

Culturing experiments to determine the nutrient requirement for exopolymer production in *Lentisphaera araneosa*

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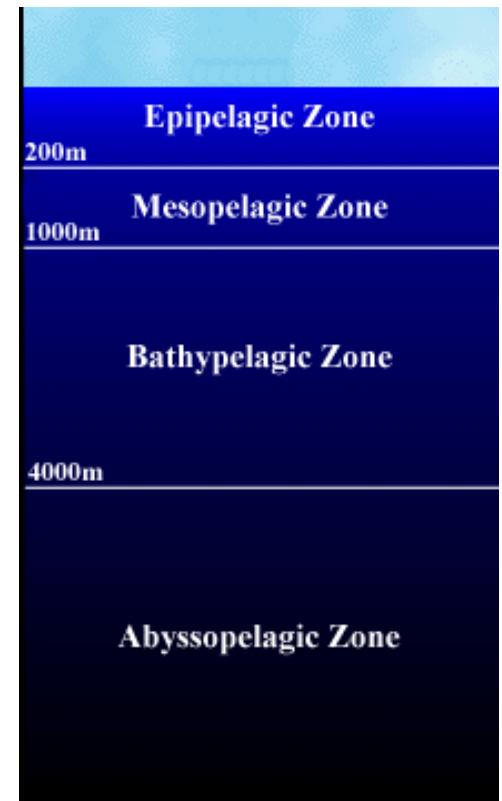
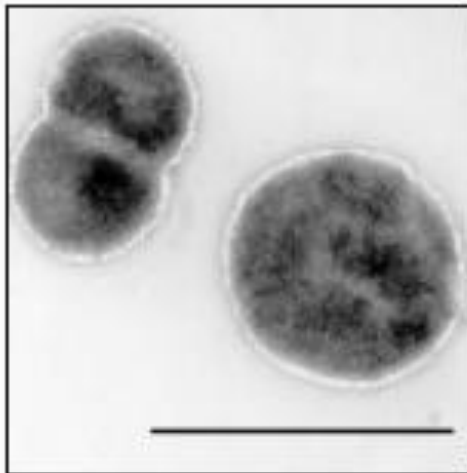
Significance

- Marine transparent exopolysaccharides (TEP)
- Marine snow
 - Recycle organic compounds
 - Movement of organic matter
- More efficient culture medium
- Foundation for further investigations
- Symbiotic relationship
- Ecological role
- Potential use in industries



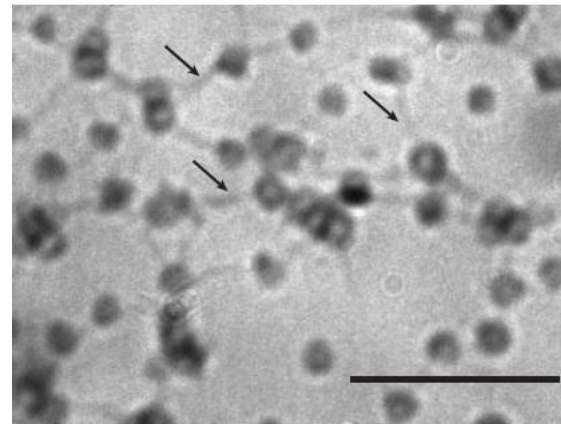
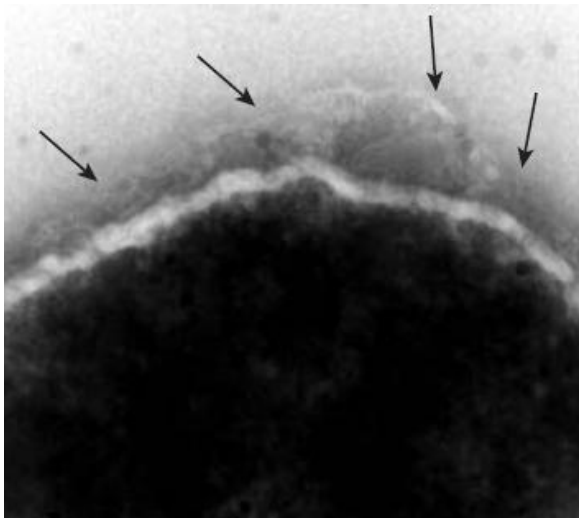
Lentisphaera araneosa

- Isolated using High Throughput Microbial Cultivation
- Obligate aerobe
- Chemoheterotroph
- Facultative oligotroph
- Found in surface and mesopelagic zones



