prehrambeno biotehnološki fakultet
Sveučilište u Zagrebu

faculty of food technology and biotechnology
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Novel insights into monitoring, ecological requirements and methods for environmentally acceptable control of saprolegniosis

Nove spoznaje o ekološkim zahtjevima uzročnika saprolegnioze, te razvoj metoda praćenja i ekološki prihvatljivih metoda suzbijanja bolesti

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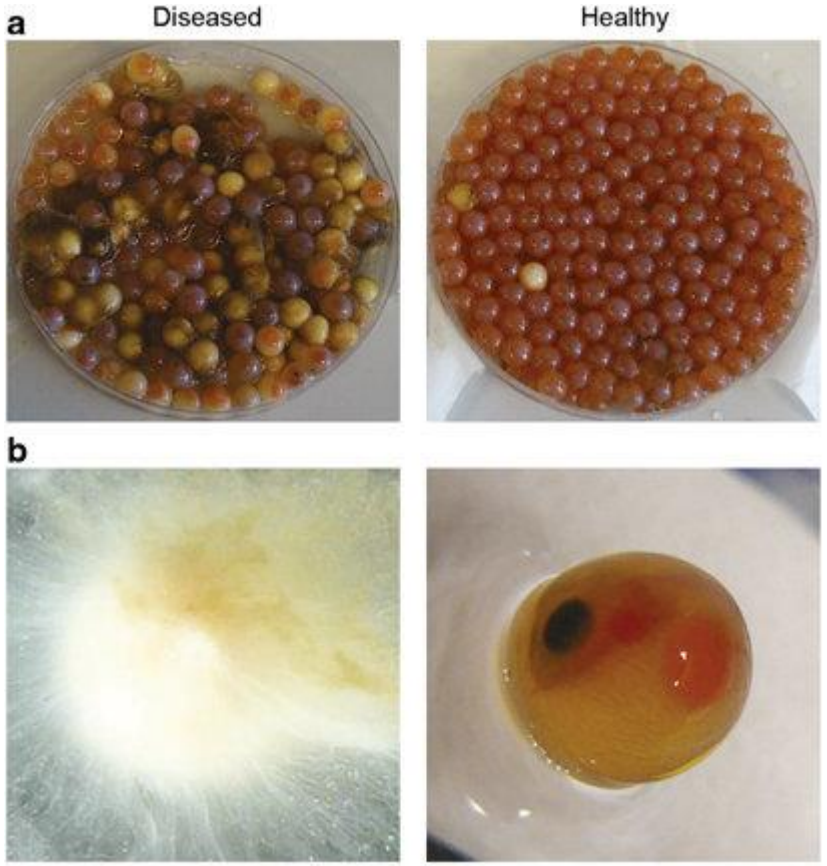
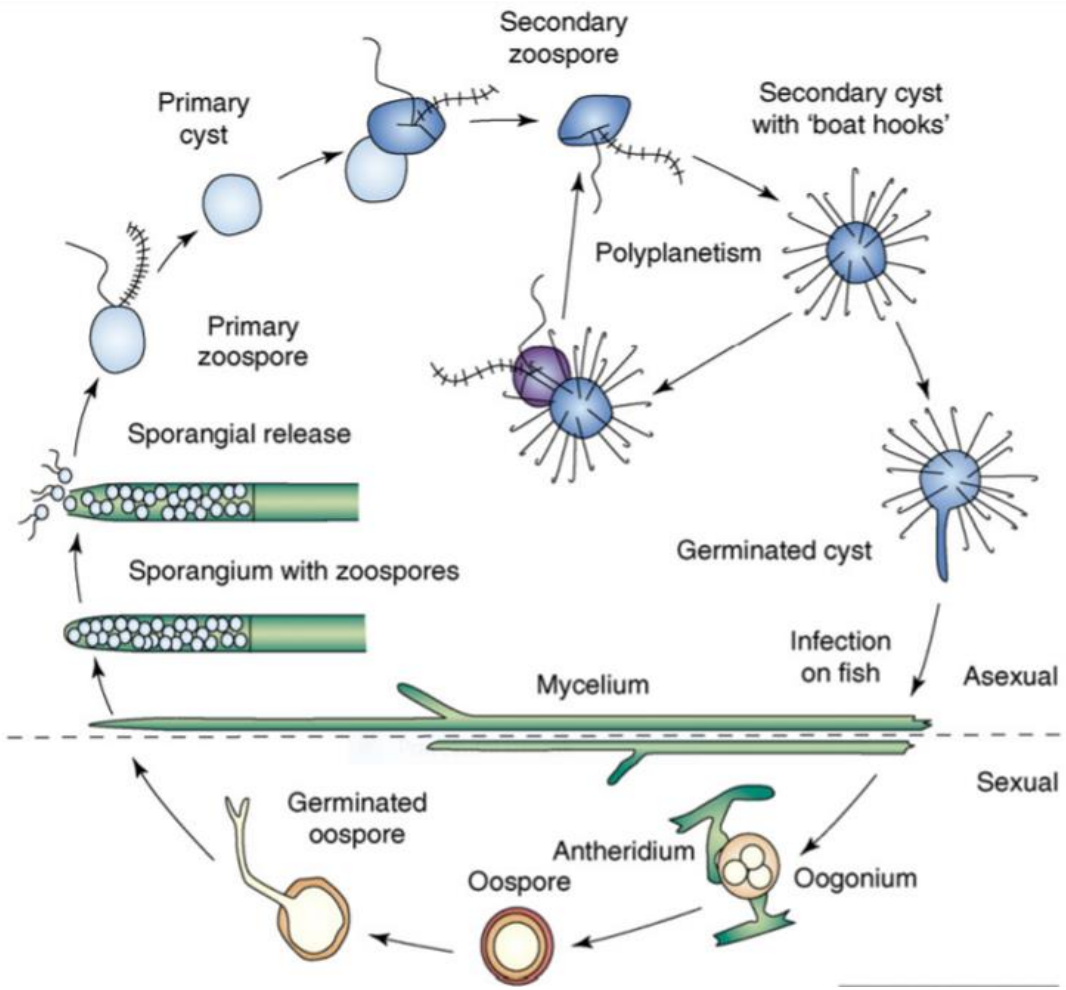
Saprolegniosis

