



# **NEW TECHNOLOGY FOR SPEED AND CONSISTENCY OF PROBIOTIC PERFORMANCE IN AQUACULTURE**

**Tom Hashman**

**Director Business Development**

**Envera LIC LLC**



# Envera Technology Presentation FENACAM

Confidential

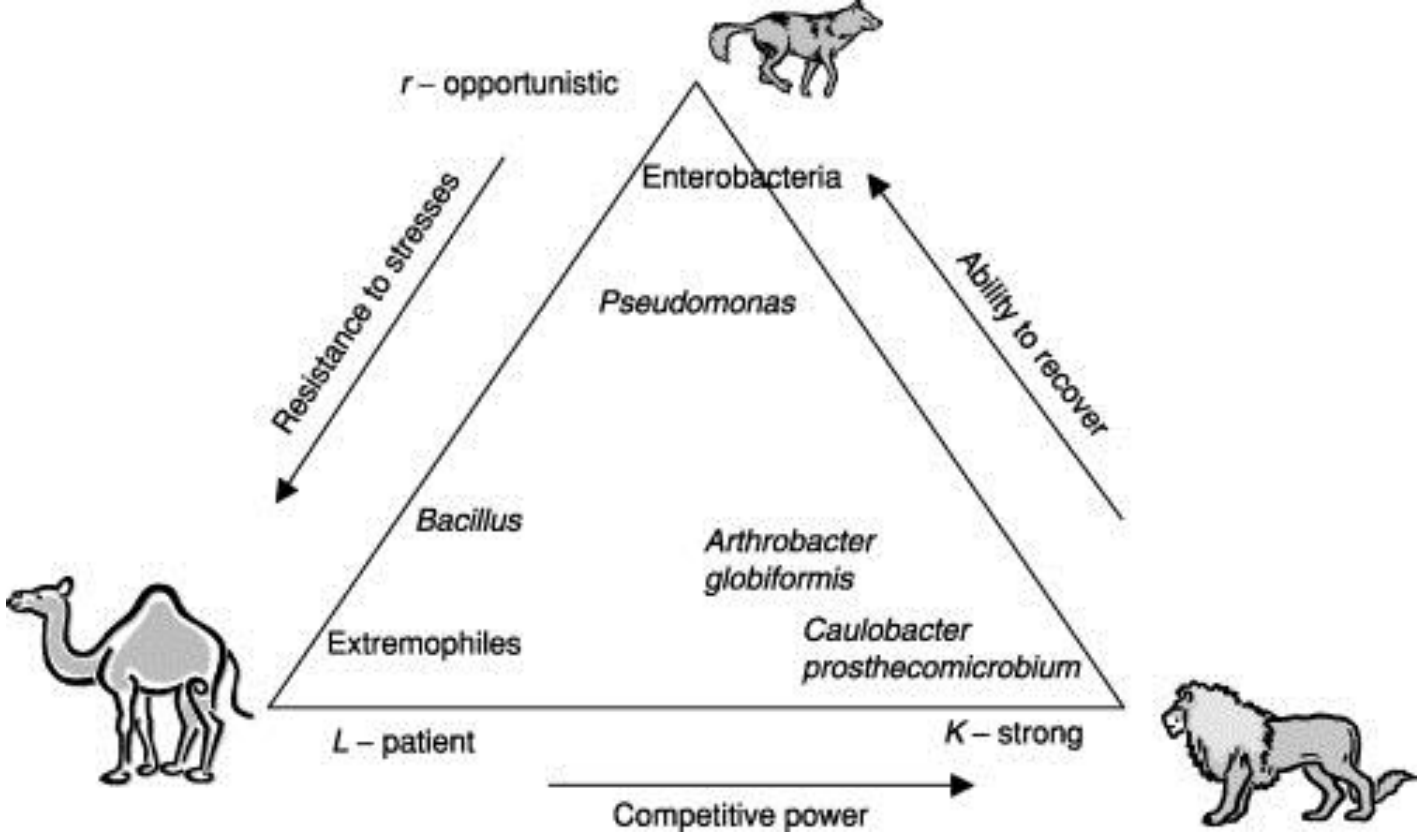
November 16<sup>th</sup>, 201

# Envera GO Technology®

---

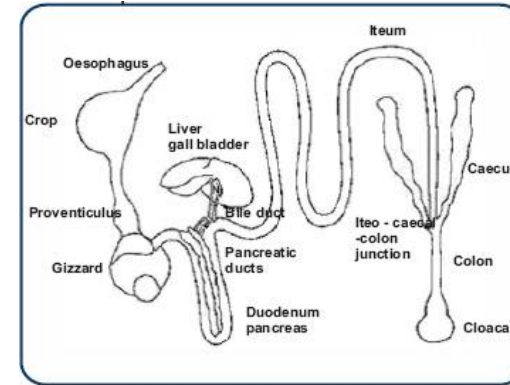
- Envera has developed a methodology to greatly speed the transition of spore forming bacteria to the vegetative state.
- Patents have been filed globally covering various processes and/or compositions involved in the technology.
- Granted US 9,447,376 & US 9,932,543 ; European Patent EP2954041B1 and Mexican Patent Application No. MX/a/2015/010124 has granted and is issuing.
- We have coined the term GO Technology® or Germination Optimization Technology and have trademarked the terminology.

