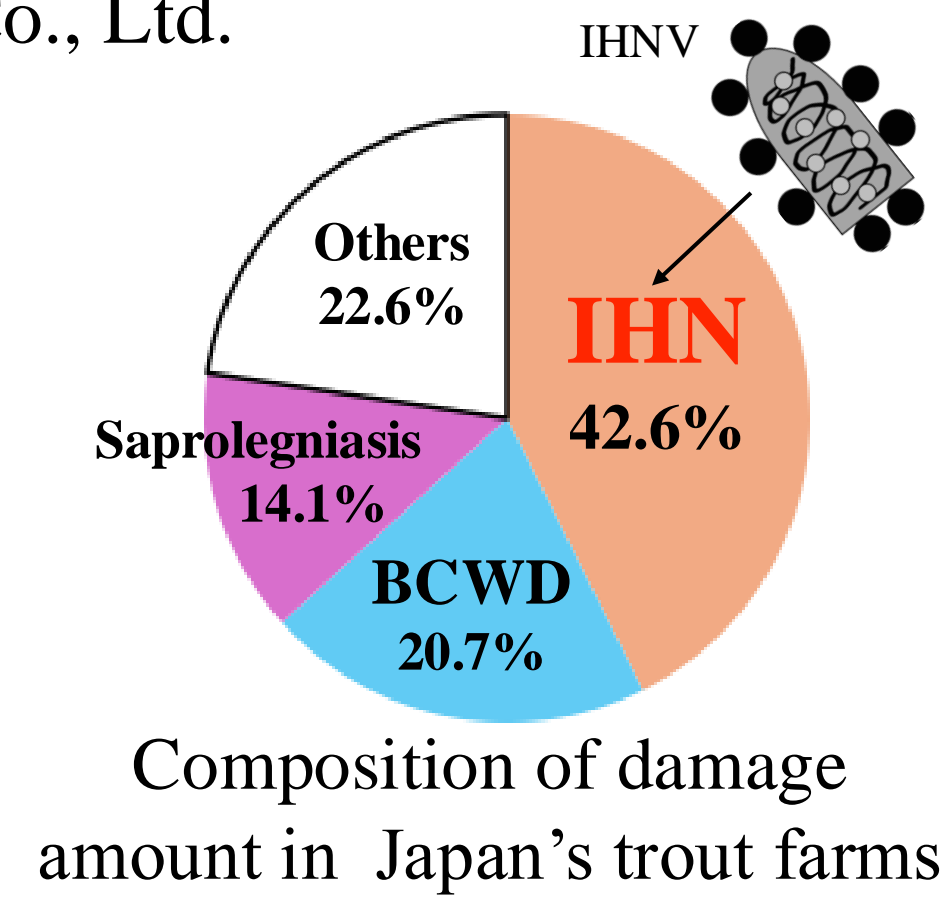




EFFICACY OF NOVEL IMMUNOSTIMULANTS AGAINST INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) INFECTION IN RAINBOW TROUT *ONCORHYNCHUS MYKISS*

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Rainbow trout *Oncorhynchus mykiss* is suitable for cultivation in cold waters, and rainbow trout now accounts for 10% of flesh water aquaculture production in Japan. However, since infectious hematopoietic necrosis virus (IHNV) is causing significant mortality in these cultures, many producers are searching for optimal measures to control communicable diseases in an effort to minimize the potential risk of viral infection. Use of **immunostimulants (IS)** may be employed by fish culturists to reduce losses due to IHN in their facilities¹. The present study was designed to investigate on the efficacy of four **novel IS** (lactic acid bacteria types a, b, yeast, and plant extract) against IHNV infection in rainbow trout, and compared them to the efficacy of high-concentration ascorbic acid supplementation (Ishikawa *et al.*, 2013), which has been previously reported to be effective.



Ishikawa *et al.* (2013)
 Fish Pathology, 48(4), 113-118.