Transparent exopolysaccharides

- Produces extracellular polysaccharide (EPS)
 - Rhamnose, galactose, mannose, glucose
- Produces transparent exopolysaccharides (TEP)



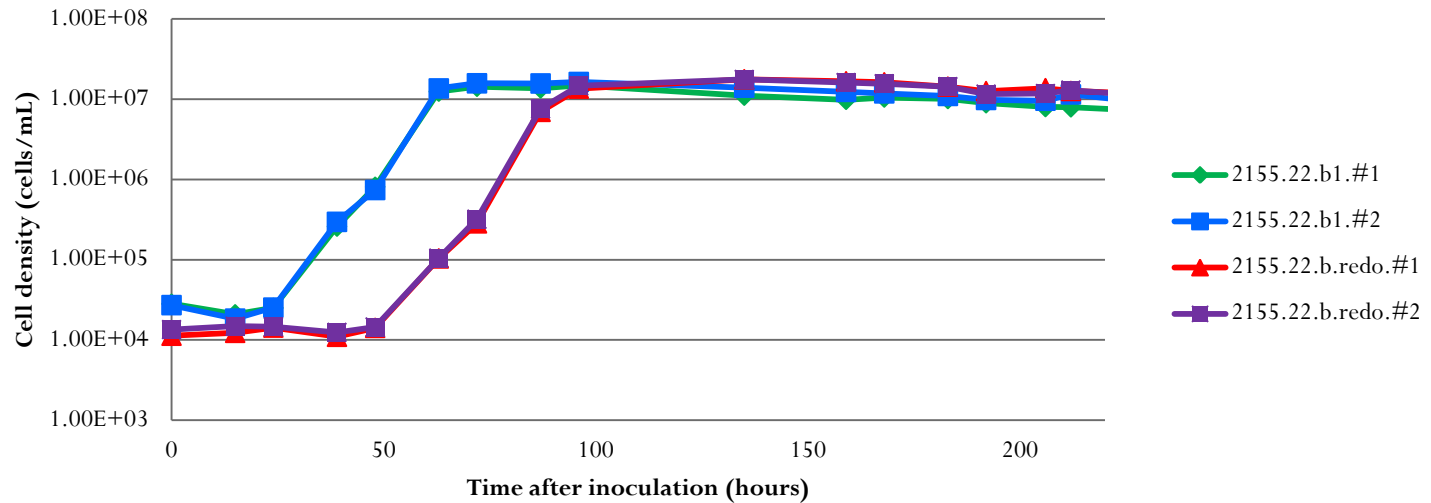
Cell Growth

- Grows in medium composed of various carbon sources
 - In low nutrient heterotrophic medium
 - In artificial seawater
- Optimum growth temperature at 16-20 degree Celsius
- Fast growth rate

