



Establishment and characterization of a novel cell line derived from spotted sea bass *Lateolabrax maculatus* and its susceptibilities to iridoviruses

Zhipeng Zhan^{a,1}, Xueqian Cao^{a,1}, Jie Su^{a,1}, Yangchi Cui^a, Yang Zheng^a, Pengli Xiao^a, Zhoutao Lu^a, Shaoping Weng^b, Changjun Guo^{a,b,*}, Jianguo He^{a,b}

^a School of Marine Sciences, State Key Laboratory for Biocontrol, Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Guangdong Provincial Key Laboratory of Marine Resources and Coastal Engineering & Guangdong Provincial Observation and Research Station for Marine Ranching of the Lingdingyang Bay, Sun Yat-sen University, 135 Xingang Road West, Guangzhou 510275, PR China

^b Guangdong Province Key Laboratory for Aquatic Economic Animals, School of Life Sciences, Sun Yat-sen University, 135 Xingang Road West, Guangzhou 510275, PR China

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ABSTRACT

The spotted sea bass (*Lateolabrax maculatus*), which holds significant commercial value, is extensively cultivated in China, Japan, and Southeast Asian countries. This study reports the establishment and characterization of a novel continuous cell line, designated as LMK cells, derived from the kidney tissue of spotted sea bass. The LMK cell line, primarily composed of epithelial-like cells, has been successfully subcultured in Medium 199 supplemented with 10 % fetal bovine serum (FBS) for over 60 passages. Mitochondrial genome sequencing and *cytochrome c oxidase subunit I* gene amplification verified the origin of the LMK cells from *L. maculatus*, distinguishing them from *L. japonicus*. Optimal culture conditions for LMK cells at passage 35 were determined to be 10 % FBS at 27 °C. Chromosomal analysis indicated that the cell line possesses 48 chromosomes. To assess viral susceptibility, LMK cells were exposed to the red sea bream iridovirus and largemouth bass ranavirus. Both iridoviruses induced cytopathic effects within 3 days post-infection and triggered immune-related gene responses. This study demonstrates that the LMK cell line is a valuable resource for molecular, virological, and immunological research in *L. maculatus* and other sea bass species.

1. Introduction

Cell lines are indispensable tools in biological research. They provide a robust platform for investigating cell growth, development, metabolism, and function (Grimm, 2004). Compared to *in vivo* experiments, *in vitro* cell-based studies exhibit reduced variability between samples, decreased experimental time, and enhanced cost efficiency (Manciocca et al., 2009; Vitale et al., 2009). Furthermore, specific cell lines can be employed for virus amplification, vaccine development, and studies in virology, immunology, and toxicology (Collet et al., 2018; Ye et al., 2006). Therefore, the establishment of species-specific and tissue-specific cell lines is of paramount importance for research focused on particular species.

The spotted sea bass (*Lateolabrax maculatus*) is widely distributed

along the western coast of the Korean Peninsula, the mainland coast of China, the coast of Vietnam, and the coast of Kyushu, Japan (Liu et al., 2024). Historically, *L. maculatus* and the Japanese sea bass (*Lateolabrax japonicus*) were classified as a single species until enzyme studies in the 1990s differentiated them (Yokogawa et al., 1997; Han et al., 2015). While numerous cell lines have been developed from the *L. japonicus* (Huang et al., 2021; Chenet et al., 2003; Ye et al., 2006; Yan et al., 2024a; Le et al., 2017; Liu et al., 2022), only two cell lines derived from *L. maculatus* have been established to date (Yan et al., 2024a; Liu et al., 2022), and there are no reports on kidney-derived cell lines from this species.

As one of the top five marine aquaculture fish species in China, the spotted sea bass holds significant economic importance (Zhang et al., 2022). However, in recent years, viral diseases such as iridoviruses have

* Corresponding author at: School of Marine Sciences, State Key Laboratory for Biocontrol, Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Guangdong Provincial Key Laboratory of Marine Resources and Coastal Engineering & Guangdong Provincial Observation and Research Station for Marine Ranching of the Lingdingyang Bay, Sun Yat-sen University, 135 Xingang Road West, Guangzhou 510275, PR China.

E-mail address: gchangj@mail.sysu.edu.cn (C. Guo).

¹ These authors contributed equally to this work.

inflicted substantial economic losses on the sea bass aquaculture industry (Seo et al., 2016; Yan et al., 2024b). The establishment of virus-sensitive cell lines has facilitated the understanding of viral pathogenesis and promoted advancements in vaccine development (Zeng et al., 2023b; Zeng et al., 2023a). In this study, we report the establishment of a novel cell line derived from the kidney of spotted sea bass and its susceptibility to iridoviruses, including red sea bream iridovirus (RSIV) and largemouth bass ranavirus (LMBV). This cell line is poised to be a valuable resource for virus amplification and vaccine development against these viral threats.

2. Materials and methods

2.1. Fish and viruses

Healthy *L. maculatus* specimens, measuring 10 ± 0.5 cm in length and weighing 50 ± 5 g, were procured from a fish farm in Guangdong Province, China. The LMBV and RSIV strains were isolated from a fish farm in Zhuhai, Guangdong Province, and stored in our laboratory for subsequent experimentation.

2.2. Primary cell culture and subculture

Primary cell culture procedures were adapted from Jia et al., 2022, with minor modifications. Fish samples were disinfected with 75 % ethanol prior to excision of kidney tissue. The tissues were washed thoroughly with phosphate-buffered saline (PBS) supplemented with antibiotics (200 IU/mL penicillin and 200 µg/mL streptomycin; Gibco, USA), and subsequently minced into fine pieces (2 mm^3). Digestion was performed using 0.25 % trypsin solution at 27°C for 30 min, followed by centrifugation at 1300 rpm for 3 min. The resultant pellet was resuspended in 1 mL Medium 199 (M199; Elgbio, CN) containing 20 % fetal bovine serum (FBS; Hyclone, USA), antibiotics (100 IU/mL penicillin and 100 µg/mL streptomycin), and seeded into six-well plates. Cells were maintained at 27°C under 5 % CO_2 , with medium changes occurring twice weekly. Monolayer cells were subcultured using 0.25 % trypsin solution at a 1:2 split ratio. From the 10th passage, FBS

concentration was reduced to 15 %, and to 10 % at the 20th passage.