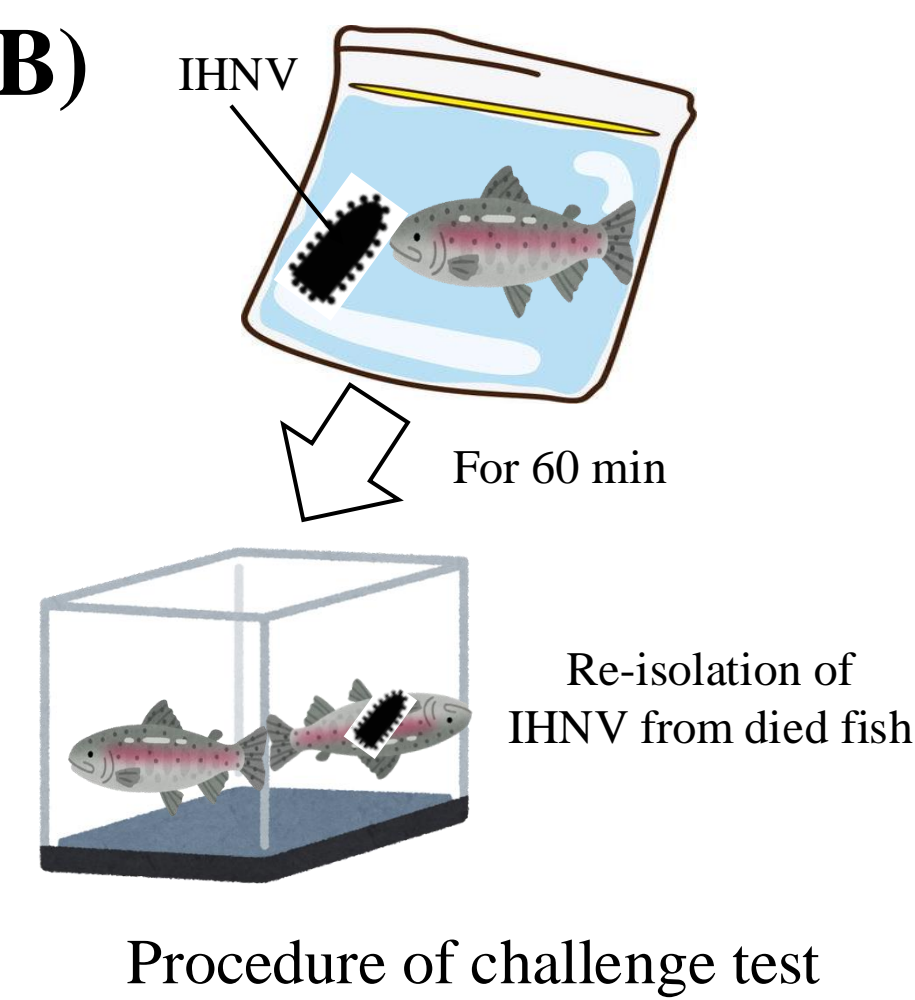
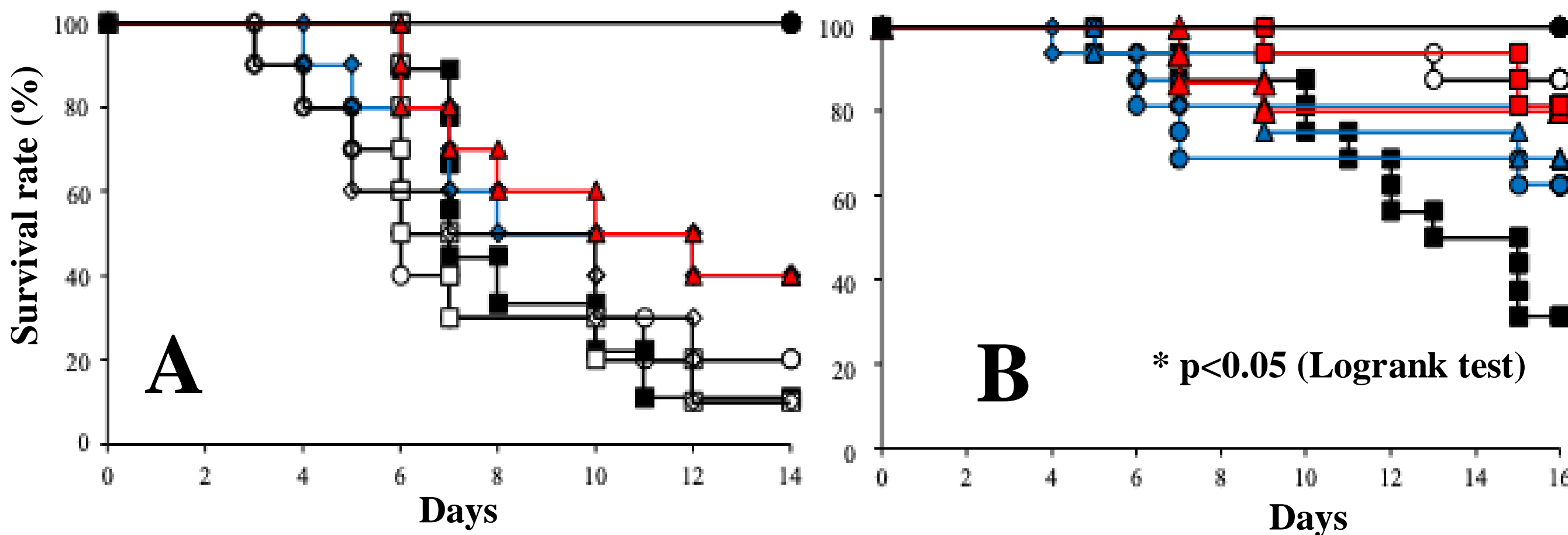