- Caused by *Saprolegnia* spp. (Saprolegniales, Oomycota)
- Huge economic losses in freshwater aquaculture, primary salmonids
- Ubiquitous in freshwaters
- Wide host range
- Opportunistic secondary pathogens
- Primary pathogen – *Saprolegnia parasitica*

Symptoms of saprolegniosis: grey patches of cottony mycelium (photo by Dora Pavić).



Atlantic salmon eggs infected with saprolegniosis vs. healthy eggs (photo from Liu et al., 2014)



Saprolegnia parasitica life cycle (Phillips et al., 2008).

In vitro we can follow…

Zoospore motility

- spreading of the pathogen in the environment

Germination of cysts

- penetration through the host skin

Mycelium growth

- disease progression within the host tissues

1. Sensitive and cultivation-free detection and quantification of *Saprolegnia parasitica* by droplet digital PCR (ddPCR)

2. New insights into biology/ecology of *Saprolegnia* spp.

3. Development of environmentally friendly control strategies for saprolegniosis in aquaculture

Sensitive and cultivation-free detection and quantification of *Saprolegnia parasitica* by droplet digital PCR (ddPCR)

Droplet digital PCR: advanced tehnology capable of quantifiying small amounts of target DNA by fractionating a PCR reaction into more than 20 000 droplets.