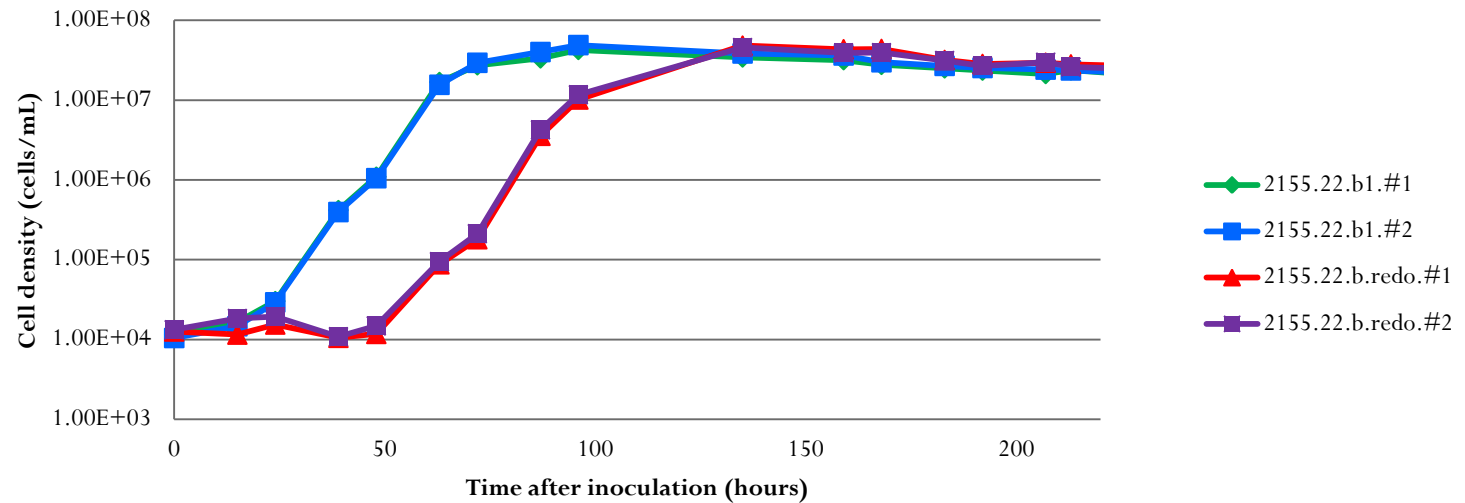
Composition of current medium

- Glycine
- Methionine
- Serine
- Pyruvate
- Taurine
- Oxaloacetic acid
- Glucose

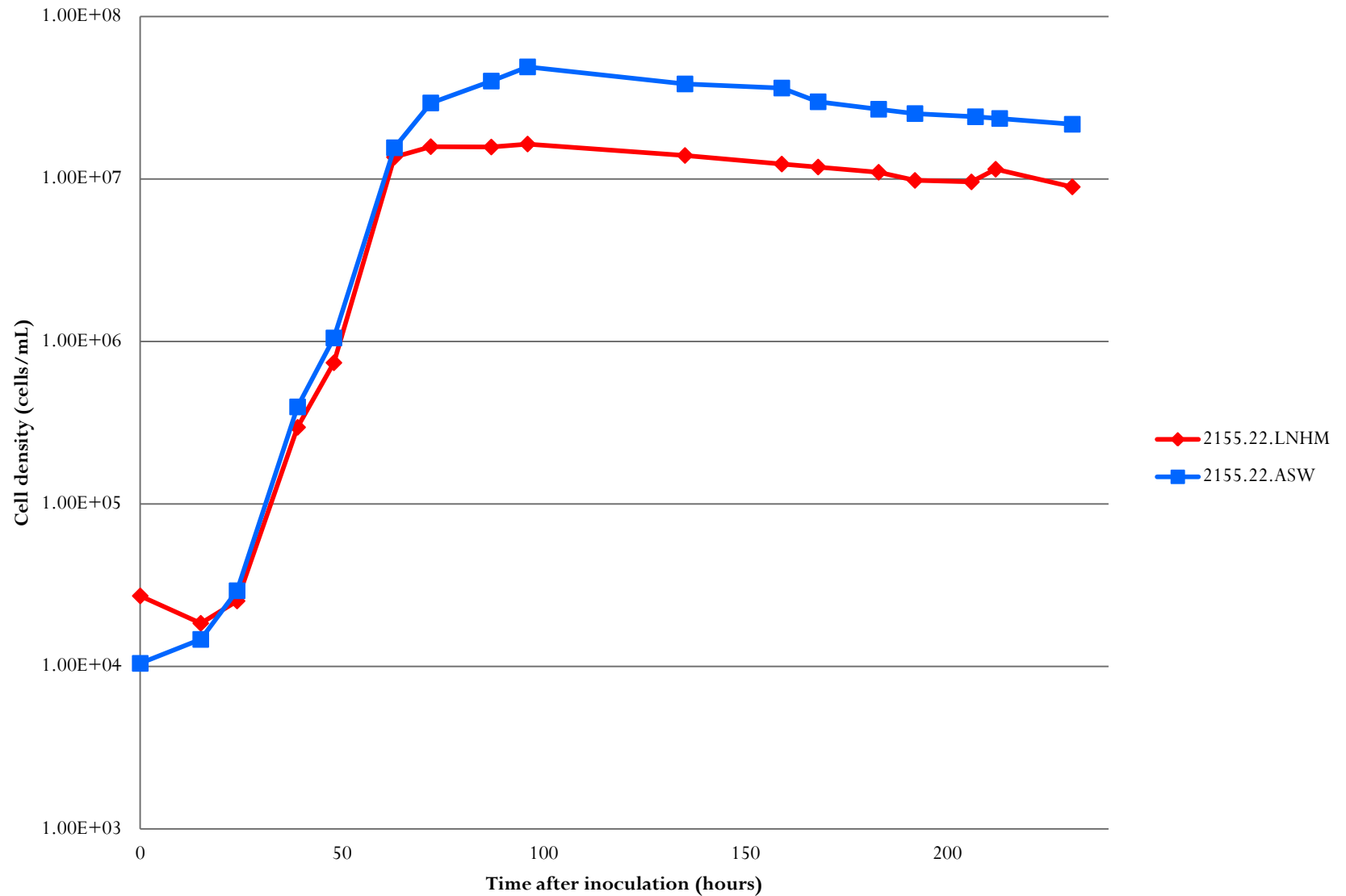
Lentisphaera araneosa 22nd generation growth curve (in LNHM)



Lentisphaera araneosa 22nd generation growth curve (in ASW)



Growth of *Lentisphaera araneosa* in different seawater media



Hypothesis

The unusual property of TEP production in *Lentisphaera araneosa* is triggered by general nutrient input and growth condition.