Experimental designs of the present study

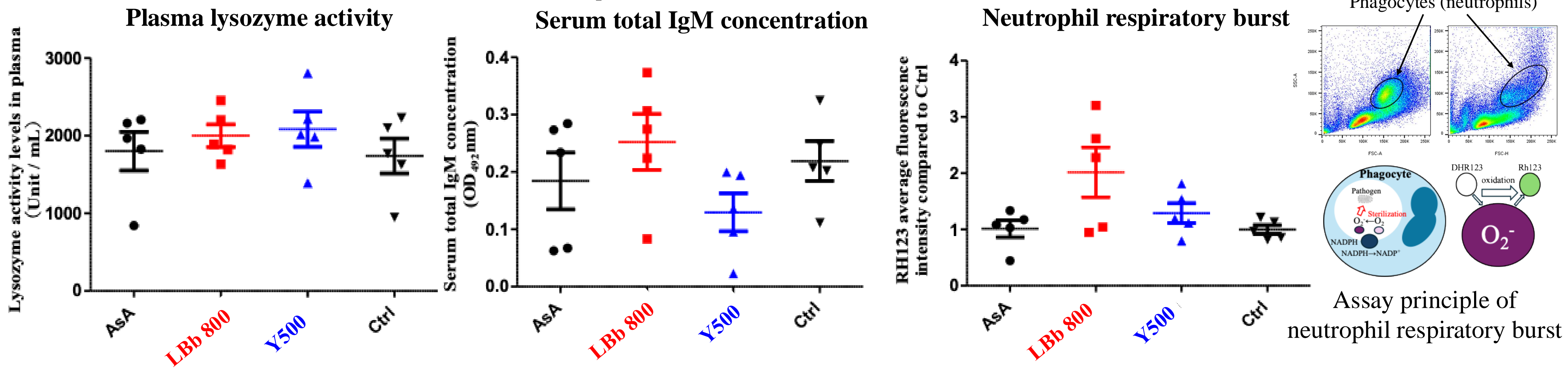
| Experiments | Groups | Added IS (mg/kg diet) | Feeding trial days | Number of fish per group | Mean weight (g) | AsA concentrations in liver (mg/100g) | Water temperature (°C) | Methods and experimnet parameters |
|---|-----------------------------------|-----------------------|--------------------|--------------------------|-----------------|---------------------------------------|------------------------|---|
| Challenge test for screening of IS | Control (Ctrl) | — | 11 days | 20 | 3 | 13.6±2.9 | 14±1 | ✓ Bath exposure for 60 min using IHNV NT1304 strain (10 ^{4.5} TCID ₅₀ /ml) |
| | Ascorbic acid (AsA) | 5,000 | | | | 21.0±3.6* | | |
| | Plant extract (Pe) | 5,000 | | | | | | |
| | Lactic acid bacteria type a (LBa) | 500 | | | | | | |
| | Lactic acid bacteria type b (LBb) | 200 | | | | | | |
| Yeast (Y) | 7,000 | | | | | | | |
| Challenge test for evaluation of optimal concentration | Ctrl | — | 11 days | 20 | 4 | 14.46±5.5 | 14±1 | |
| | AsA | 5,000 | | | | 26.06±2.8* | | |
| | LBb 200 | 200 | | | | | | |
| | LBb 800 | 800 | | | | | | |
| | Yeast 500 (Y500) | 500 | | | | | | |
| | Yeast 2000 (Y2000) | 2,000 | | | | | | |
| Yeast 7000 (Y7000) | 7,000 | | | | | | | |
| Monitoring nonspecific immune responses | Ctrl | — | 11 days | 20 | 20 | NM | 14±1 | ✓ Plasma lysozyme activity ✓ Serum total IgM concentration ✓ Neutrophil respiratory burst |
| | AsA | 5,000 | | | | | | |
| | LBb 800 | 800 | | | | | | |
| Effects of long-term supplementation | Ctrl | — | 100 days | 20 | 1 | NM | 14±1 | ✓ Thermal tolerance test ^a ✓ Growth performance ✓ Histological observation |
| | AsA | 5,000 | | | | | | |
| | LBb 800 | 800 | | | | | | |
| | Y500 | 500 | | | | | | |