2.3. Cell cryopreservation and recovery

LMK cells were cryopreserved at regular intervals of every 10 passages. Prior to cryopreservation, cells were digested, centrifuged at 1300 rpm for 3 min, counted, and resuspended in cryopreserving medium (90 % FBS and 10 % DMSO). Cells were then transferred to cryovials, cooled overnight at -80°C , and subsequently stored in liquid nitrogen at -196°C . For recovery, cryovials were recovered in a 37°C water bath, resuspended in M199 complete medium, and seeded into culture flasks. The medium was renewed after 24 h to eliminate residual DMSO. The post-thaw survival rate was assessed using Countess 3 FL (ThermoFisher, USA) after staining with trypan blue.

2.4. Mitochondrial genome sequencing and annotation

Total genomic DNA was extracted from LMK cells at the 35th passage and kidney tissue using a DNA isolation mini kit (Vazyme, CN). The mitochondrial genome was sequenced, and the assembly was facilitated using MitoZ v3.6 based on the short-reads data. The assembled mitochondrial genome was compared to known sequences in the National Centre for Biotechnology Information (NCBI) database via BLAST. Comparative species included *L. maculatus* (NC_029318.1), *L. japonicus* (NC_042503.1), *L. latus* (NC_030375.1), *Dicentrarchus labrax* (NC_026074.1), *D. punctatus* (NC_026075.1), *Morone saxatilis* (NC_014353.1), *Lates calcarifer* (NC_007439.1) and *Decapterus maruadsi* (NC_024556.1).

2.5. Chromosome analysis

Chromosomal counts were performed on LMK cells at the 35th passage. Cells at 80 % confluence were treated with 5 µg/mL colchicine (Sigma, USA) for 4 h at 27°C , followed by fixation and staining protocols standard for cytogenetic analysis as previously described (Zhan et al., 2023). Chromosome spreads were prepared, stained with 10 % Giemsa solution, and counted under a light microscope (Leica, Germany). A total of 100 spreads were analyzed.

2.6. Cell growth characteristics

The growth curve of LMK cells at the 35th passage was determined by seeding cells into 12-well plates at 1×10^5 cells per well. Cells were cultured in various media conditions including DMEM (Elgbio, CN), M199 (Elgbio, CN), Leibovitz's L-15 (Elgbio, CN), or DMEM/F-12 (Elgbio, CN) within 10 % FBS, under varying temperatures (27°C , 32°C , and 37°C) and CO_2 conditions (5 % CO_2 for all media except Leibovitz's L-15). Cells proliferation was monitored every 2 days, using Countess 3 FL.

2.7. Ethynyl-2'-deoxyuridine (EdU) click chemistry assays

Cell proliferation was evaluated using the BeyoClick™ EdU Cell Proliferation Kit with Alexa Fluor 555 (Beyotime, CN). LMK cells at the 35th passage were incubated with EdU solution for 2 h, fixed with 4 % paraformaldehyde for 10 min, washed with PBS, and permeabilized with 0.5 % Triton-X 100 in PBS for 20 min. Cells were incubated with a reaction cocktail containing Alexa Fluor 555, washed, stained with Hoechst 33342 for 10 min, and imaged using a fluorescent microscope (Leica, Germany) under 405 and 488 nm excitation.

2.8. Transfection assays

LMK cells at the 35th passage were cultured in six-well plates, and then were transfected with 2 µg of pEGFP-N1 plasmid (Takara, CN) using Lipofectamine 2000 transfection reagent (ThermoFisher, USA) following the manufacturer's protocols. For green fluorescent protein

Table 1
The primers used in this study for PCR.

Primers	Sequences (5' - 3')
<i>lmCOX1-518F</i>	CCCTATTTGTCAGCGT
<i>lmCOX1-518R</i>	GTTAAGCCCCGACTGTGAA
<i>lmβ-actin-F</i>	CAACTGGATGACATGGAGAAG
<i>lmβ-actin-qR</i>	TTGGCTTGGGGTTCAAG
<i>LMBV-MCP-qF</i>	GTCACCCCTGCCCTAACAGAAA
<i>LMBV-MCP-qR</i>	CACGATGGGTTGACTTCTCC
<i>LMBV-018L-qF</i>	AGTTTGACGCCAGCTTCACG
<i>LMBV-018L-qR</i>	TGCCATACCGCTGCACCTCG
<i>LMBV-052L-qF</i>	GGGCTCAAGTGCATCAG
<i>LMBV-052L-qR</i>	CAGCGAGACAGCTTCCAAT
<i>RSIV-MCP-qF</i>	GAACAGCTACATCCGCTGGT
<i>RSIV-MCP-qR</i>	AGGGGTTCCAGAACGCAAGG
<i>RSIV-011R-qF</i>	TGACCTGTGCCATTAGATGATAAC
<i>RSIV-011R-qR</i>	AGAGCCAGAGCAGCAGCATGAG
<i>RSIV-054L-qF</i>	TGGCAAATGCGGTCTATGA
<i>RSIV-054L-qR</i>	TGGCCCCAGCGTTGGTAT
<i>lmIL1-qF</i>	GCGGTGGAGGACAGCAGTTG
<i>lmIL1-qR</i>	AGCCCAGTGGTCTGTTGTTGAAG
<i>lmISG15-qF</i>	GTATCAGCAGATGCGGGACA
<i>lmISG15-qR</i>	GGCCTGAAGTTCTGCTGAA
<i>lmIRF3-qF</i>	CAAGAACGACGCTGCTAACCC
<i>lmIRF3-qR</i>	TGTCAGCCCCCTTACAGACTCC
<i>lmTNFα-qF</i>	GATCGTCATCCCACAAACCG
<i>lmTNFα-qR</i>	GCTTTGCTGCCATTGGAGTC
<i>lmMAVS-qF</i>	GTCCAAAACATTCCAAAGGCC
<i>lmMAVS-qR</i>	GCAATAACCAAAGAAATCCCCA
<i>lmIRF7-qF</i>	TCACCCAGACCCAAGAAAG
<i>lmIRF7-qR</i>	TGTAGTCAGGAGAGCCACCAT