# Concept of Life Strategies



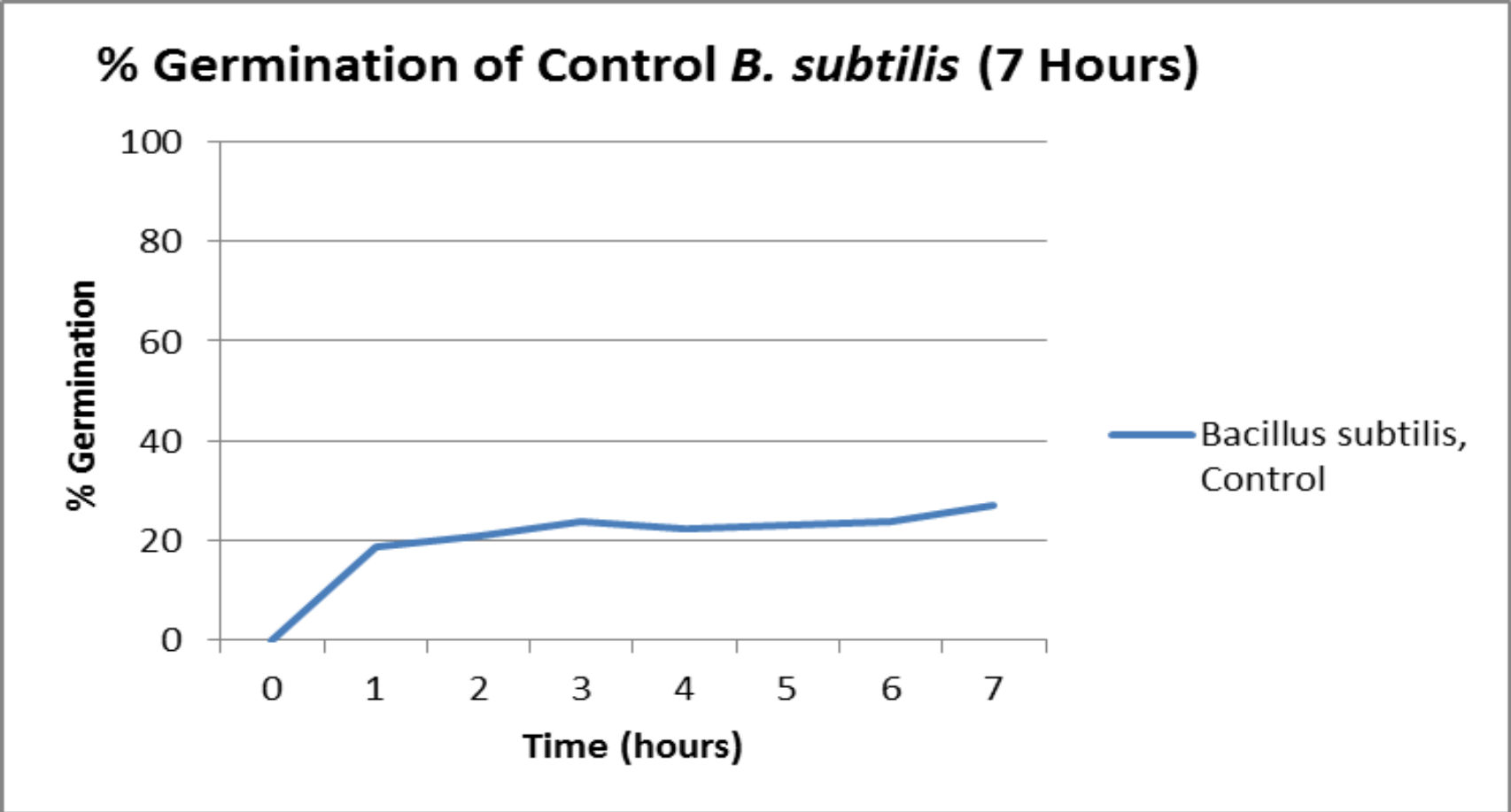
# Transit Time and pH

## Running the Gauntlet

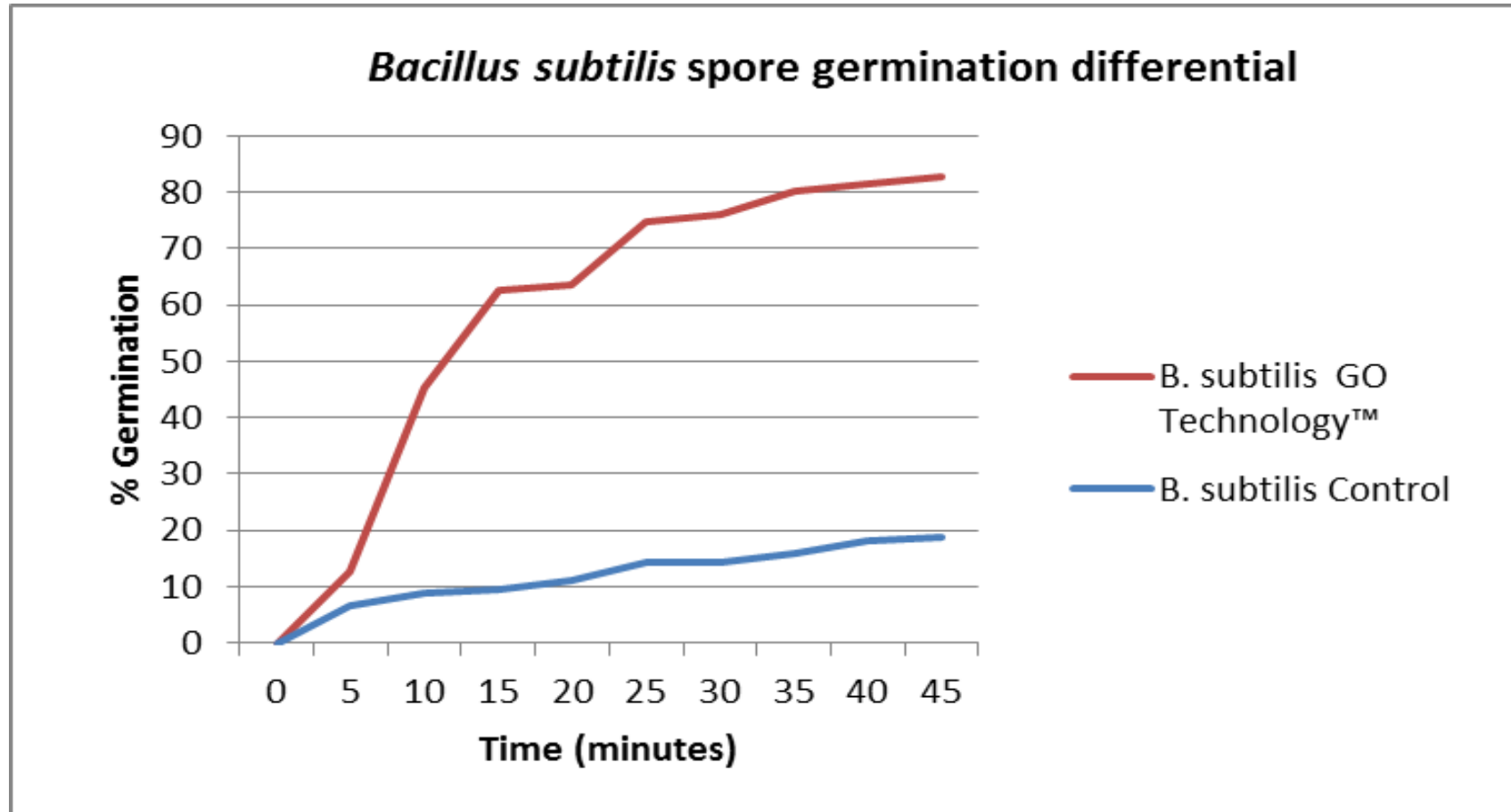


GIT Segment	Transit Time(Min)	pH
Crop	50	5.5
Proventriculus / Gizzard	90	2.5-3.5
Duodenum	5-8	5-6
Jejunum	20-30	6.5-7.0
Ileum	50-70	7.0-7.5
Colon	25	8.0

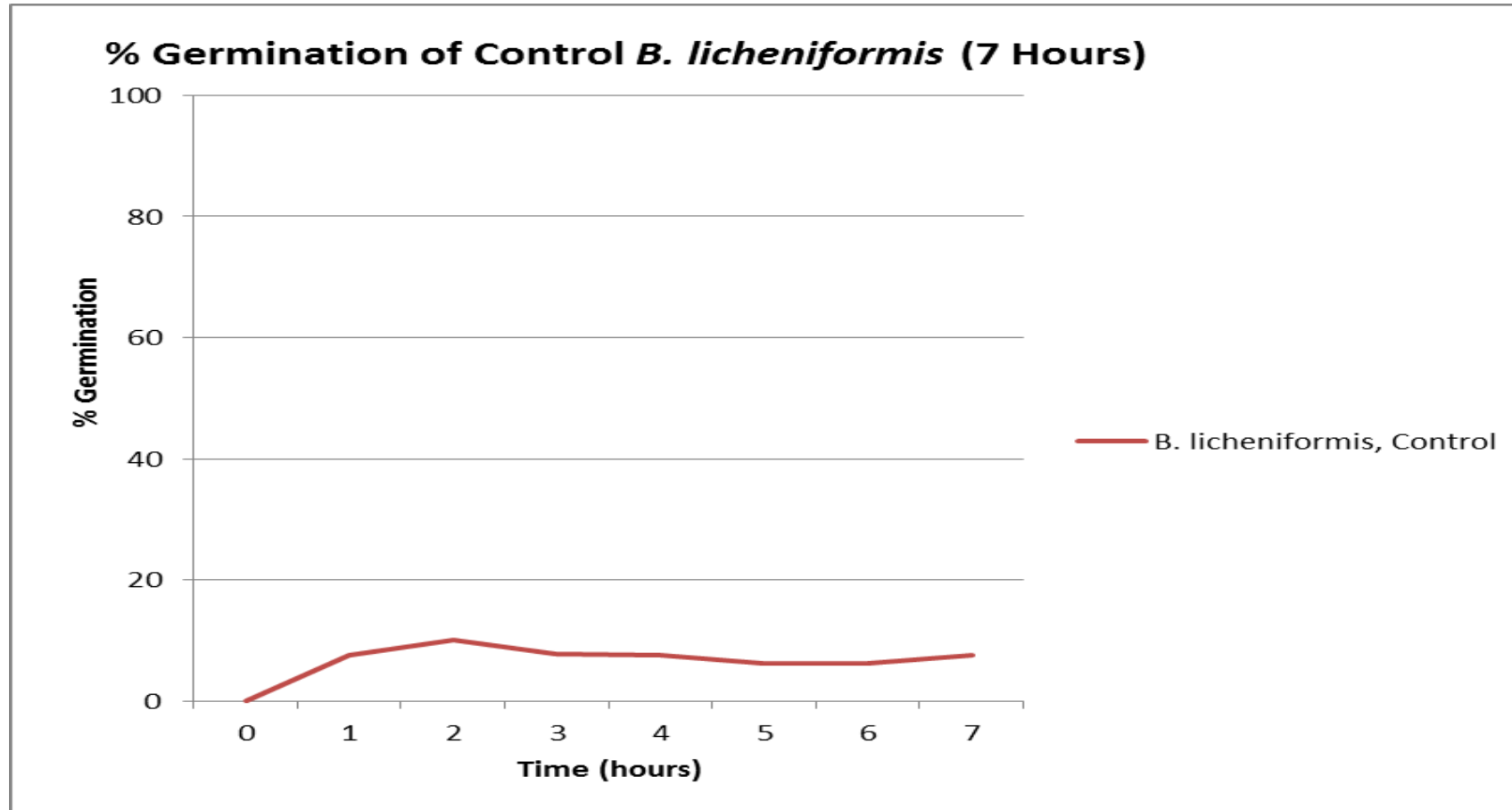
# Bacillus subtilis Germination Without GO Technology®