Prediction

By manipulating the nutrient input and growth condition, the production of TEP in *Lentisphaera araneosa* is affected.

Experimental procedures

- Inoculate fresh cultures
- Cultures in different media
- Monitor cell growth
- Measure TEP production by viscosity

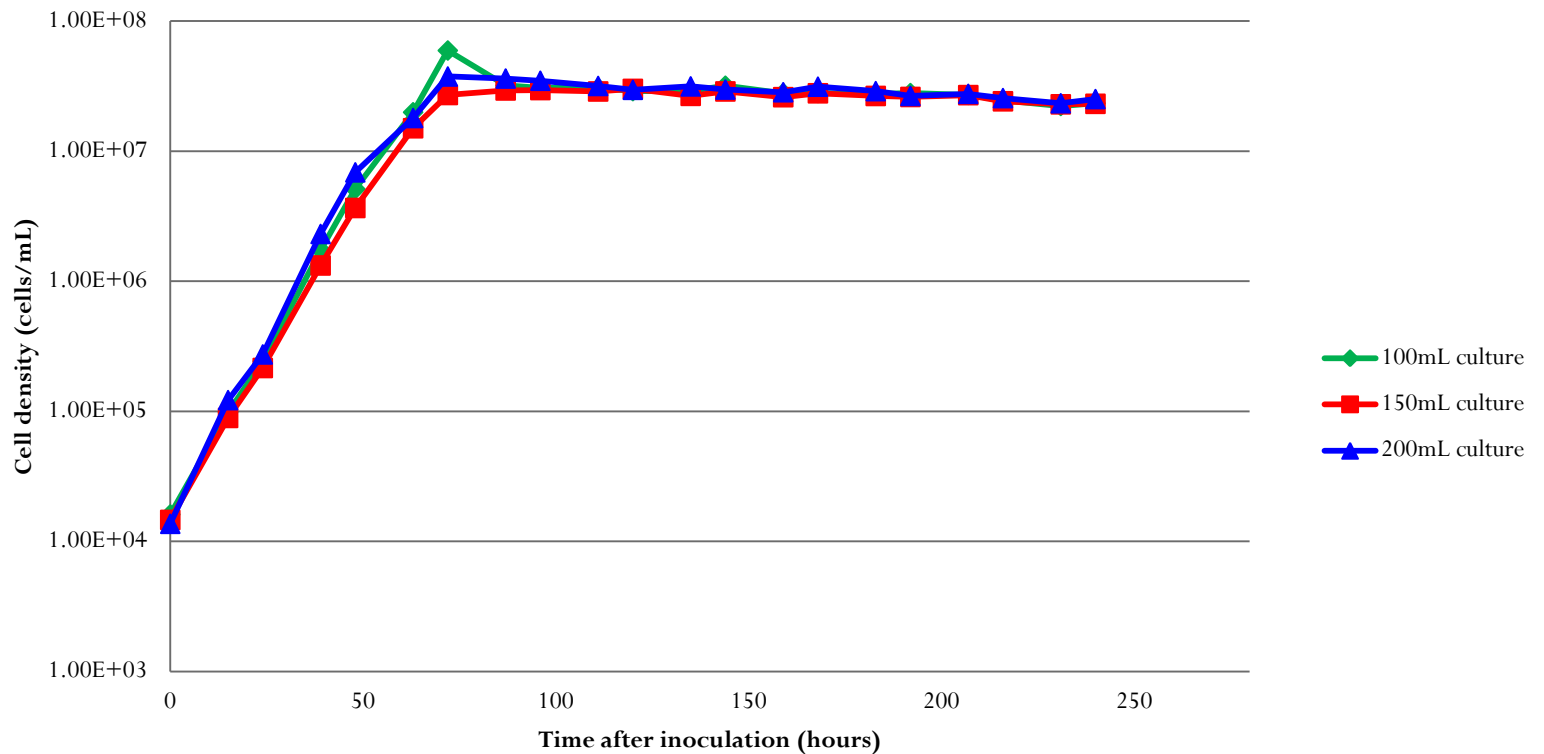
Composition of current medium

- Glycine
- Methionine
- Serine
- Pyruvate
- Taurine
- Oxaloacetic acid
- Glucose