NM: not measured. Asterisk (*) indicates p < 0.05, student's t-test. ^a Test was performed after 100 days of feeding period.

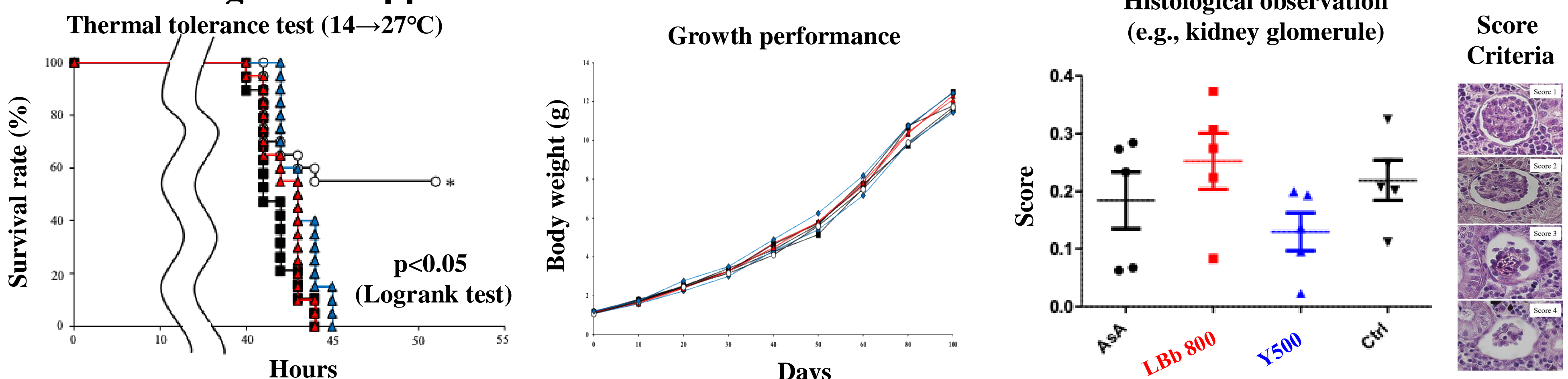
Challenge test for screening (A) and evaluation of optimal concentration (B)



Monitoring nonspecific immune responses



Effects of long-term supplementation



The survival rate of rainbow trout fed either **lactic acid bacteria types b** or **yeast** increased against IHNV infection, and they do not induce any adverse effects in rainbow trout. These results indicate that both materials can be used in rainbow trout farms as **novel immunostimulants**.