High specificity

Species	Isolate/code	Detection
Saprolegnia parasitica	BF1	+
Saprolegnia parasitica	BF2	+
Saprolegnia parasitica	Z42	+
Saprolegnia parasitica	Z46	+
Saprolegnia australis	Z25	-
Saprolegnia delica	BF5	-
Saprolegnia diclina	SAP-1	-
Saprolegnia ferax	Z106	-
Saprolegnia litoralis	SAP-2	-
Saprolegnia sp. 1	SAP-3	-
Aphanomyces astaci	PEC8	-
Pythium sp.	VU3 3	-
Oncorhynchus mykiss	T-DNA	-
Pacifastacus leniusculus	C-DNA	-

High sensitivity: down to 14 fg of pathogen DNA (1 genome)

Pavić et al., *Scientific reports*, 2022

Sensitive and cultivation-free detection and quantification of *Saprolegnia parasitica* by droplet digital PCR (ddPCR)

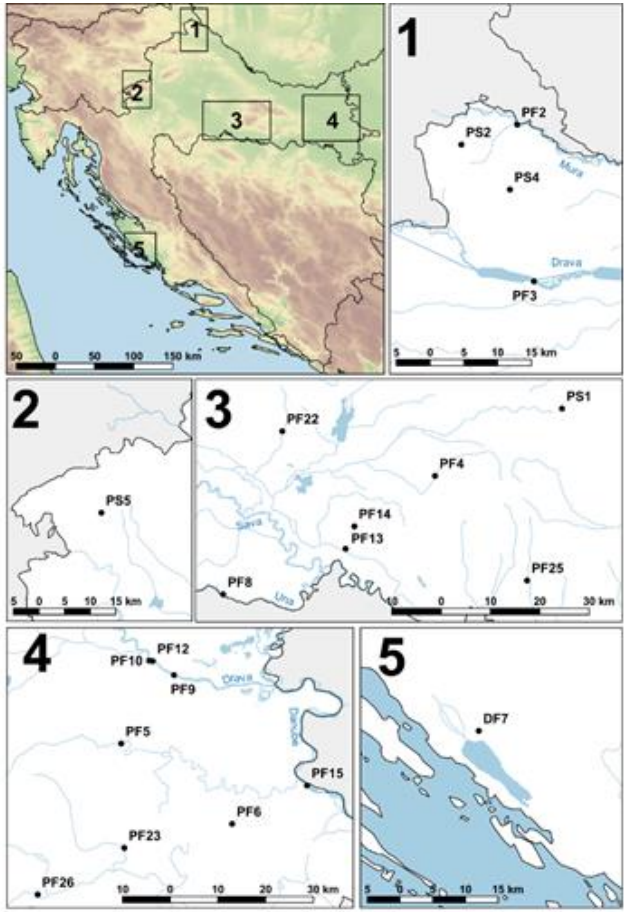
Applicability of the developed assay tested on:

Swab samples

Swab samples taken from the infected eggs and injured trout had a significantly higher *S. parasitica* load.