# *Bacillus subtilis* Germination With GO Technology®

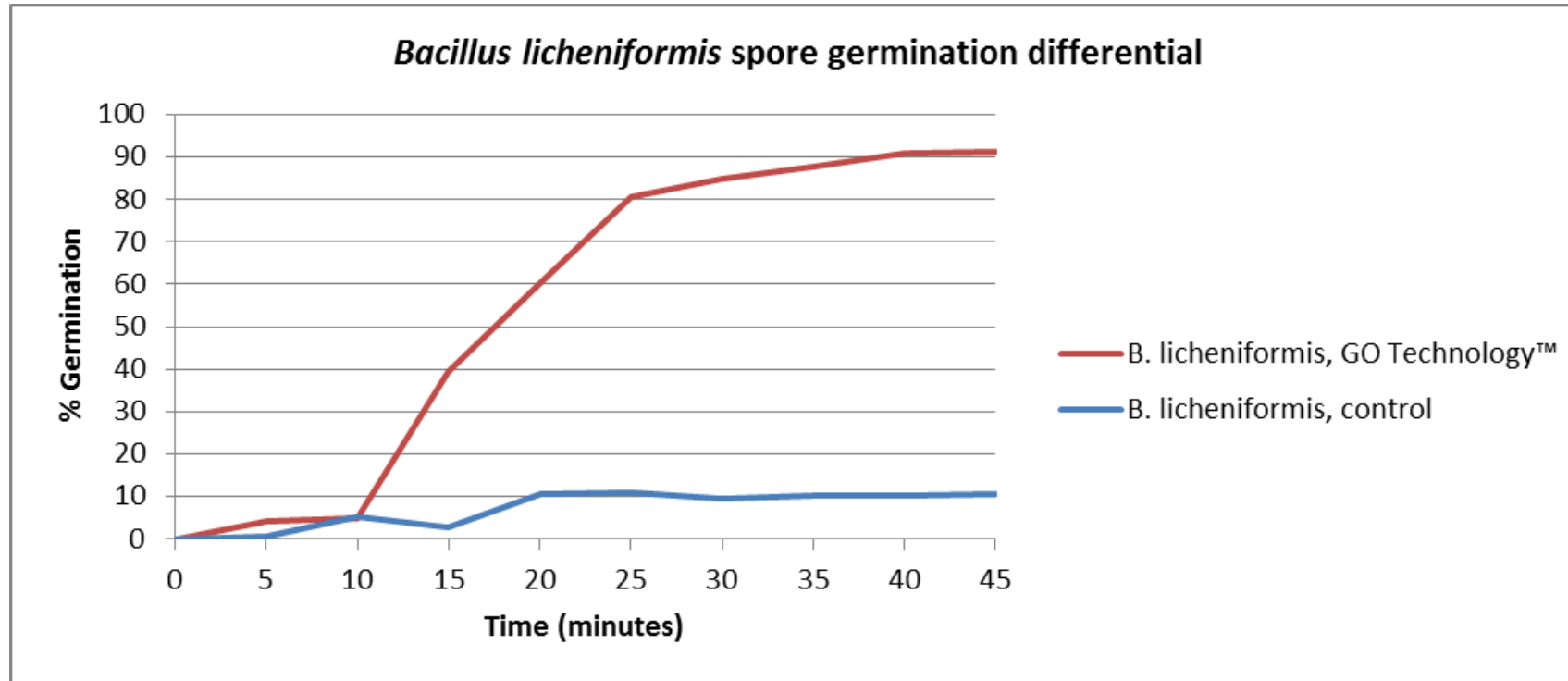


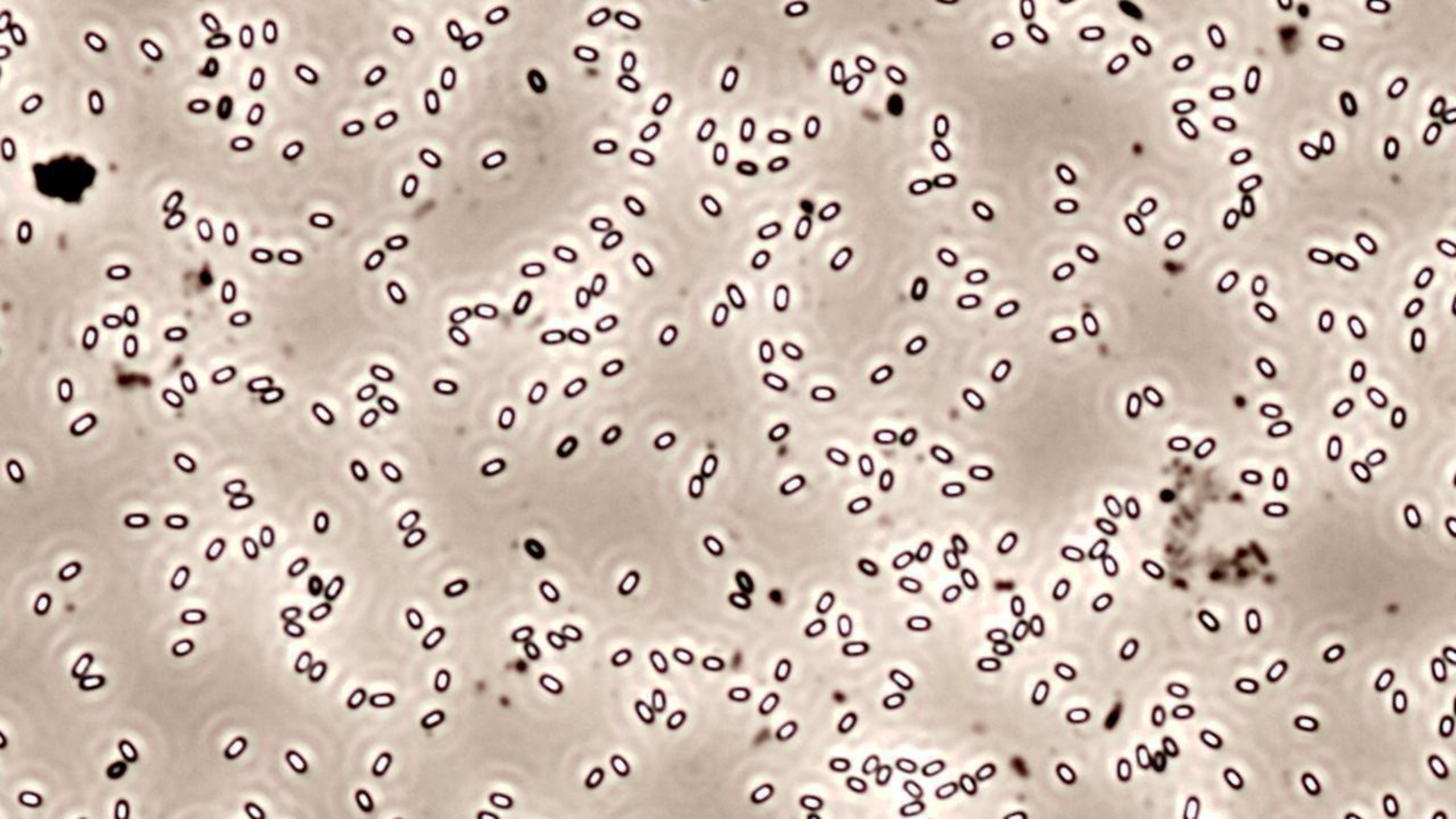
# *Bacillus licheniformis* Extended Time Without GO Technology®