Oxygen requirement

Oxygen requirement of *Lentisphaera araneosa*



Positive controls

- Two positive controls
- Both in artificial seawater

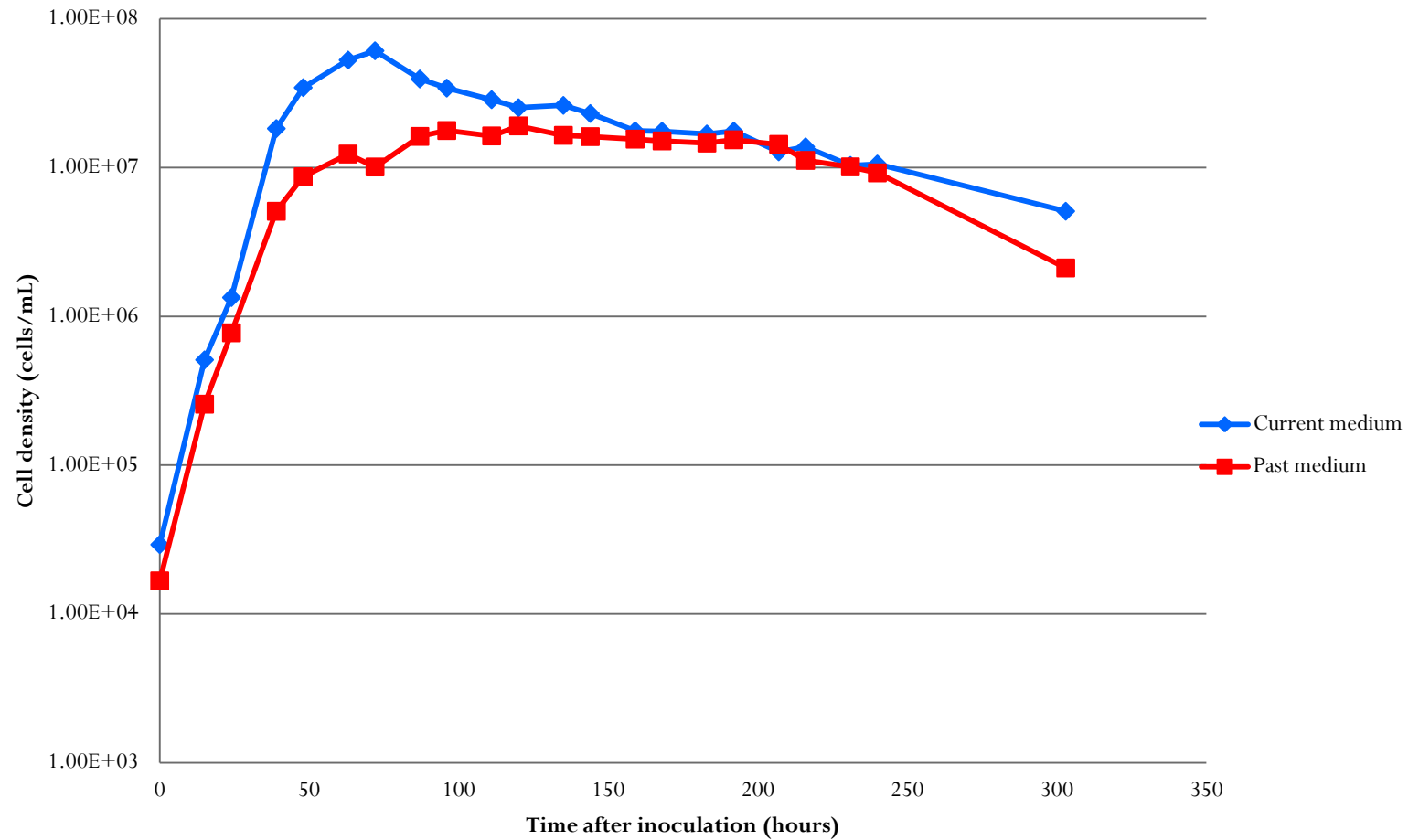
Composition of current medium

- Glycine
- Methionine
- Serine
- Pyruvate
- Taurine
- Oxaloacetic acid
- Glucose