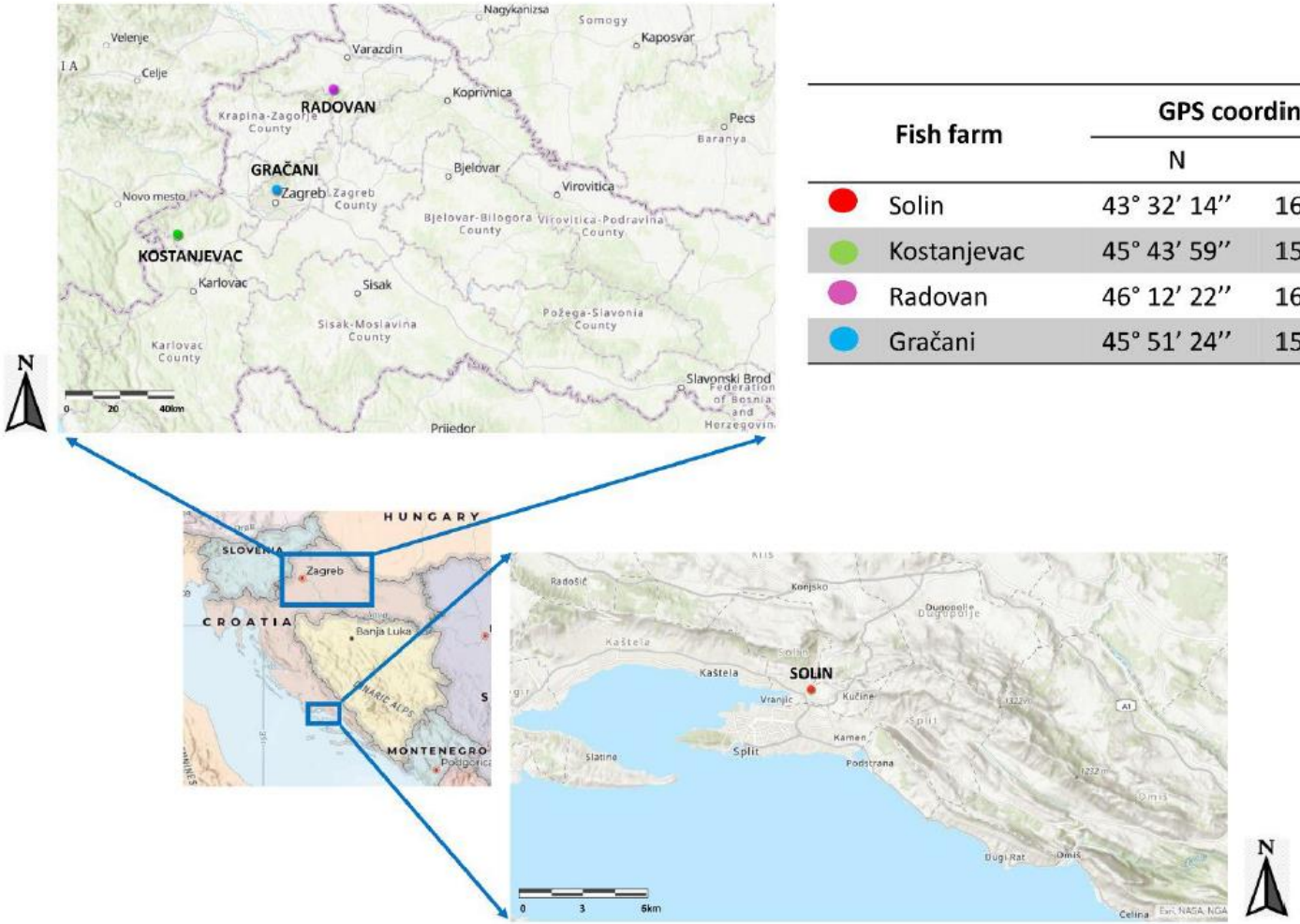
Water samples

The omnipresence of *S. parasitica* was demonstrated by ddPCR in natural waters in Croatia, where the pathogen was detected at 13 out of 21 sites (**62%**).



Pavić et al., *Scientific reports*, 2022

New insights into biology/ecology of *Saprolegnia* spp.: trout farms can act as hot spots of *Saprolegnia* release in natural environment