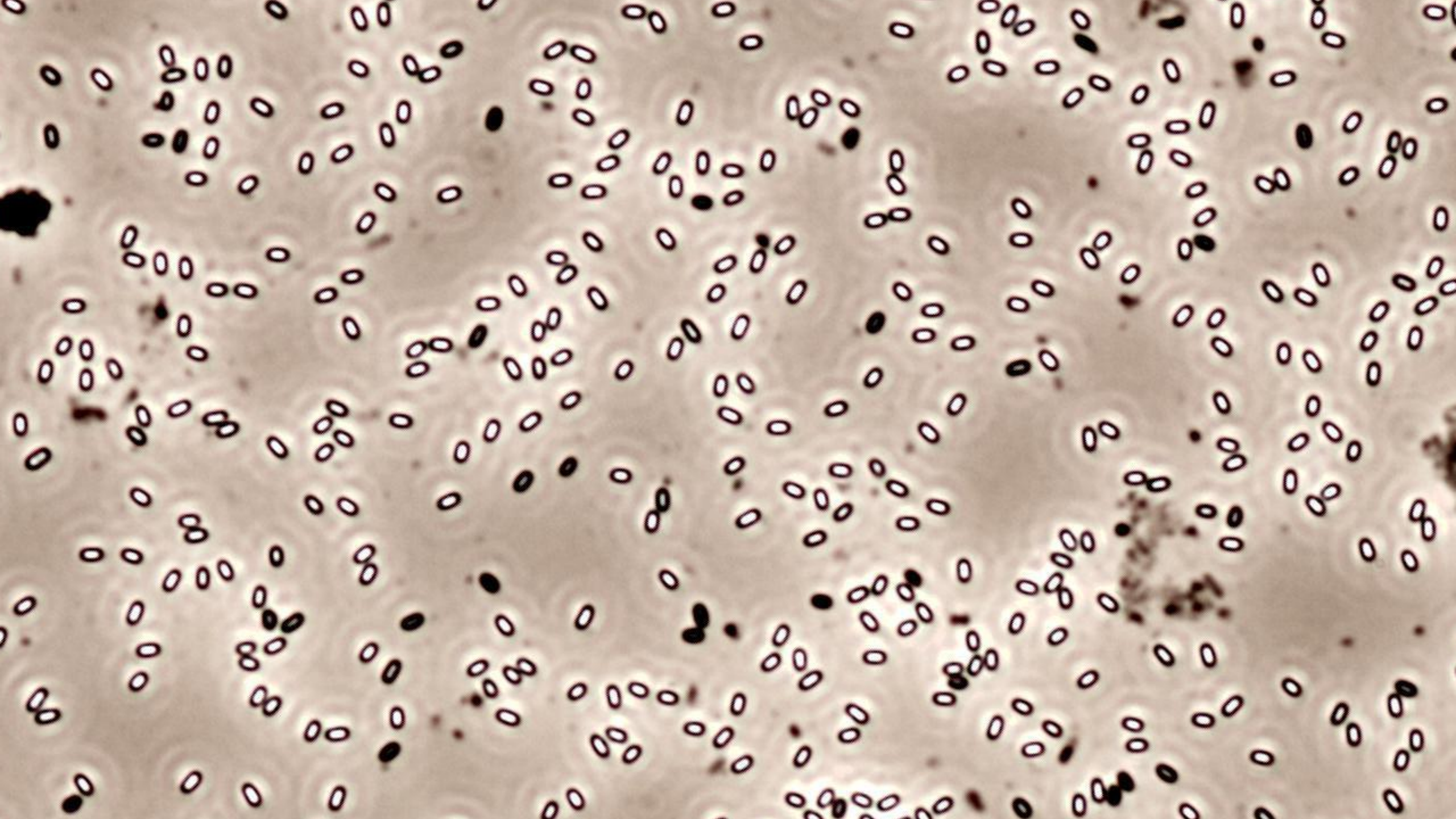


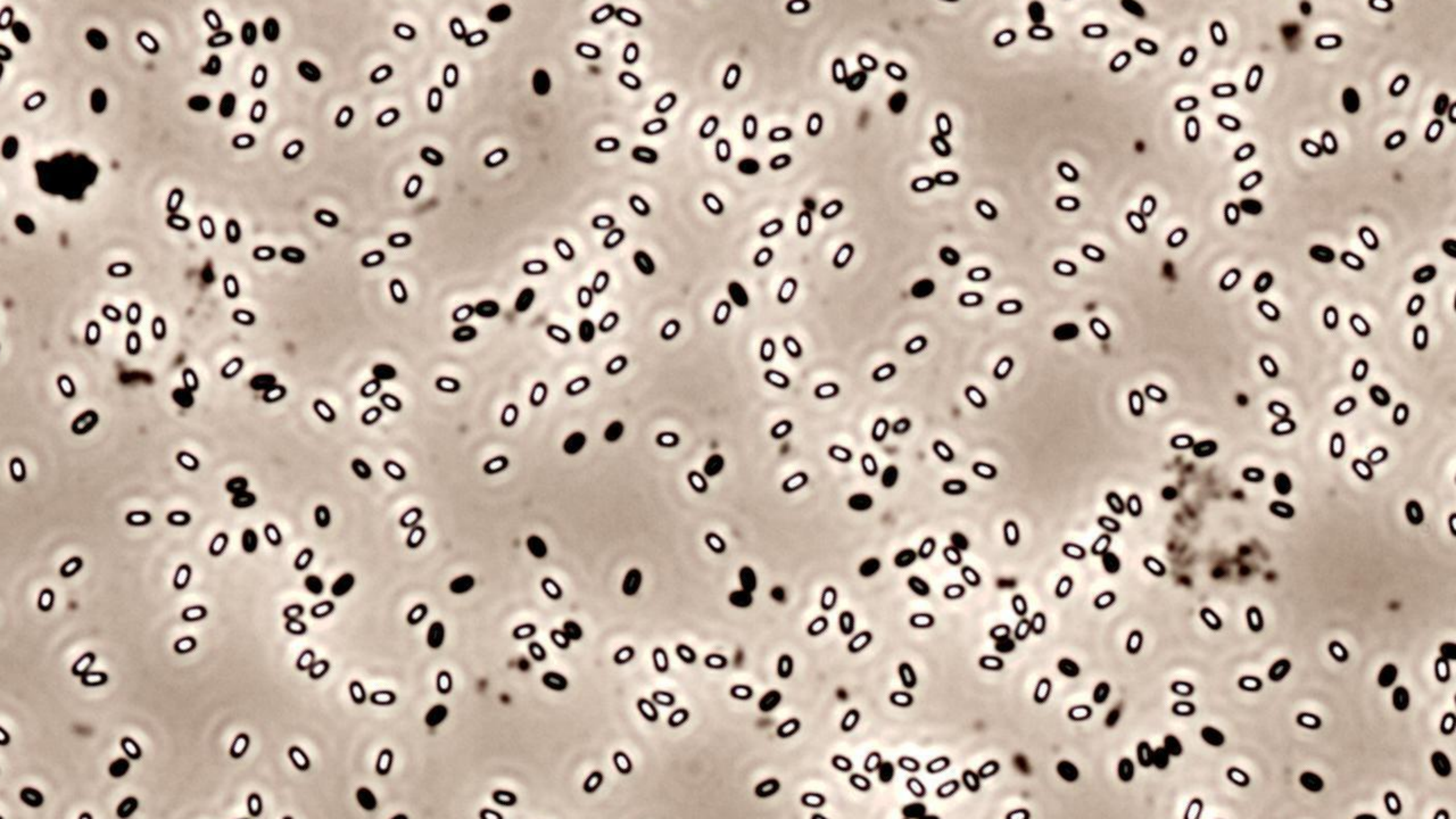


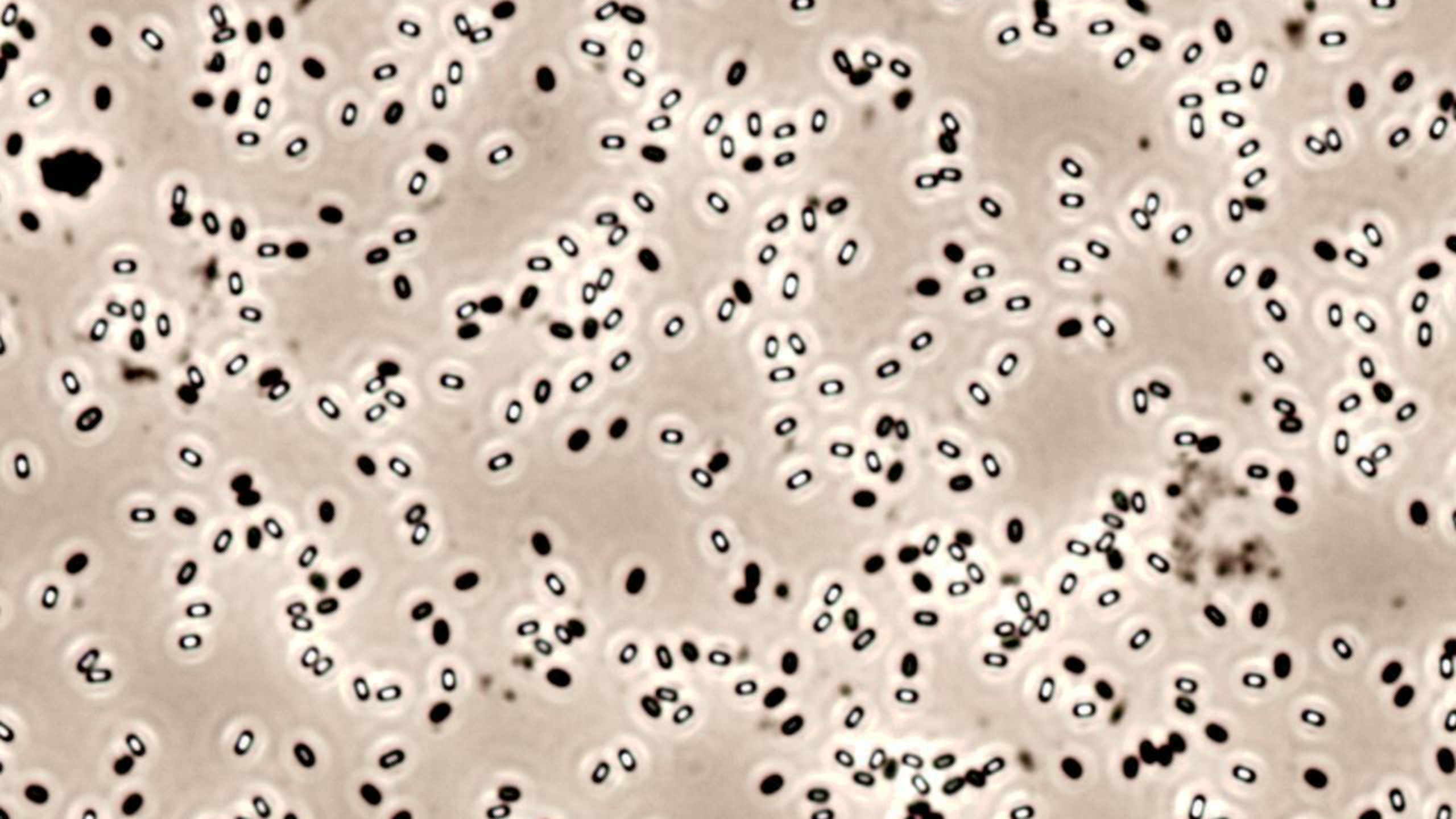
# *Bacillus licheniformis* Germination With GO Technology®