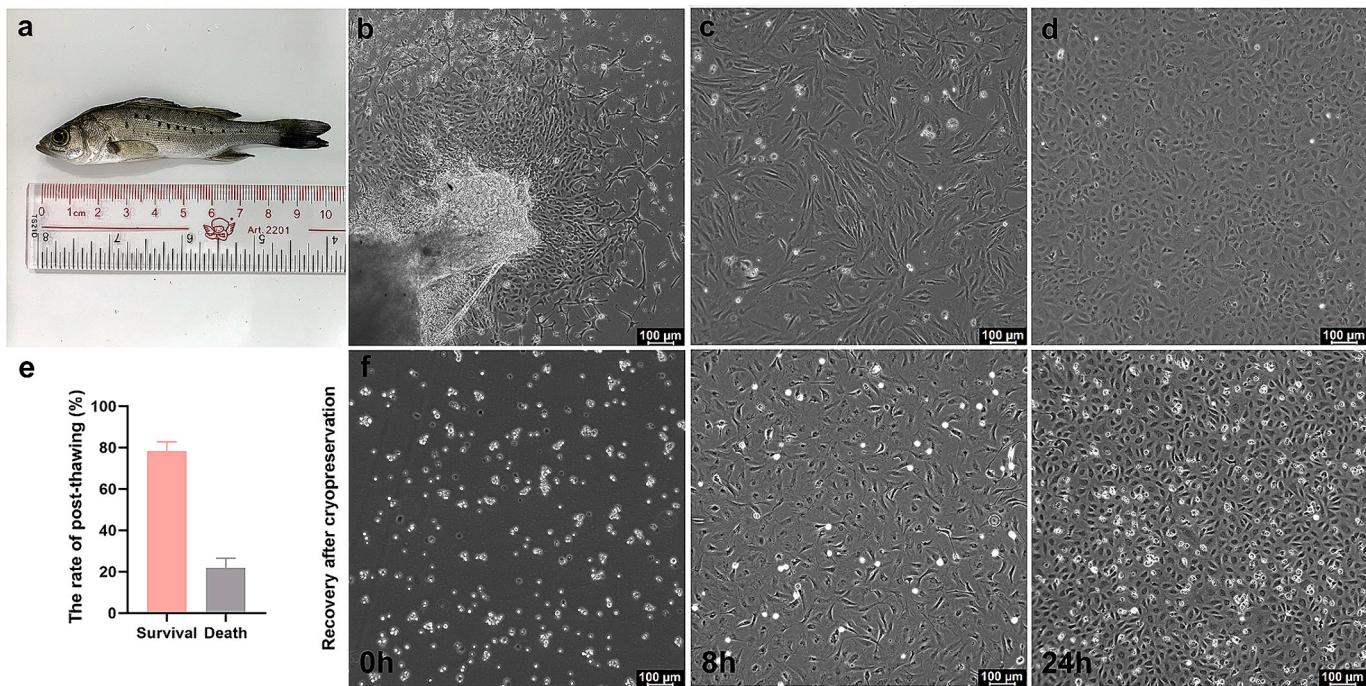


Fig. 1. Morphology characteristics and identification of the LMK cells. (a) Sea bass used for primary cell culture. (b) Primary cells observed. (c) Cells at the 20th passage. (d) Cells at the 40th passage. (e) Survival rate after cryopreservation. (f) the morphological photograph after thawing. Scale bar represents 100 μm . Data presented as mean \pm SD ($n = 3$).

(GFP) mRNA transfection, Hieff Trans mRNA Transfection Reagent (YEASEN, CN) was employed. Green fluorescent protein (GFP) expression was recorded under a fluorescent microscope 24 h post-transfection. Transfection efficiency was assessed by using Countess 3 FL with matching fluorescent accessories. Three biological replicates were analysis in each transfection.

2.9. Viral challenge experiments

LMK cells at the 35th passage were seeded into 12-well plates and infected with RSIV or LMBV at a multiplicity of infection of 2. Infected cells were maintained at 27 °C, and cytopathic effects (CPEs) were monitored on the 3rd day post-infection under a light microscope. Triplicate samples for DNA and RNA analysis were collected at 1, 3, 5, and 7 days post-infection (dpi). RNA samples were also collected at 48 h post-infection (hpi) for immune-related genes analysis.

2.10. Viral load quantification by absolute quantitative PCR (qPCR)

Viral genomic copies were quantified via qPCR using gene-specific primers targeting the major capsid protein genes of RSIV (*rsiv-mcp*) and LMBV (*lmbv-mcp*). DNAs were extracted from infected cells using a DNA isolation mini kit (Vazyme, CN), and qPCR was performed using as previously described (Luo et al., 2023). The gene-specific primers are listed in Table 1, and a standard curve was generated for absolute quantification.