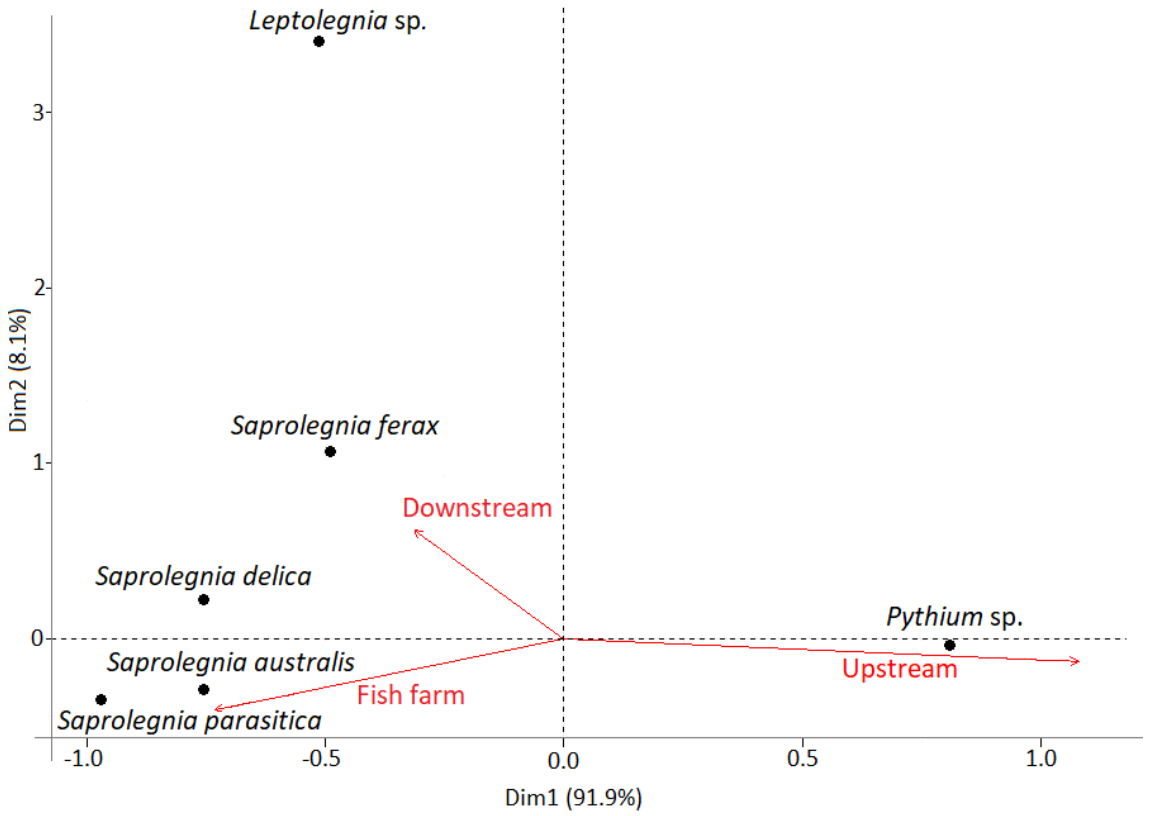
Fish farm	GPS coordinates	
	N	E
Solin	43° 32′ 14″	16° 29′ 33″
Kostanjevac	45° 43′ 59″	15° 25′ 18″
Radovan	46° 12′ 22″	16° 15′ 12″
Gračani	45° 51′ 24″	15° 57′ 53″

Sampling of Oomycota

I. In the farm (F)
II. Upstream (U)
III. Downstream (D)

Pavić et al., *Aquaculture*, 2021

New insights into biology/ecology of *Saprolegnia* spp.: trout farms can act as hot spots of *Saprolegnia* release in natural environment



Correspondence analysis (CA) biplot displaying the associations of Oomycota species isolated from water (hempseed baits) with the sampling location (upstream, fish farm, or downstream).

- *Saprolegnia* species were more abundant in the fish farms and downstream locations.
- Upstream sites were strongly associated with *Pythium* species (plant pathogens or soil saprotrophs)

Pavić et al., *Aquaculture*, 2021

Development of environmentally friendly control strategies for saprolegniosis in aquaculture

vs

Environmentally friendly solutions

plant extracts
propolis
bacteria from the genus *Pseudomonas*

Development of environmentally friendly control strategies for saprolegniosis in aquaculture: propolis and essential oils of Mediterranean wild plants

Sample	EC_{50} for mycelium growth	EC_{50} for germination of cysts	MIC for zoospore motility
Rosemary essential oil	>1000 µL/L*	63.0 µL/L	N.A.
Sage essential oil	40.5 µL/L	11.6 µL/L	N.A.
Bay laurel essential oil	>1000 µL/L	12.6 µL/L	N.A.
Propolis sample 1	206.20 mg/L	23.62 mg/L	61.88 mg/L
Propolis sample 2	206.60 mg/L	19.01 mg/L	38.67 mg/L
Malachite green (pos. control)	0.120 mg/L	0.032 mg/L	0.08 mg/L