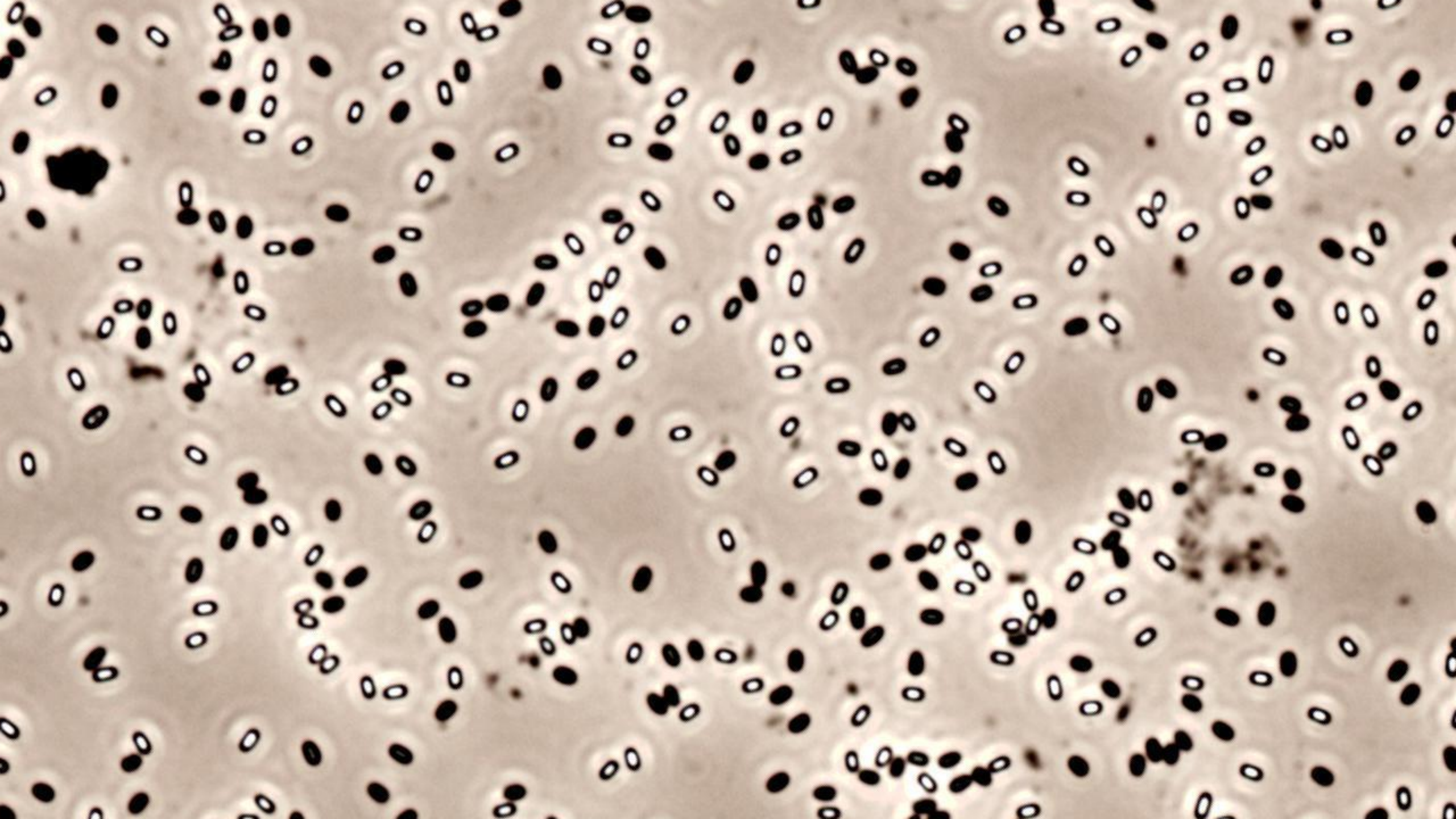


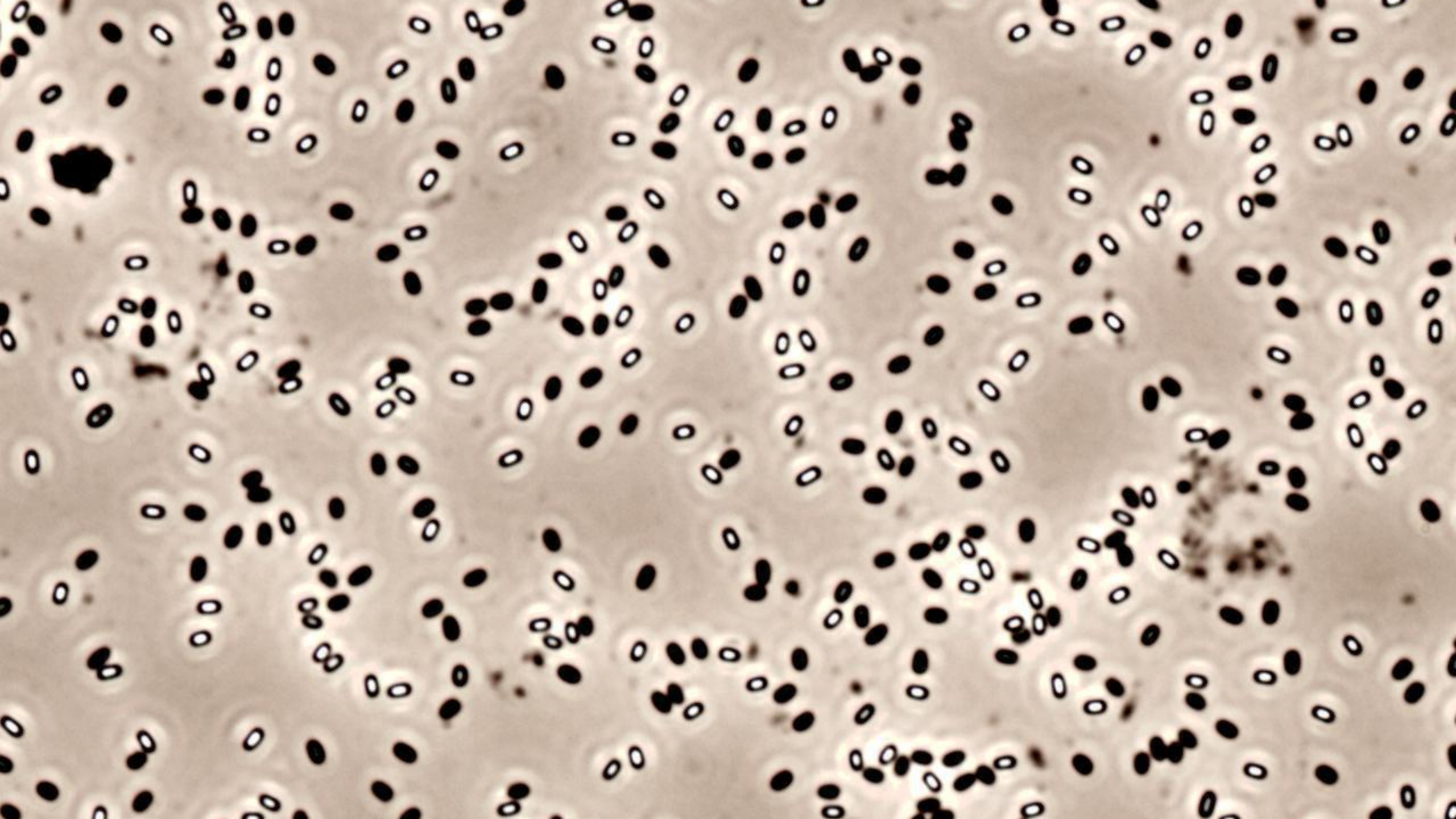


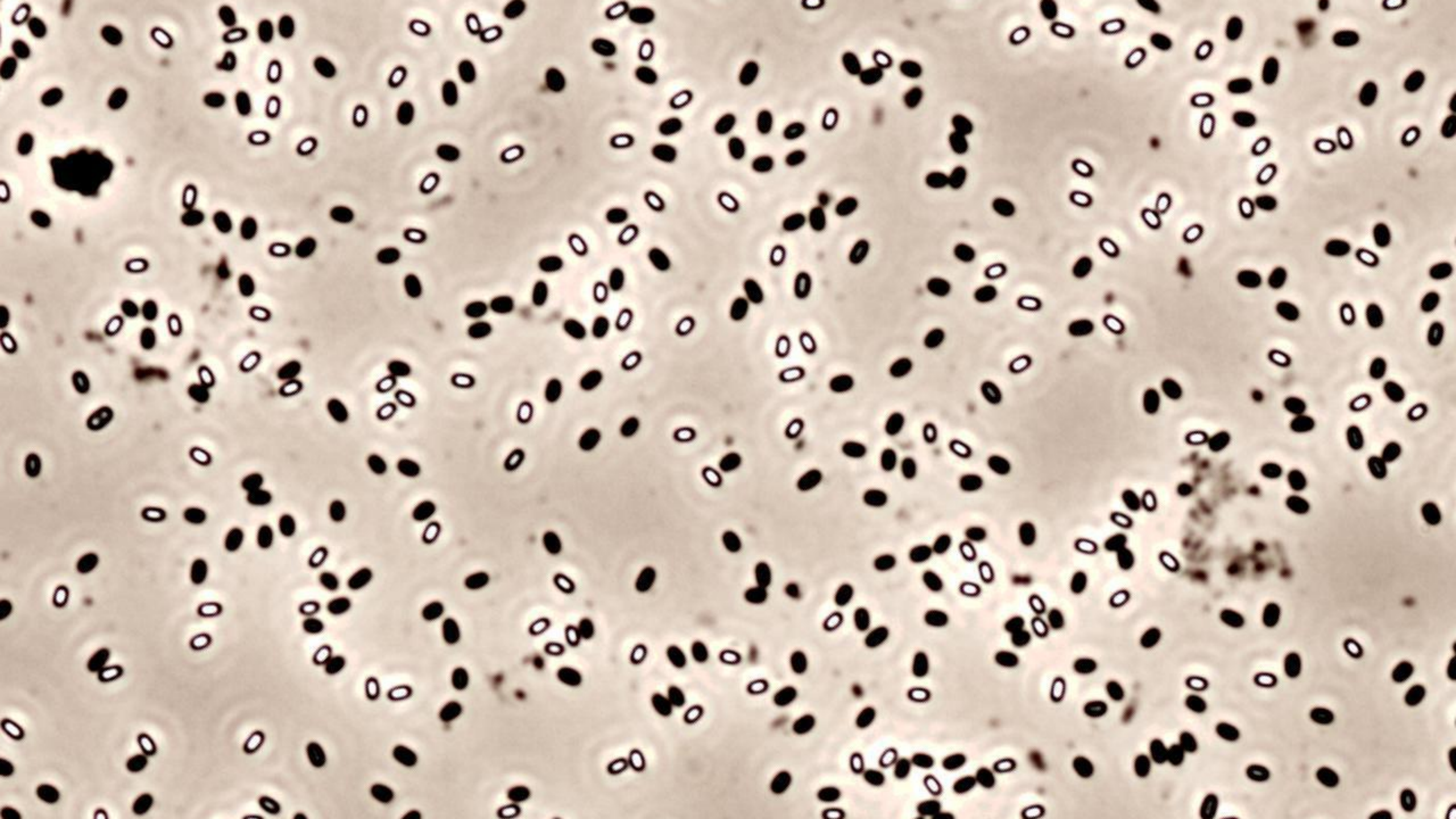




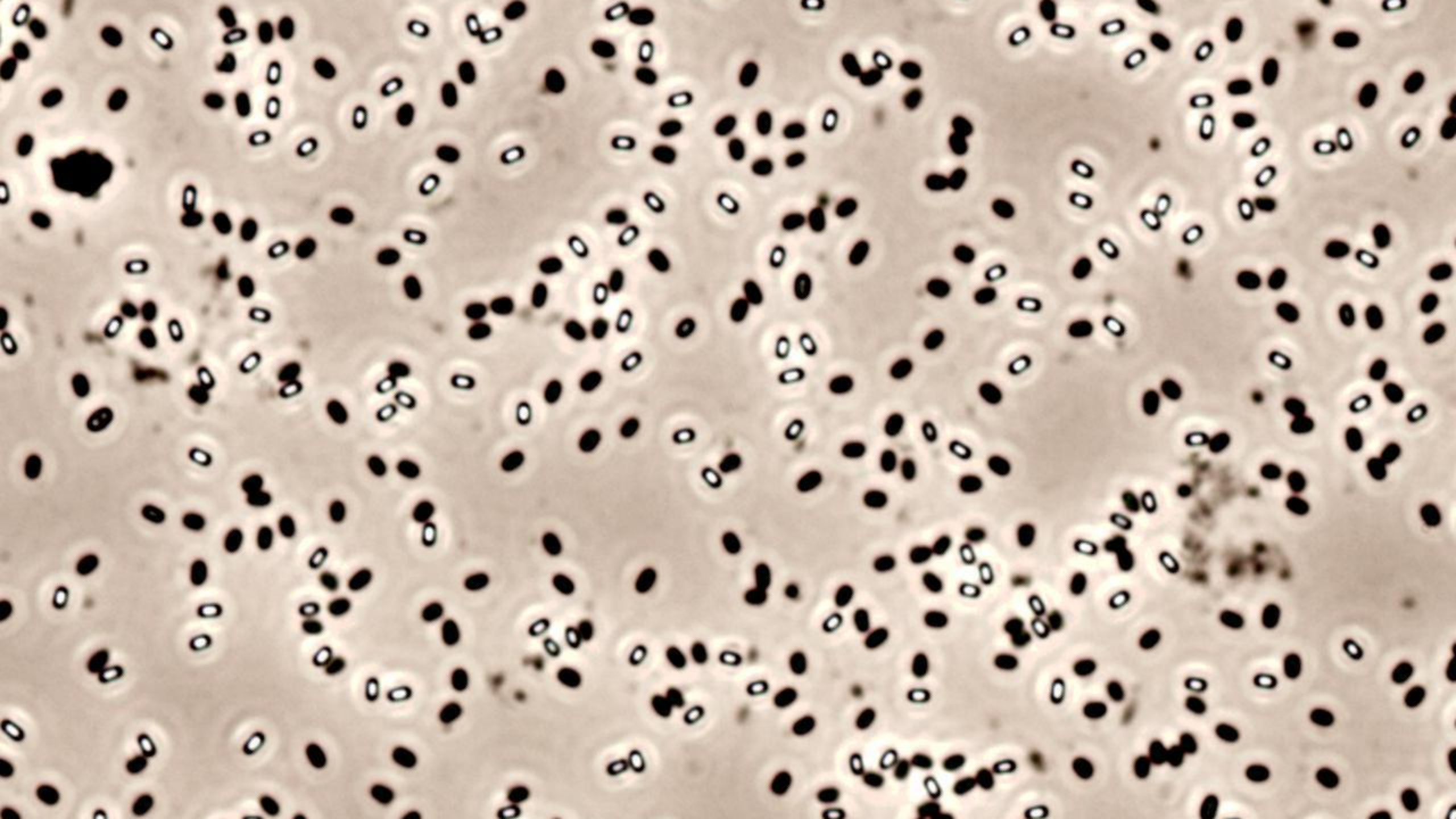