Composition of past medium

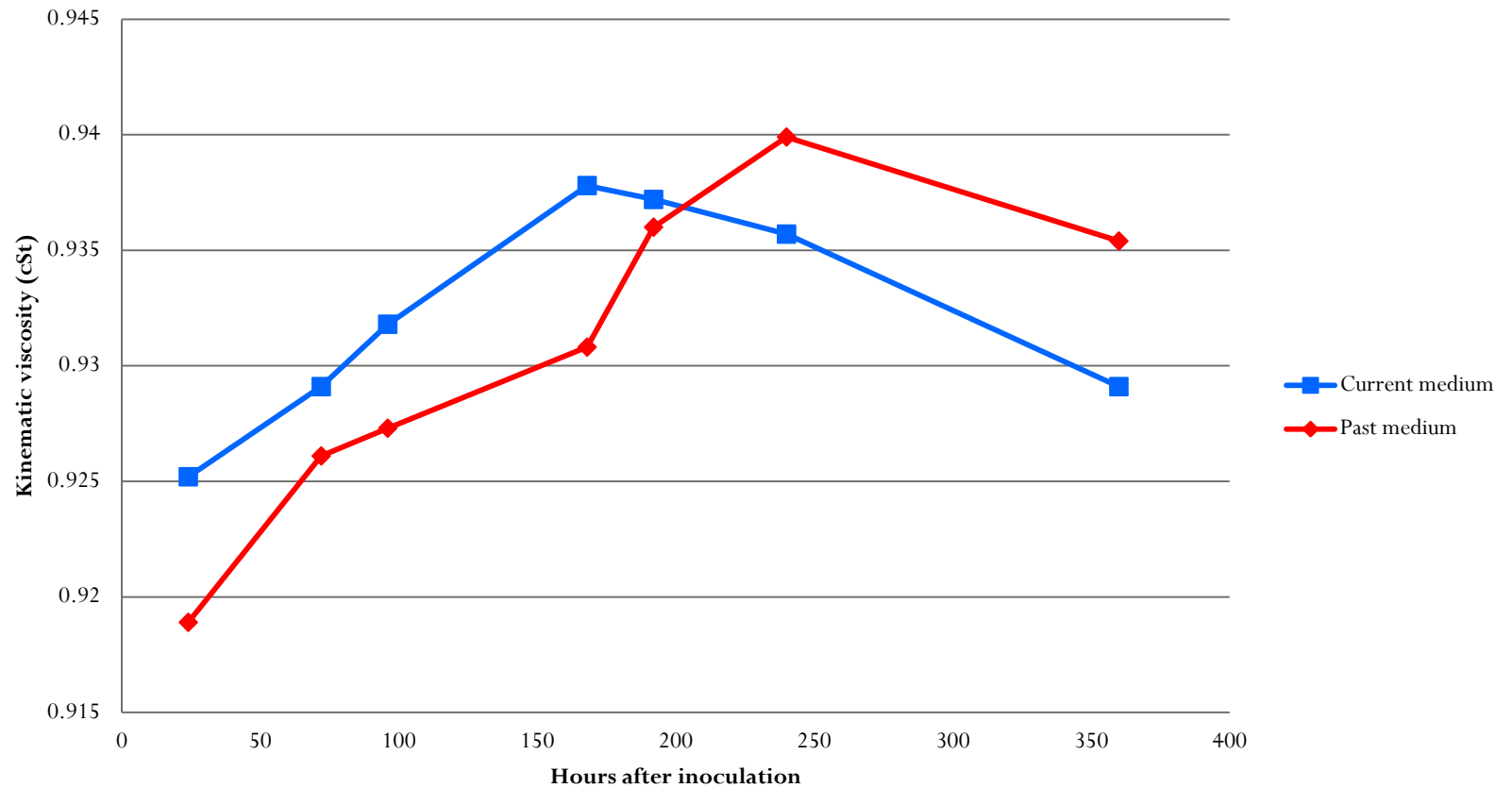
- Glucose
- Ribose
- Succinic acid
- Pyruvate
- N-acetyl D-glucosamine