2.11. Gene expression analysis by quantitative reverse transcription PCR (RT – qPCR)

The total RNA was isolated from infected cells using a total RNA extraction kit (Promega, CN) in accordance with the manufacturer's protocol. RNA samples were reverse-transcribed to cDNA by using the PrimeScript RT Reagent Kit (Takara, CN) as previously described (Zeng

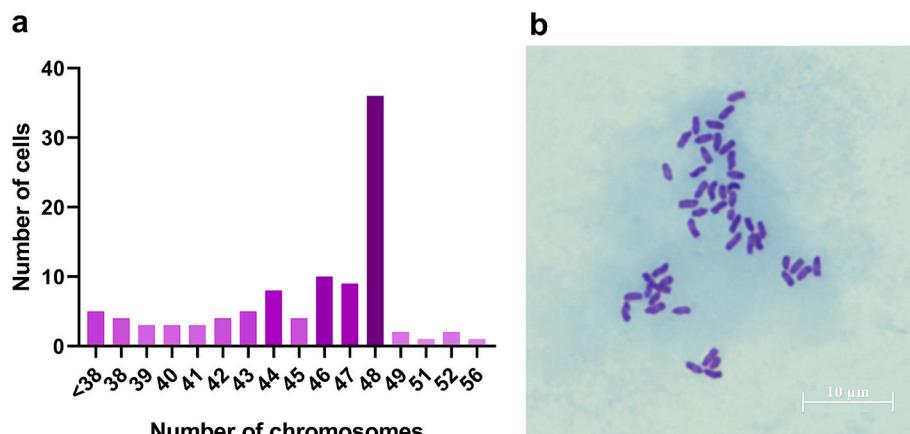


Fig. 2. Karyotype analysis of the LMK cells. (a) Chromosome number distribution in 100 metaphase LMK cells at the 35th passage. (b) Metaphase chromosomes stained with Giemsa. Scale bar represents 10 μm .

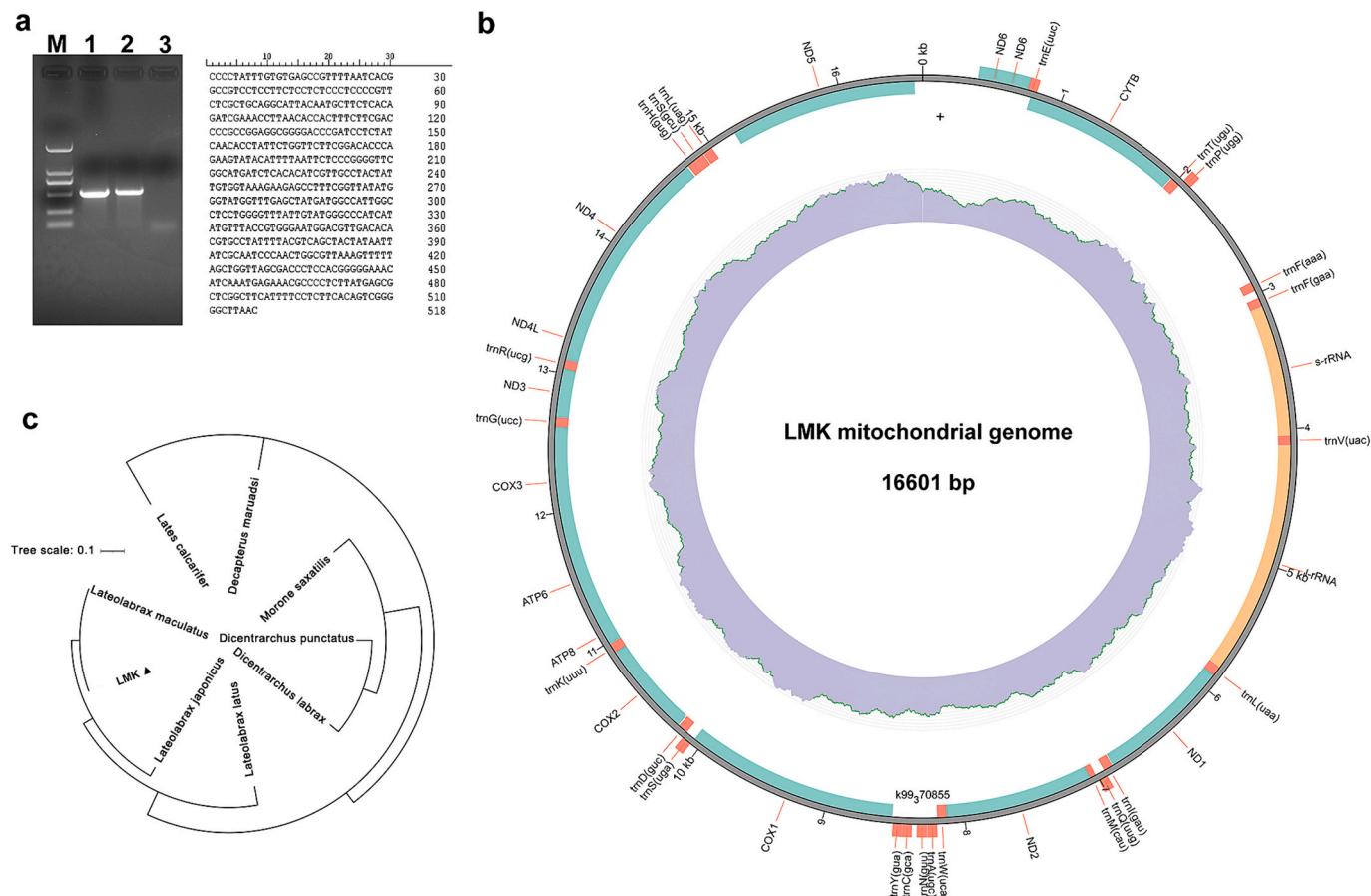


Fig. 3. Species identification of the LMK cell line. (a) PCR amplification of the *cox1* fragment from LMK cells and tissue, accompanied by the fragment sequence. Lane assignments: 2000 bp marker (M), LMK cells (lane 1), kidney tissue of *L. maculatus* (lane 2), negative control (lane 3). (b) Distribution of annotated genes on the mitochondrial genome, with the inner ring indicating relative read coverage. (c) Phylogenetic analysis based on mitochondrial sequences.

et al., 2023a, 2023b). The gene-specific primers used are listed in Table 1. The results of RT – qPCR were calculated according to the $2^{-\Delta\Delta CT}$ using β -actin gene as an internal control.

2.12. Statistical analysis

Data analyses were performed using GraphPad Prism software, and results are presented as mean \pm standard deviation (SD). Experiments were conducted in triplicate unless stated otherwise. Statistical significance was determined using two-tailed unpaired Student's *t*-tests, with $p < 0.05$ considered significant and $p < 0.01$ considered highly significant.