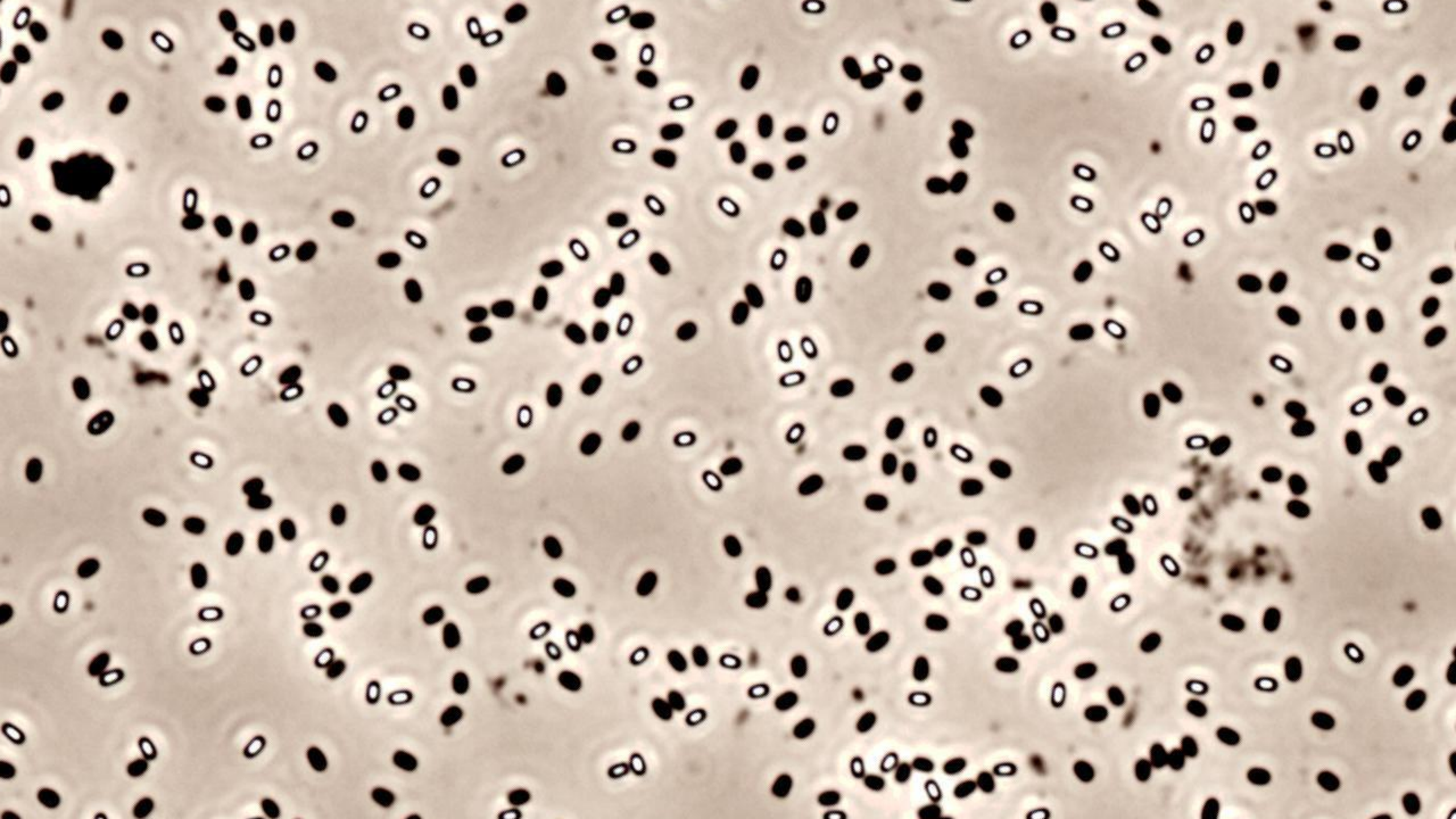


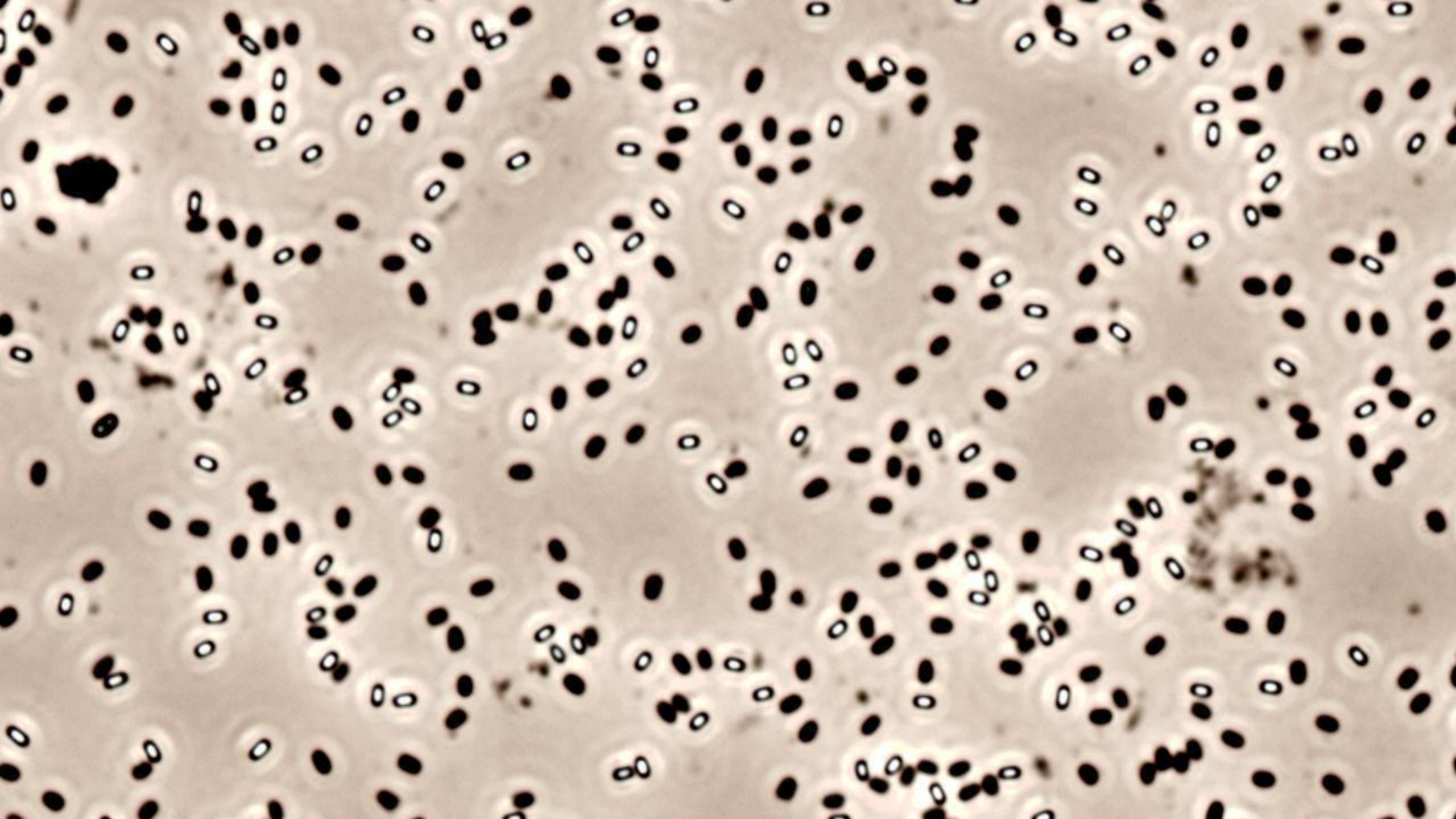


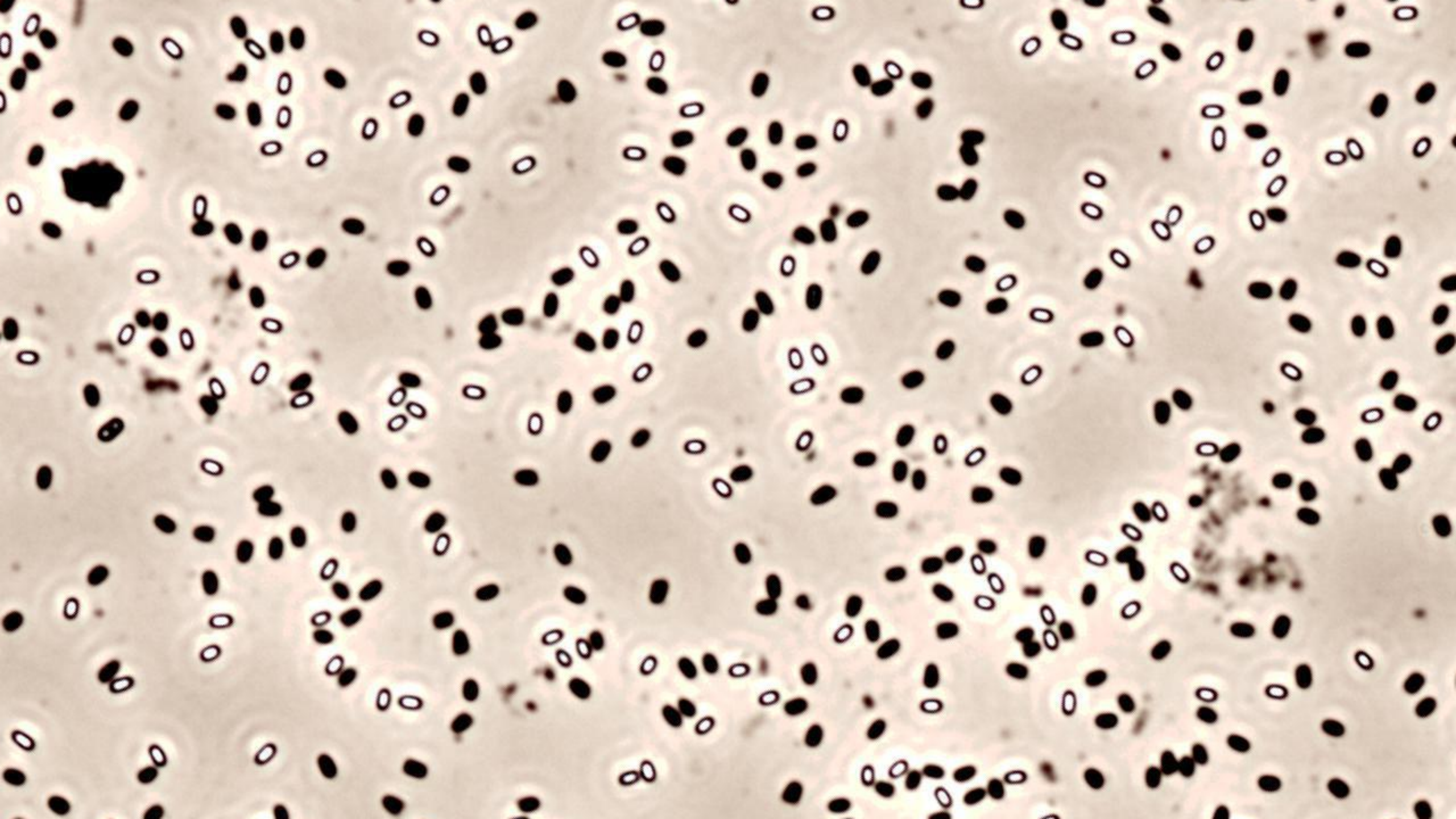






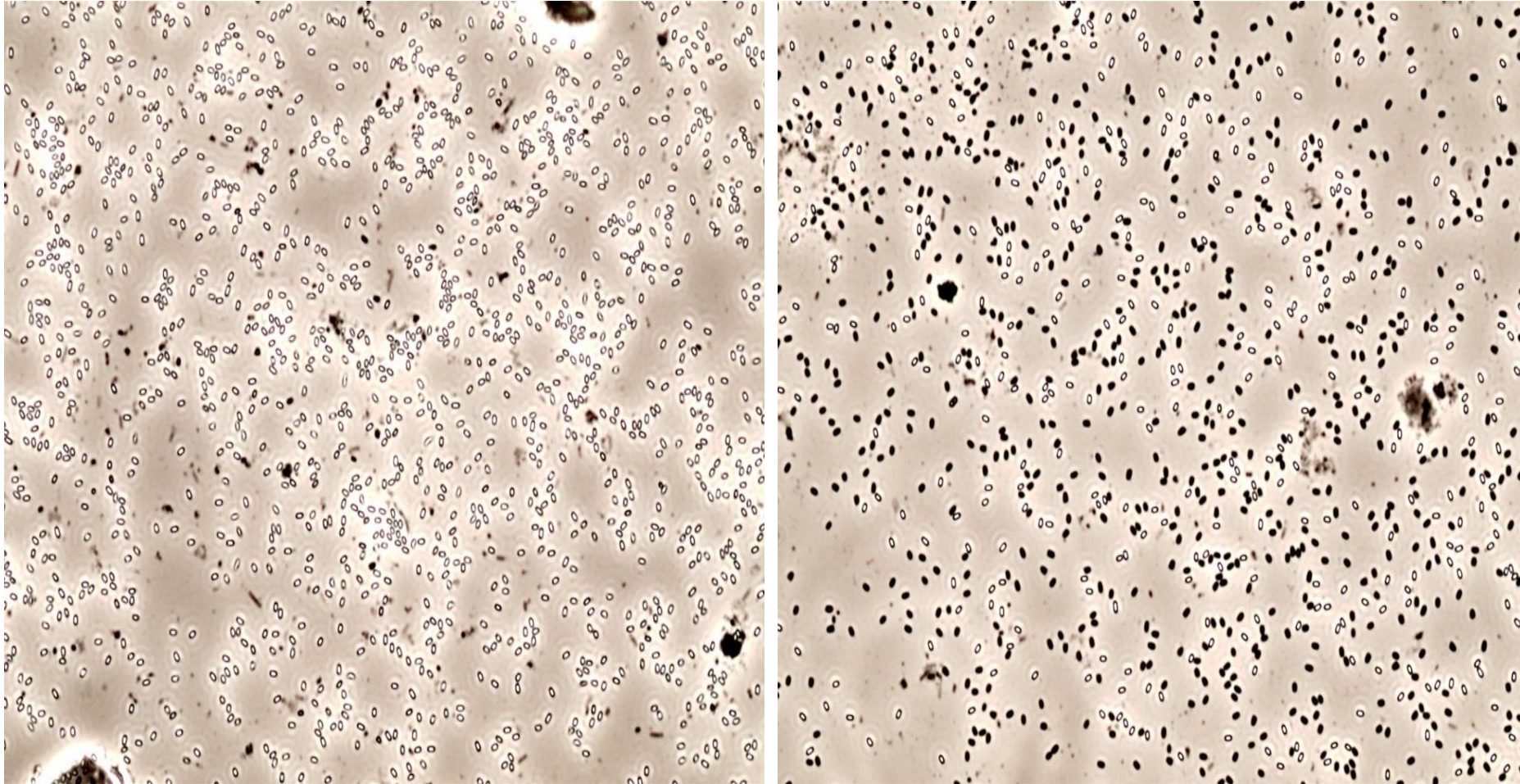




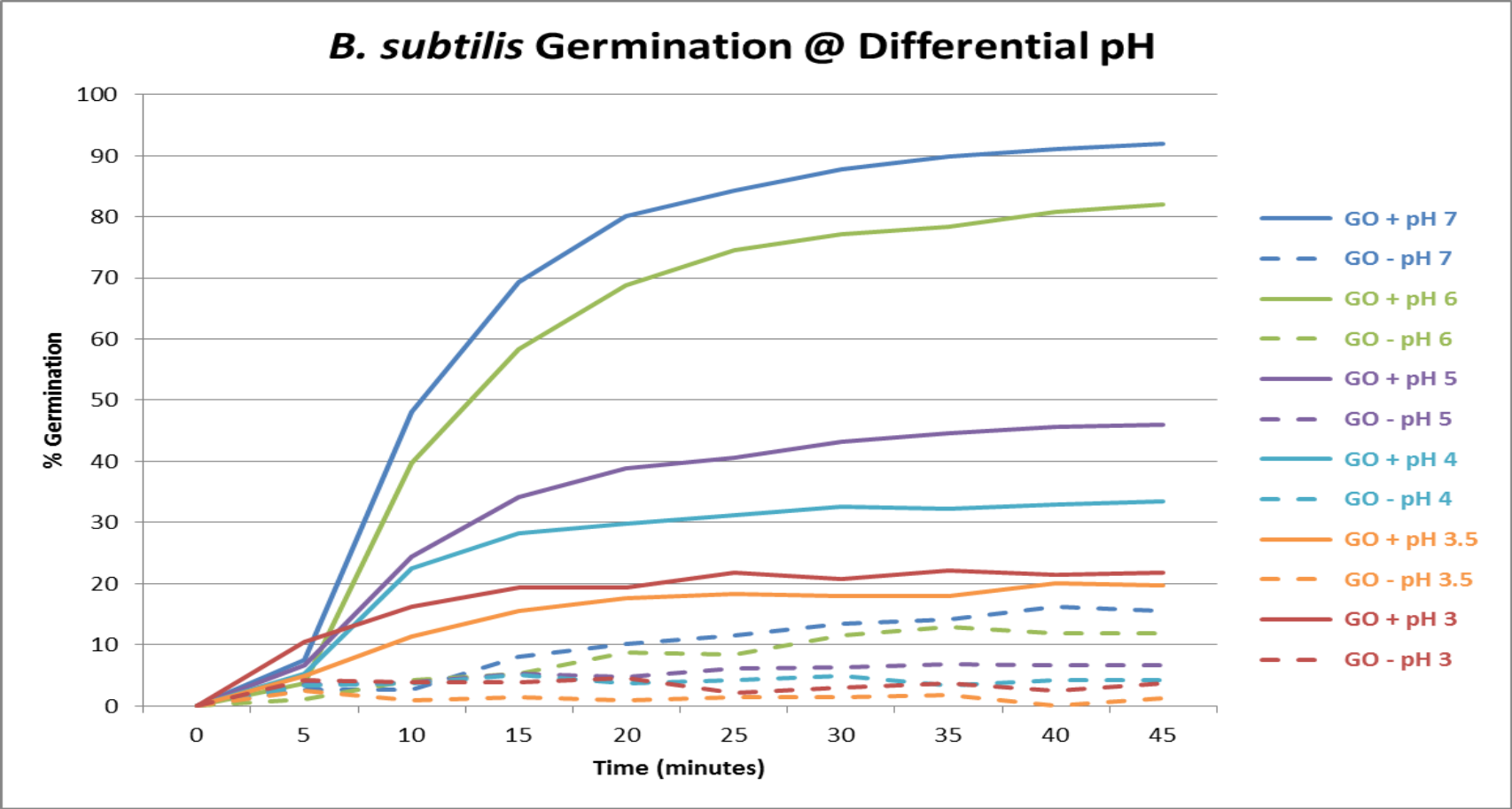