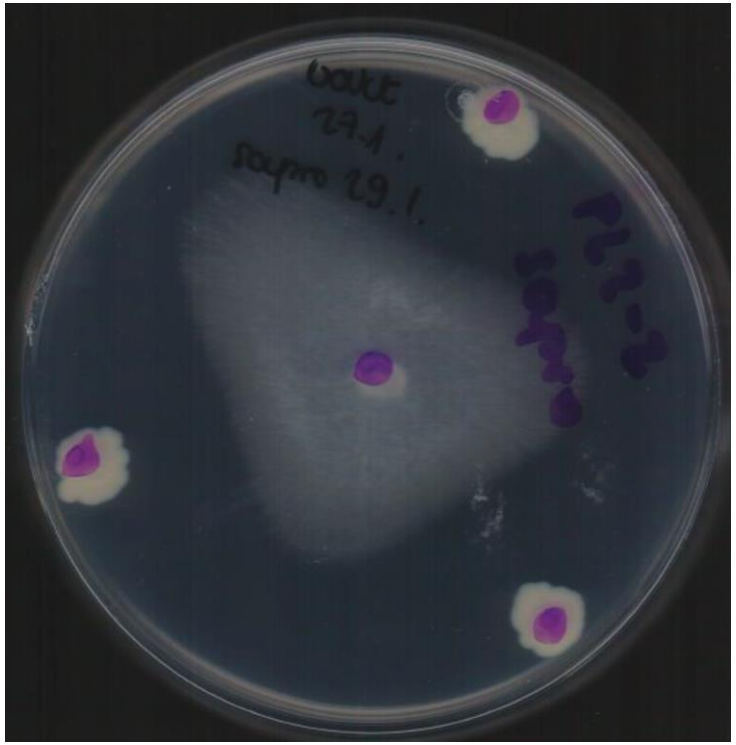
EC_{50} value: sample concentration causing 50% inhibition; MIC: minimum inhibitory concentration

*The highest concentration tested that did not cause 100% inhibition. Thus, EC_{50} value could not be determined.

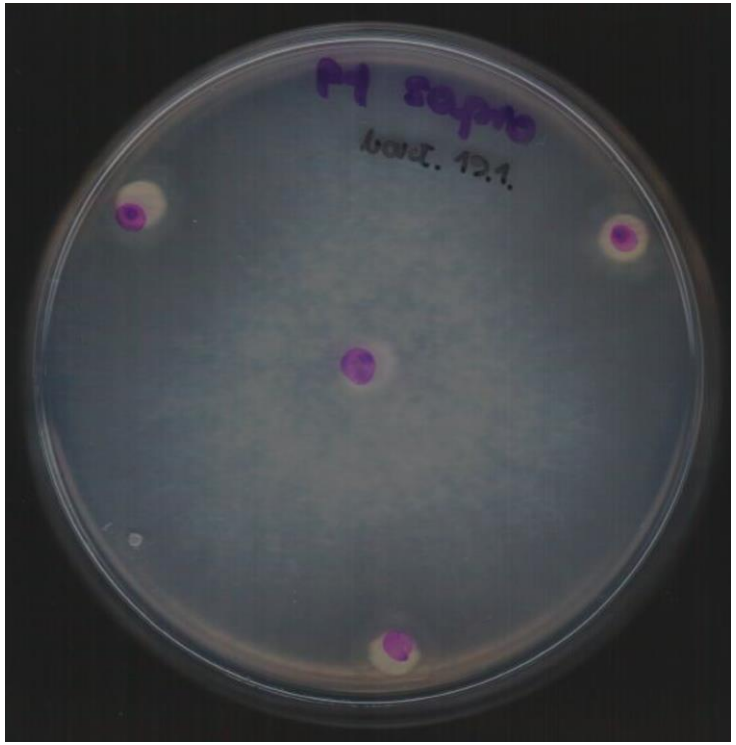
Miljanović et al., *Plants*, 2021; Miljanović et al., *Aquaculture*, 2022