3. Results

3.1. Cell growth and morphology

Healthy spotted sea bass were selected for kidney tissues extraction, which was subsequently minced and digested to establish primary cell culture (Fig. 1a). During primary culture, cells migrated continuously from the tissue explants, forming a complete monolayer by day 7 (Fig. 1b). Initially, M199 with 20 % FBS was used, with a gradual decrease in FBS concentration by 5 % every 10 passages until reaching 10 %. Subculturing was performed at a 1:2 ratio every 2–3 days (Fig. 1c). Following extensive passaging (>40 passages), the cells adapted, and antibiotics were omitted from the culture media. Up to now, the cells have undergone over 60 passages, while maintaining an epithelial-like morphology for over 30 passages (Fig. 1d). Post-thaw survival rates remained at 80 % after three months of cryopreservation (Fig. 1e), without morphological alterations (Fig. 1f). These cells were designated

as the LMK cell line.

3.2. Chromosome analysis

Chromosome analysis of the 35th passage LMK cell line revealed a range of chromosome numbers from 36 to 56, with a modal number of 48 observed in 36 % of the counted metaphase cells (Fig. 2a). Giemsa stain of the LMK cell metaphase chromosome spread showed the mode (Fig. 2b), which is consistent with the typical chromosome count of *L. maculatus* ($2n = 48$) (Shao et al., 2018).

3.3. Characterization of the LMK Cell Line

The species origin of the LMK cell line was confirmed through *cytochrome c oxidase subunit I* (*cox1*) gene amplification and mitochondrial genome sequencing. DNA was extracted from the original spotted sea bass tissue. PCR products showed a consistent base pair size of 516 bp (Fig. 3a). The assembled mitochondrial genome spanned 16,601 bp, annotating mitochondrial-specific genes such as *cytochrome oxidase* and *ATP synthase* genes. It encompassed 14 protein-coding genes, 23 tRNAs, and 2 rRNAs (Fig. 3b). Phylogenetic tree analysis and sequence comparison with the NCBI database revealed 99.86 % similarity to *L. maculatus*, distinguishing the LMK cells from *L. japonicus* (Fig. 3c).

3.4. Optimization of culture condition

Optimal culture conditions for the LMK cell line were determined using the 35th passage cells. Cells were cultured in various media conditions, including DMEM, M199, Leibovitz's L-15, or DMEM/F-12 each

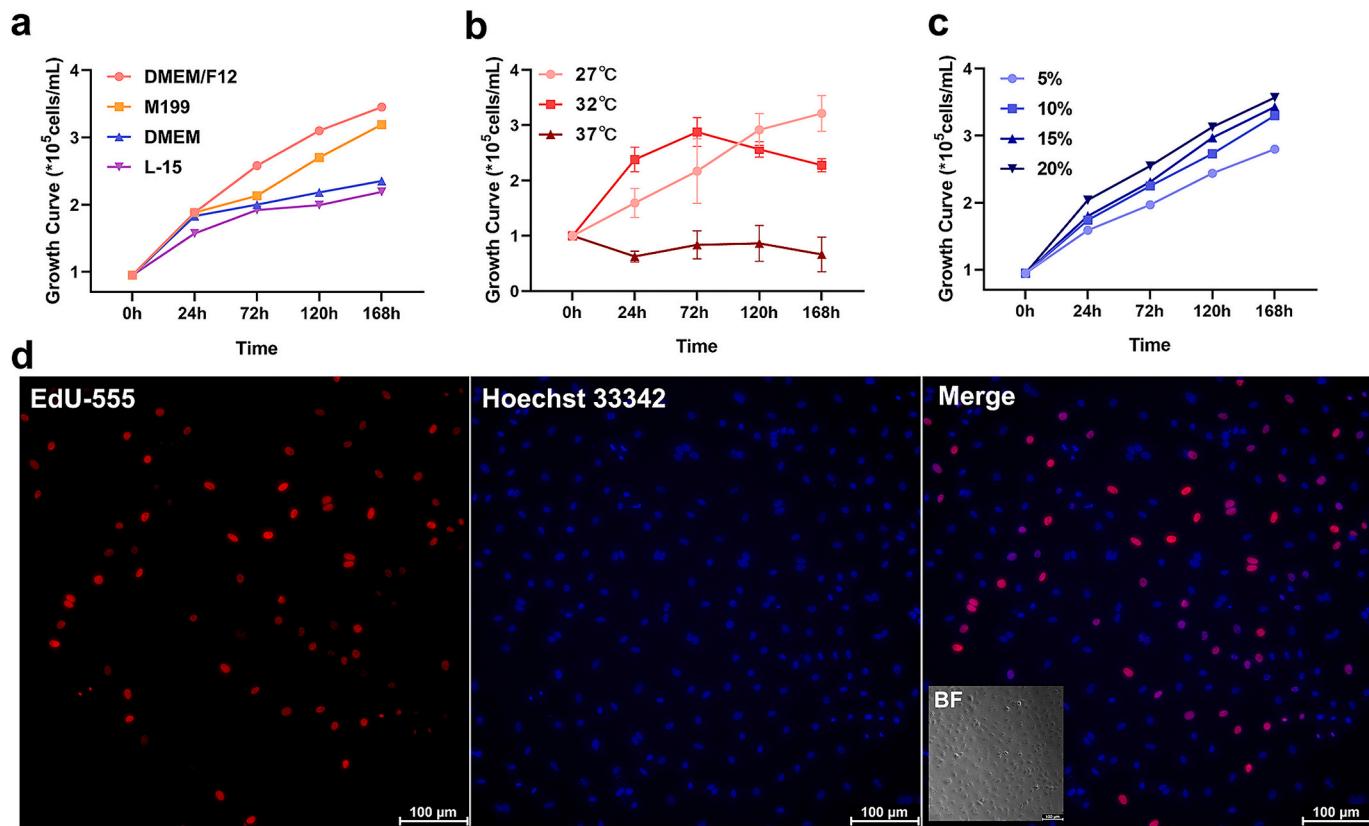


Fig. 4. Growth kinetics of the 35th passage LMK cells. (a) Impact of different media on cell growth (with 10 % FBS). (b) Effect of varying temperatures on cell growth in M199 medium with 10 % FBS. (c) Influence of different FBS concentrations (5 %, 10 %, 15 %, and 20 %) on cell growth in M199 medium at 27 °C. (d) EdU-555 and Hoechst 33342 labeling of LMK cells. Scale bar represents 100 µm. Data presented as mean ± SD ($n = 3$).