# With and Without GO Technology® After 90 Minutes

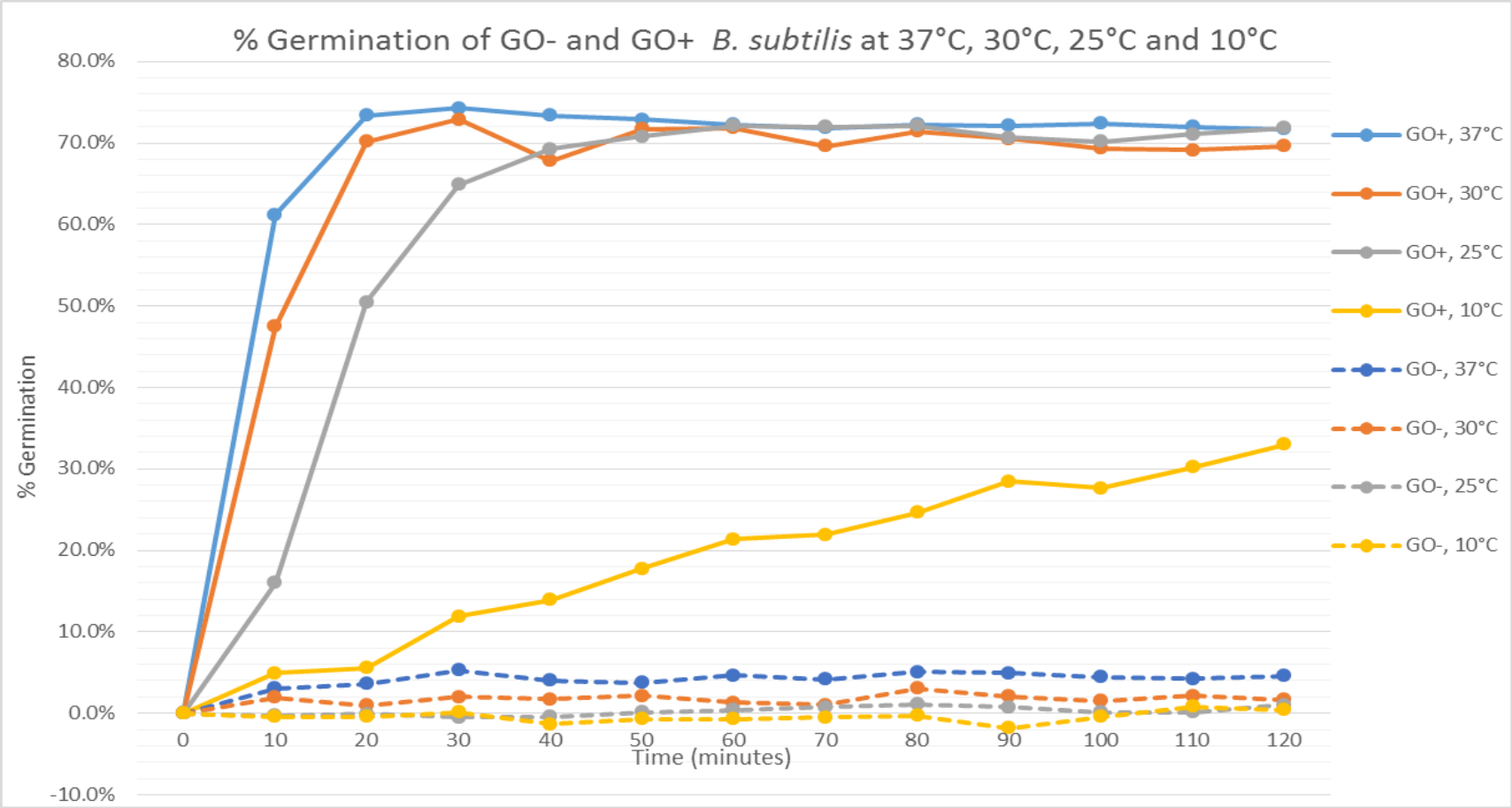
---



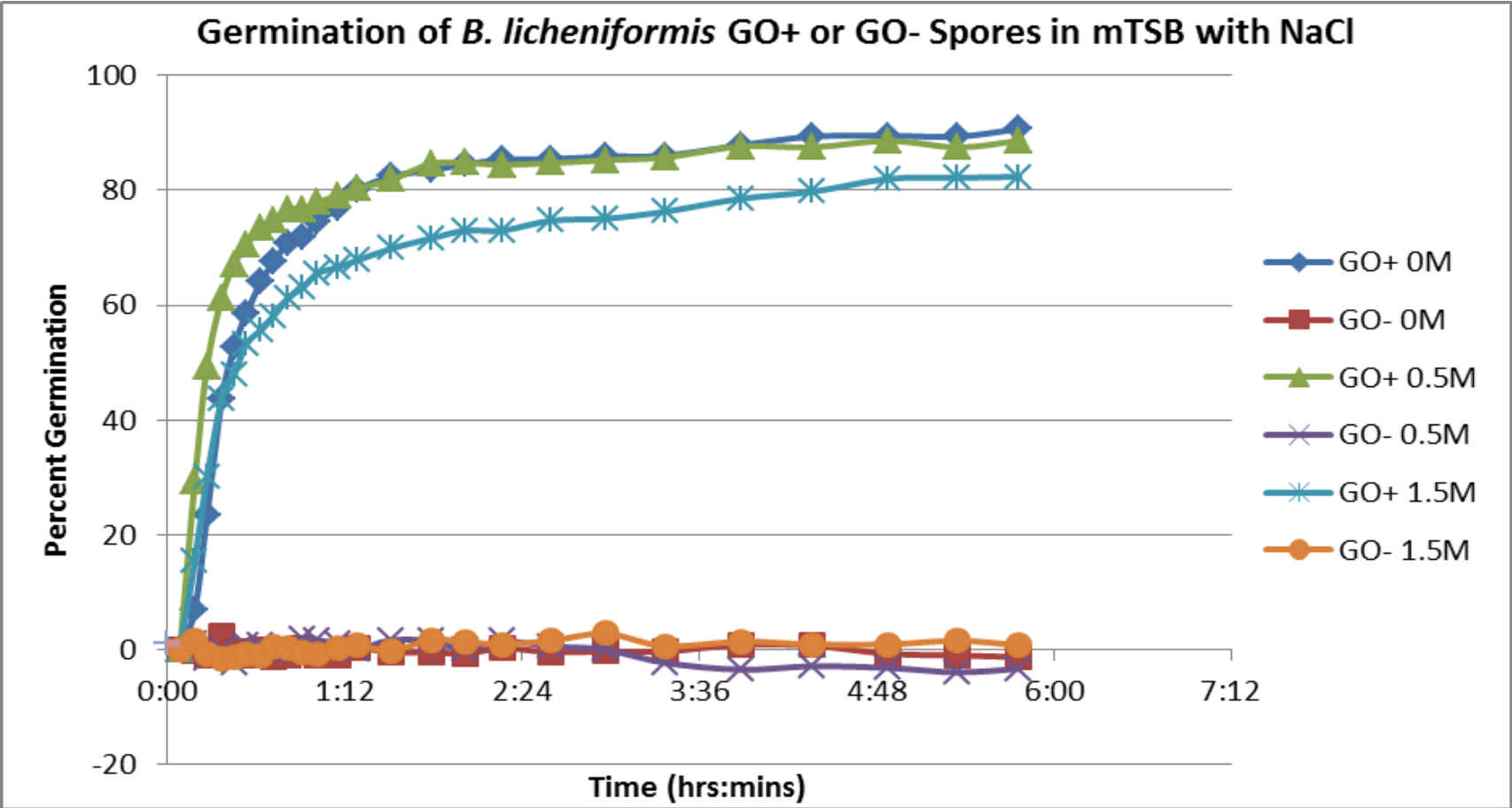
