeutics.

## V.Conclusion

The discovery and production of naturally occurring bioactive peptides constitute a multidisciplinary field. Traditional isolation methods, omics-based discovery, and computational design form the three pillars of modern peptide discovery. Meanwhile, chemical synthesis, microbial fermentation, and enzymatic conversion provide comprehensive technical solutions spanning from laboratory research to industrial-scale production. With the continuous advancement and integration of these technologies, more highly efficient and safe natural bioactive peptides will undoubtedly be discovered and applied in the future, providing sustained momentum for the development of the pharmaceutical, healthcare, and food industries.