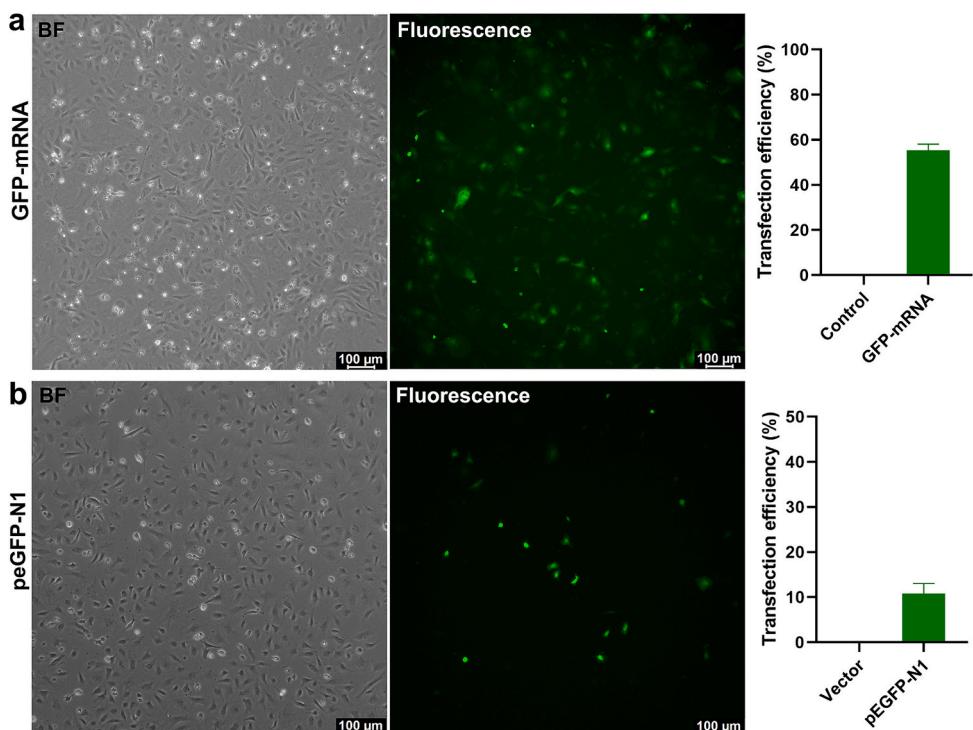


Fig. 5. Gene transfection of the 35th passage LMK cells. (a) Visualized and transfection efficiency with GFP-mRNA. (b) Visualized and transfection efficiency with pEGFP-N1. Scale bar represents 100 µm. Data presented as mean ± SD ($n = 3$).