Growth curve of positive controls



Viscosity of positive controls

Kinematic viscosity of positive controls

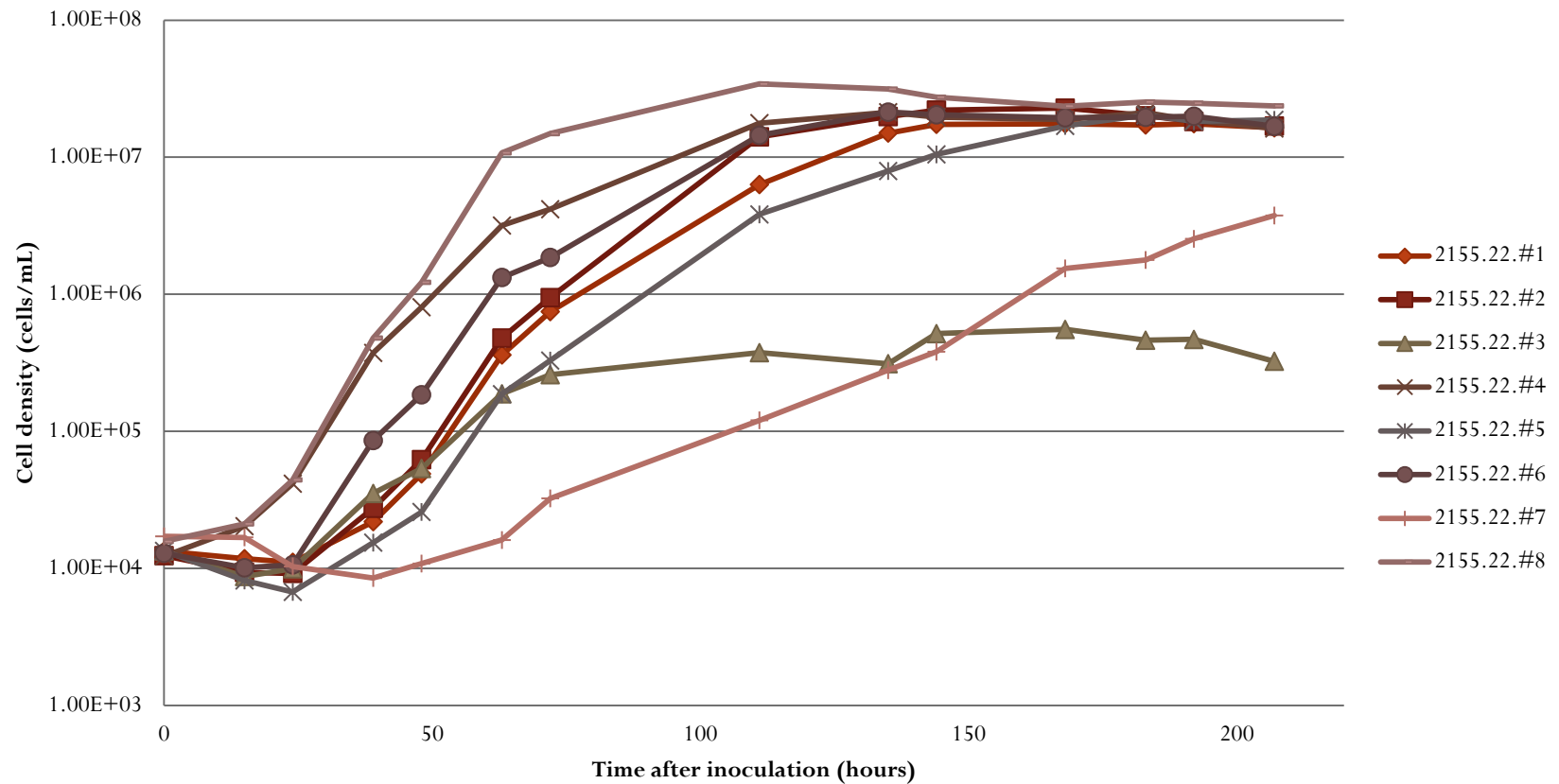


Experiment scheme

	Glycine	Methionine	Serine	Pyruvate	Taurine	OAA	Glucose
2155.22.#1	○	○	○	○	○	○	○
2155.22.#2	X	○	○	○	○	○	○
2155.22.#3	○	X	○	○	○	○	○
2155.22.#4	○	○	X	○	○	○	○
2155.22.#5	○	○	○	X	○	○	○
2155.22.#6	○	○	○	○	X	○	○
2155.22.#7	○	○	○	○	○	X	○
2155.22.#8	○	○	○	○	○	○	X

