# Lack of Acute Toxicity and Mutagenicity from Recombinant *Epinephelus lanceolatus* Piscidin Expressed in *Pichia pastoris*

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**Abstract**

The study aims to evaluate the safety of recombinant piscidin (rEP) from groupers as a potential eco-friendly alternative to antibiotics in aquaculture.
Researchers conducted six standardized OECD tests covering oral and respiratory toxicity, eye irritation, skin sensitization, and genetic mutations.
The results showed that rEP has no acute toxicity, no skin sensitization, and no mutagenicity, only causing mild, temporary eye irritation.
Consequently, rEP is considered a safe feed additive for livestock and aquatic animals.

**Winpact Fermentation System**

## Introduction

In response to the global crisis of multidrug-resistant "superbugs" and the stagnation in antibiotic development, there is an urgent need for alternatives such as **antimicrobial peptides (AMPs)**. This study focuses on **piscidin (rEP)** derived from the giant grouper, which eliminates bacteria by disrupting their cell membranes. Experimental results demonstrate that rEP not only enhances animal growth and immunity but has also passed rigorous **toxicological safety assessments**—including oral, respiratory, dermal, and genetic tests—proving it to be a safe and promising antibiotic substitute for use as a feed additive in livestock and aquaculture.

**Materials and Methods**

A yeast expression vector with the DNA sequence code for piscidin inserted after a methanol-inducible AOX promoter was cloned.

After transformation into Pichia pastoris, a single colony was inoculated into 200 mL buffered glycerol-complex medium (BMGY) with PTM4 medium for 36 h at 28 °C, 200 rpm.

The culture was transferred to a 5 L fermenter unit (Winpact, Major Science, Taoyuan, Taiwan) containing 3 L commercial culture medium (BMGY and PTM4 medium).

During fermentation, the temperature was maintained at 30 °C.

**Results**

Superbug resistance in aquaculture causes massive economic and food security losses.

This study used OECD guidelines to prove that the antimicrobial peptide piscidin has negligible toxicity.

Piscidin is a safe and powerful eco-friendly alternative to antibiotics for aquaculture fodder.

**References**

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