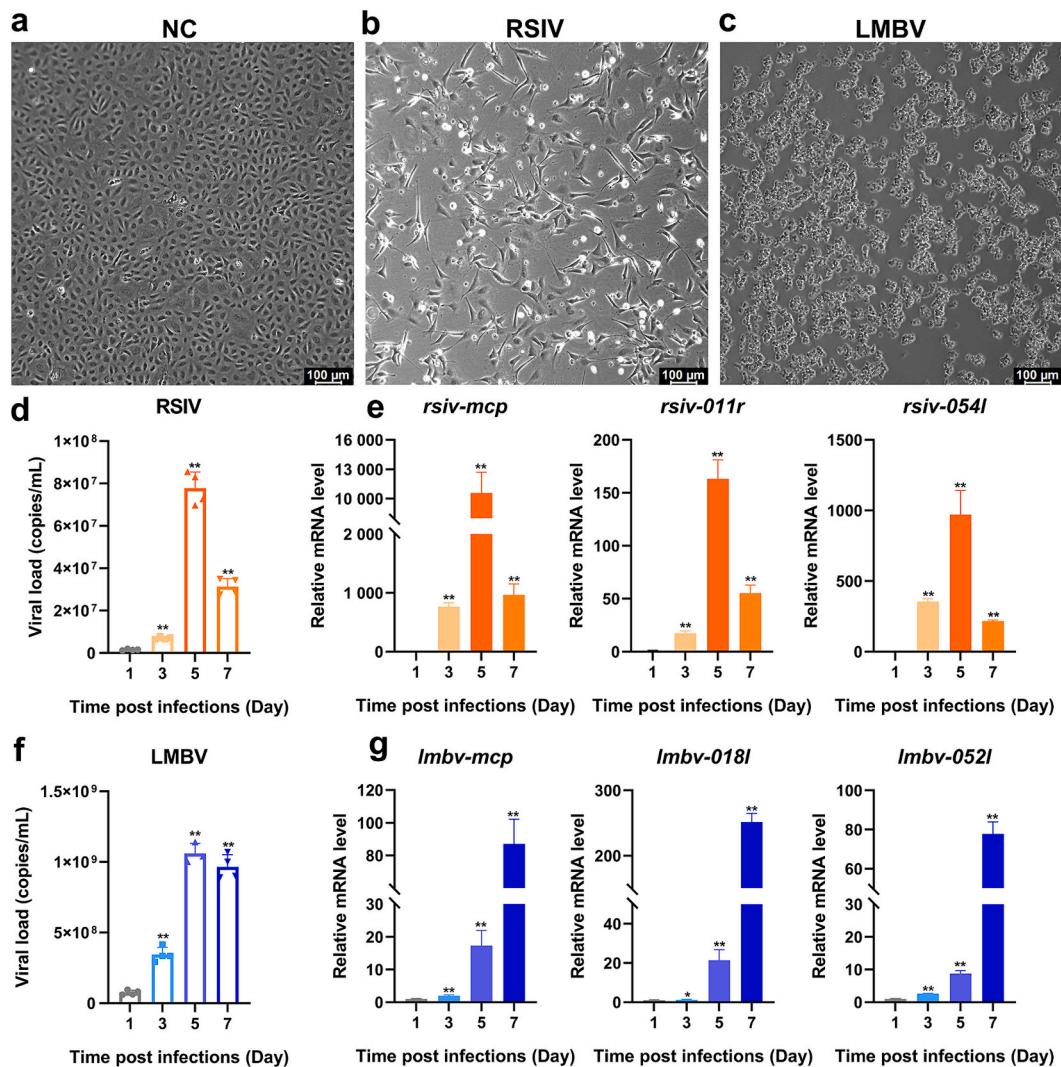


Fig. 6. Susceptibility of LMK cells to RSIV and LMBV. (a – c) Representative images of control (a) and CPEs in LMK cells infected with RSIV (b) and LMBV (c) at 3 dpi. (d) qPCR determination of RSIV viral load (genome copies). (e) RT – qPCR analysis of RSIV transcription levels at different dpi. (f) qPCR determination of LMBV viral load (genome copies). (g) RT – qPCR analysis of LMBV transcription levels at different dpi. Statistical significance denoted by asterisks: **p* value<0.05, and ***p* value<0.01.

containing 10 % FBS, under 27 °C with CO₂ conditions (5 % CO₂ for all media except Leibovitz's L-15). The cells grew well in all tested media, with superior growth observed in M199 and DMEM/F12 (Fig. 4a). As for the temperatures experiment, M199 with 10 % FBS was used in all groups, and cell growth was robust at temperatures ranging from 27 to 32 °C but ceased at 37 °C (Fig. 4b). Using M199 and a 27 °C culture condition, the cells were capable of growing in media with serum concentrations ranging from 5 % and 20 %, with growth rates positively correlating with serum concentration (Fig. 4c). Using EdU labeling, 30 % of the 35th passage cells exhibited active division (Fig. 4d). The above results indicated that M199 with 10–20 % FBS under 27 °C was appropriate for the growth of LMK.

3.5. Transfection efficiency

To assess the suitability of the LMK cell line for genetic applications, cells were transfected with GFP mRNA or the pEGFP-N1 plasmid. At 24 h post-mRNA transfected, a bright green fluorescent signal was detected in LMK cells (Fig. 5a), estimating 55 % of cells. The pEGFP-N1 plasmid resulted in detectable GFP expression in approximately 10 % of cells after 24 h (Fig. 5b). These results indicated the LMK cell line's potential for *in vitro* genetic studies.

3.6. Viral susceptibility of LMK cells

At 3 dpi, both RSIV and LMBV induced clear CPEs in LMK cells (Fig. 6a–c). By 48 h post-infection (hpi), cells exhibited rounding and typical pathological changes. By 72 hpi, significant cell death was observed, with a notable difference in refractive index between infected and normal cells. qPCR analysis revealed a gradual increase in viral loads over time, peaking on day 5 for RSIV (Fig. 6d) and day 7 for LMBV post-infection (Fig. 6f). To further investigate the viral susceptibilities of LMK cells, the levels of viral mRNAs were determined. The results showed that the levels of viral mRNAs (*rsiv-mcp*, *rsiv-011r*, and *rsiv-054l*) significantly increased in cells at 1–7 dpi with RSIV and peaked on day 5 (Fig. 6e). Similarly, the levels of viral mRNAs (*lmbv-mcp*, *lmbv-018l*, and *lmbv-052l*) were also significantly increased in cells at 1–7 dpi with LMBV and peaked on day 7 (Fig. 6g). These observations suggest that LMK cells are susceptible to RSIV and LMBV.

3.7. Expression levels of immune-related genes

Six immune-related genes, *interleukin-1 beta* (*il-1*), *interferon regulatory factor 3* (*irf3*), *irf7*, *interferon-stimulated gene 15* (*isg15*), *tumor necrosis factor alpha* (*tnfa*) and *mitochondrial antiviral signaling protein*