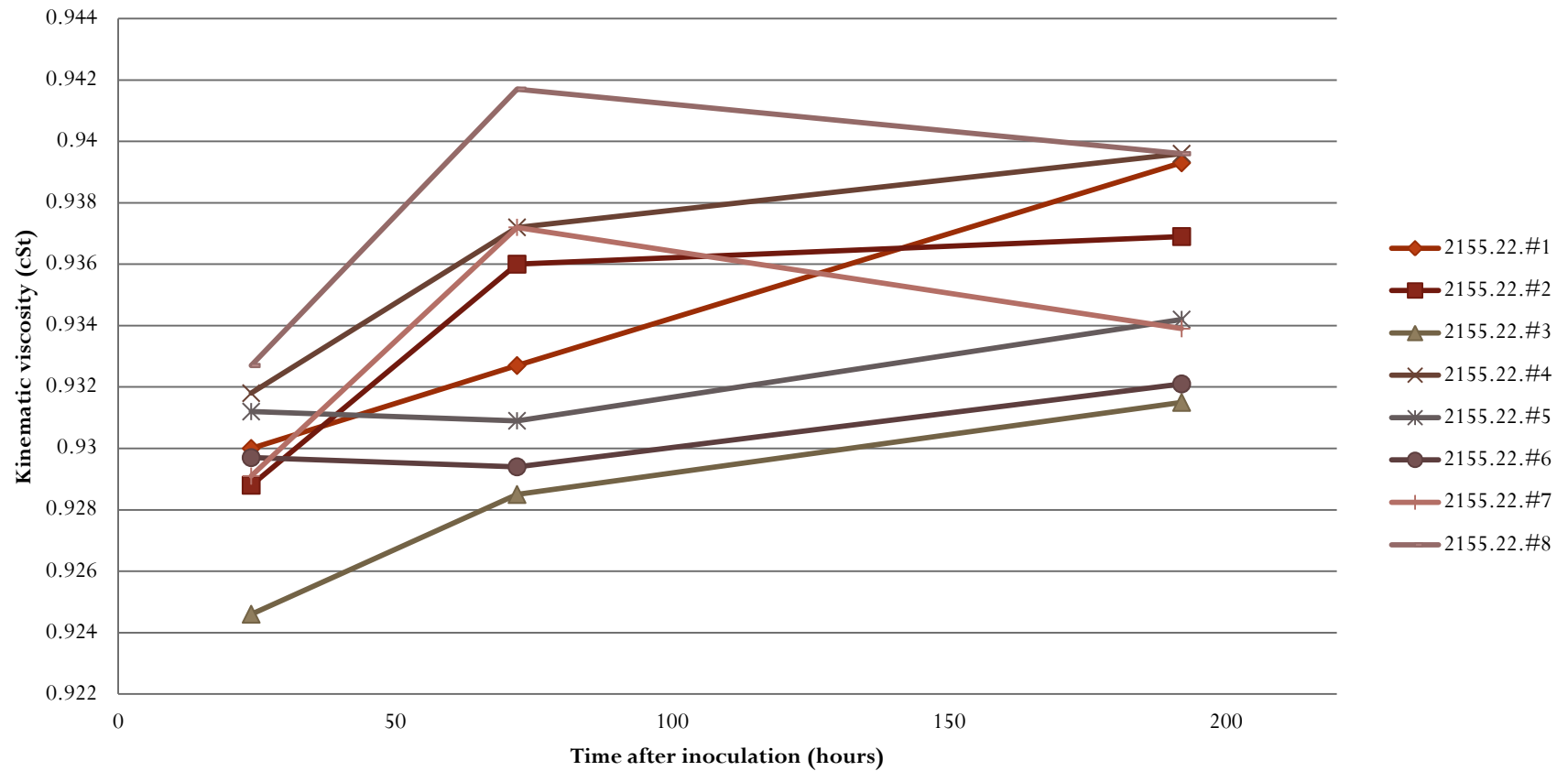
Growth curve

Growth curve of *Lentisphaera araneosa* under different treatments



Viscosity

Kinematic viscosity of *Lentisphaera araneosa* under different treatments



Conclusion

- TEP production — positive
 - Late exponential and stationary phase
- Cultures without serine or without glucose

Composition of new medium #1

- Glycine
- Methionine
- ~~Serine~~
- Pyruvate
- Taurine
- Oxaloacetic acid
- Glucose

Composition of new medium #2

- Glycine
- Methionine
- Serine
- Pyruvate
- Taurine
- Oxaloacetic acid
- ~~Glucose~~

Future work

- Culturing at different concentration and different temperature
- Further investigation on the effects of different carbon compounds based on *Lentisphaera araneosa*'s genome sequences.
- Effects of nitrogen, phosphorus, and sulfur on *Lentisphaera araneosa*'s growth and TEP production

Acknowledgement

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