Development of environmentally friendly control strategies for saprolegniosis in aquaculture: *Pseudomonas* bacteria

Effects of pseudomonads on *Saprolegnia parasitica* growth



INHIBITOR
Pseudomonas chlororaphis PL2-2

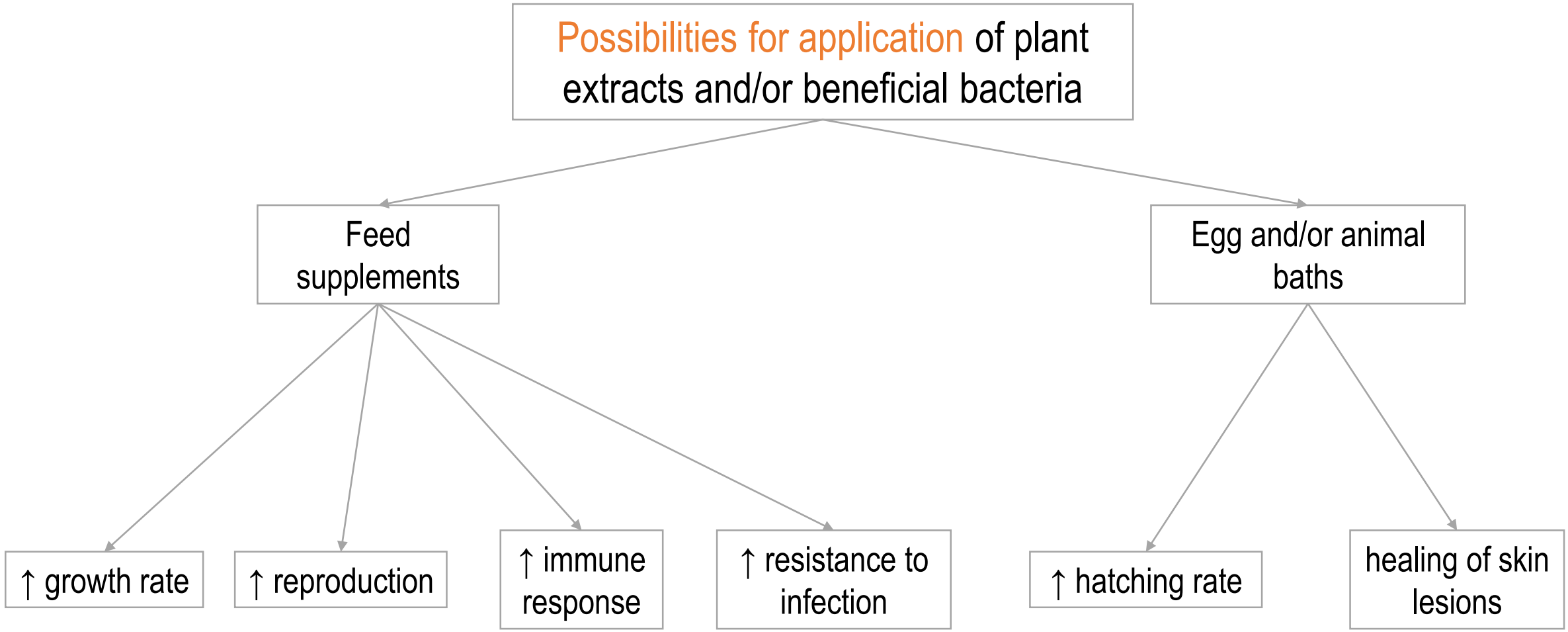


NON-INHIBITOR
Pseudomonas peli P1

Future prospects:

- Genome sequencing to identify gene cluster for production of secondary metabolites with anti-*Saprolegnia* effect
- Chemical analyses

Development of environmentally friendly control strategies for saprolegniosis in aquaculture: future prospects



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