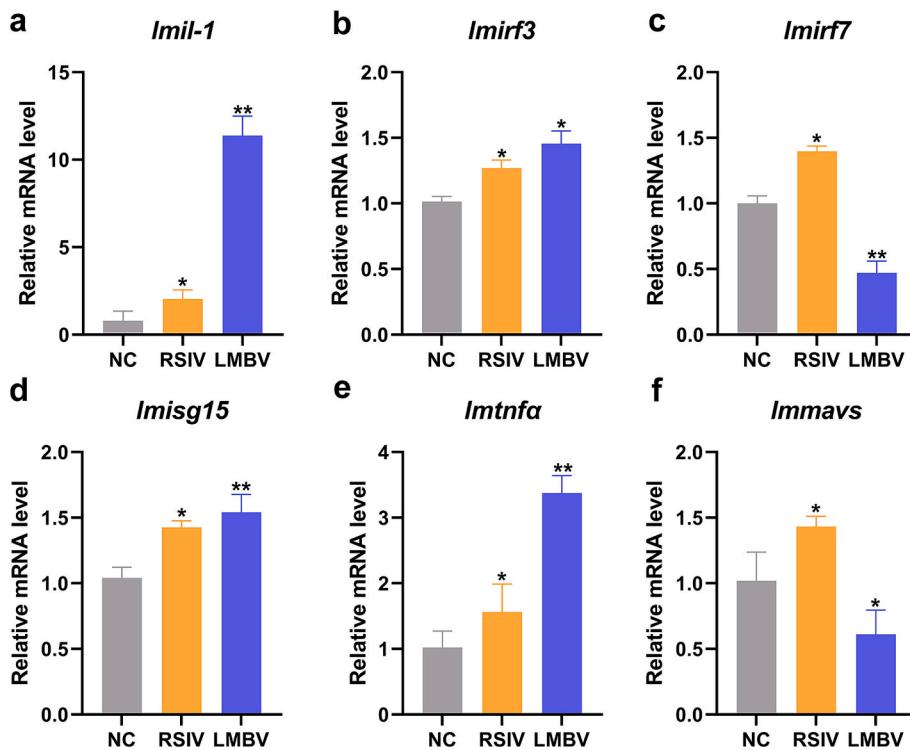


Fig. 7. Expression levels of immune-related genes in LMK cells infected with RSIV or LMBV. Gene analyzed: (a) *lml-1*; (b) *lmirf3*; (c) *lmirf7*; (d) *lmisg15*; (e) *lmtnfa*; (f) *lmmav*. Data presented as mean \pm SD ($n = 3$). Statistical significance denoted by asterisks: * p value <0.05 , and ** p value <0.01 .

(*mav*s), were validated using RT – qPCR (Fig. 7). At 48 hpi with RSIV or LMBV, most genes expressions showed significant upregulation from that in the non-infected group, while the expression levels of *irf7* and *mav*s genes were significantly downregulated in LMBV infected cells. These results indicated that LMK cells could respond to immune stimuli and were appropriate for immunological studies.

4. Discussion

In this study, we successfully established and characterized the first kidney cell line derived from the spotted sea bass *L. maculatus*. Currently, eight cell lines from sea bass have been developed (Chen et al., 2003; Huang et al., 2021; Le et al., 2017; Liu et al., 2022; Yan et al., 2024a; Ye et al., 2006), yet reports on kidney cell lines from sea bass remain scarce. Among these eight cell lines, only three are derived from *L. maculatus*, while the remaining five are reported to originate from the *L. japonicus*. This highlights the necessity to expand the repertoire of cell lines specific to particular species.

Mitochondrial DNA has been extensively used in studies involving fish phylogenetics, species identification, and population structure (Kyle and Wilson, 2007; Yang et al., 2014). Markers such as *cytochrome b* and *cox1* enable preliminary species identification (Pepe et al., 2005). However, for closely related species, comparison using multiple genes is essential. Historically, *L. maculatus* and *L. japonicus* were considered conspecific until recent studies distinguished *L. maculatus* as a separate species (Han et al., 2015; Liu et al., 2006). By sequencing the mitochondrial genome of tissues used to establish the LMK cell line and comparing these sequences with registered species, we confirmed that the LMK cell line's derivation from *L. maculatus* and its phylogenetically distinct from the *L. japonicus*.

Chromosome number and karyotyping are fundamental in cytogenetics for identify in the origin of cell lines. Analysis of 100 metaphases from the LMK cells revealed chromosome numbers ranging from 36 to 56, with a modal number of 48, consistent with the typical chromosome count of *L. maculatus* ($2n = 48$) (Shao et al., 2018). This indicates that

the majority of cells have retained their chromosome number throughout passaging, with minimal spontaneous transformation (Vincent Laizé et al., 2022).

The LMK cell line exhibited stable growth for 50 passages within one year. When cultured in M199 medium supplemented with 10 % FBS, the LMK cells demonstrated robust growth. M199 medium is well-documented for its comprehensive formulation in culturing various fish cells (Morgan et al., 1955). Furthermore, although with low efficiency, the LMK cells successfully expressed exogenous GFP following transfection. Commercial transfection reagents are primarily designed for mammalian cells, and optimal transfection conditions for fish cell lines remain to be refined.

One of the primary applications of fish cell lines lies in virological studies (Liang et al., 2024). Viral diseases impose significant economic losses on aquaculture, and cell lines are crucial for virus detection and isolation (Jyotsna et al., 2020). This study also identified the susceptibility of the LMK cell line to RSIV and LMBV. Noticeable CPEs were observed in RSIV- and LMBV-infected LMK cells, particularly at 3 dpi, where the infected cells became rounded, resembling previously reported CPEs in other cell lines (Oh and Nishizawa, 2016; Wang et al., 2023). qPCR and RT-qPCR analysis of viral loads and relative mRNA expression confirmed the potential of the LMK cell line for the isolation and propagation of RSIV and LMBV. However, it is noteworthy that RSIV replication in LMK cells decreased after 7 dpi. This decline might be attributed to the extensive cytopathological damage by day 5, which reduced the number of viable host cells available for viral replication.

5. Conclusion

In summary, a novel kidney cell line derived from *L. maculatus*, designated as LMK cells, was successfully established and characterized. The LMK cell line demonstrated favorable potential for gene manipulation. Furthermore, it exhibited susceptibility to both RSIV and LMBV, generating substantial viral loads, and responds to viral infection by upregulating immune-related genes. These findings suggest that the

LMK cell line can serve as an effective tool for vaccines development and research on virus-host interactions.

Ethics statement

The spotted sea bass samples were collected from a commercial fish farm. All animal experiments were conducted in accordance with the guidelines for the Care and Use of Laboratory Animals of Sun Yat-sen University (No. 2023032301), ensuring adherence to ethical regulations for animal use. Informed consent was obtained from the owner of the fish farm in Zhuhai, Guangdong province for the use of the fish in this study.

CRediT authorship contribution statement

Zhipeng Zhan: Formal analysis, Methodology, Visualization, Writing – original draft. **Xueqian Cao:** Writing – original draft, Methodology. **Jie Su:** Methodology, Data curation. **Yangchi Cui:** Investigation. **Yang Zheng:** Software. **Pengli Xiao:** Resources. **Zhoutao Lu:** Formal analysis. **Shaoping Weng:** Resources. **Changjun Guo:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Jianguo He:** Funding acquisition.

Declaration of competing interest

The LMK cell line has been granted a national invention patent from the P.R. China (No. CN202410870150.1).

Data availability

The authors affirm that no new genes or proteins were generated in this study, and all analyses were conducted existing data from the databases. The data supporting the findings of this study are provided within the article.

Acknowledgement

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