# GO Technology® Performance Across pH Ranges



# GO Technology® Performance Across Temperature Ranges

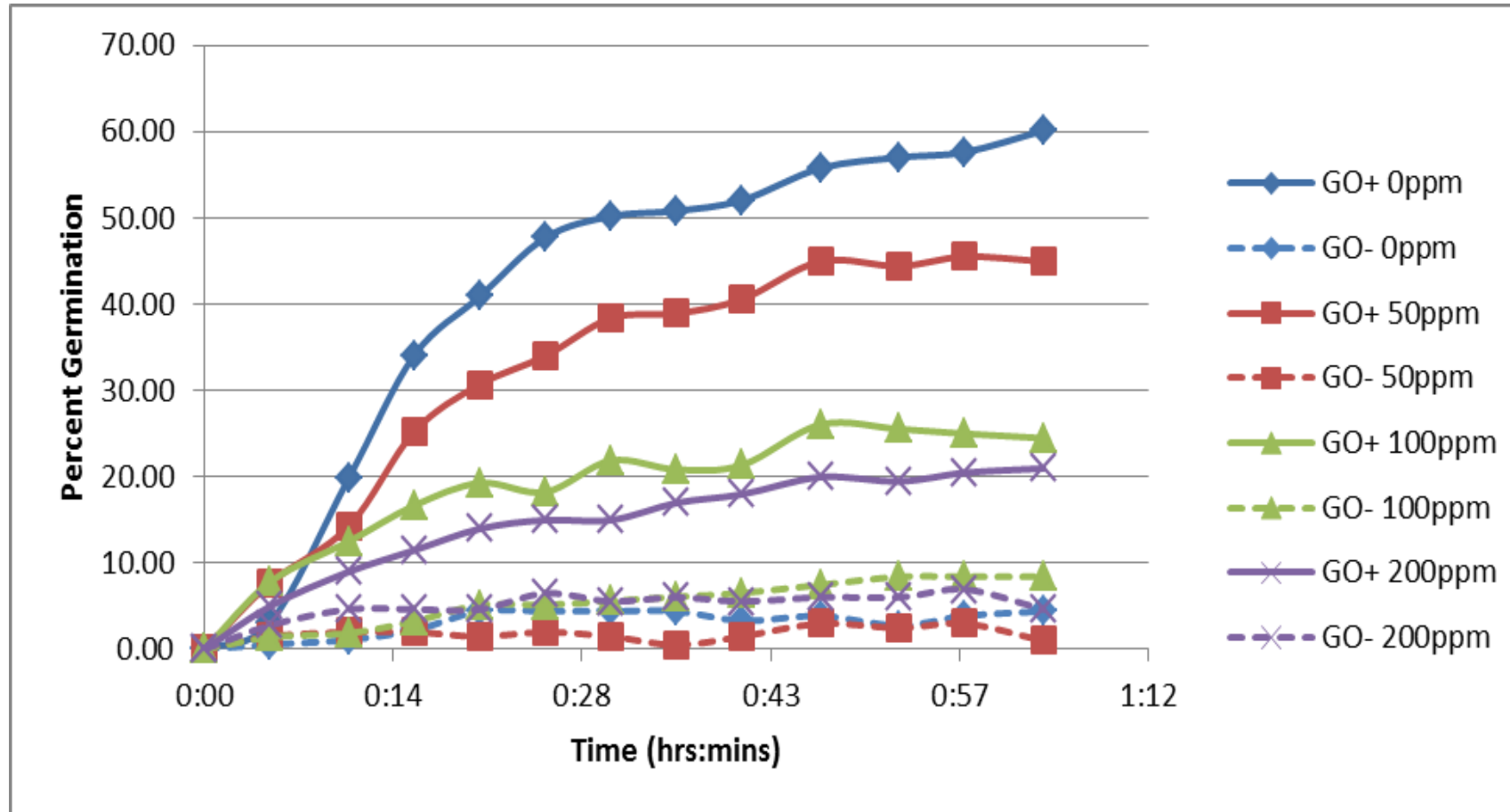


# GO Technology® Enables Germination Under Salt Stress (6 hr.)

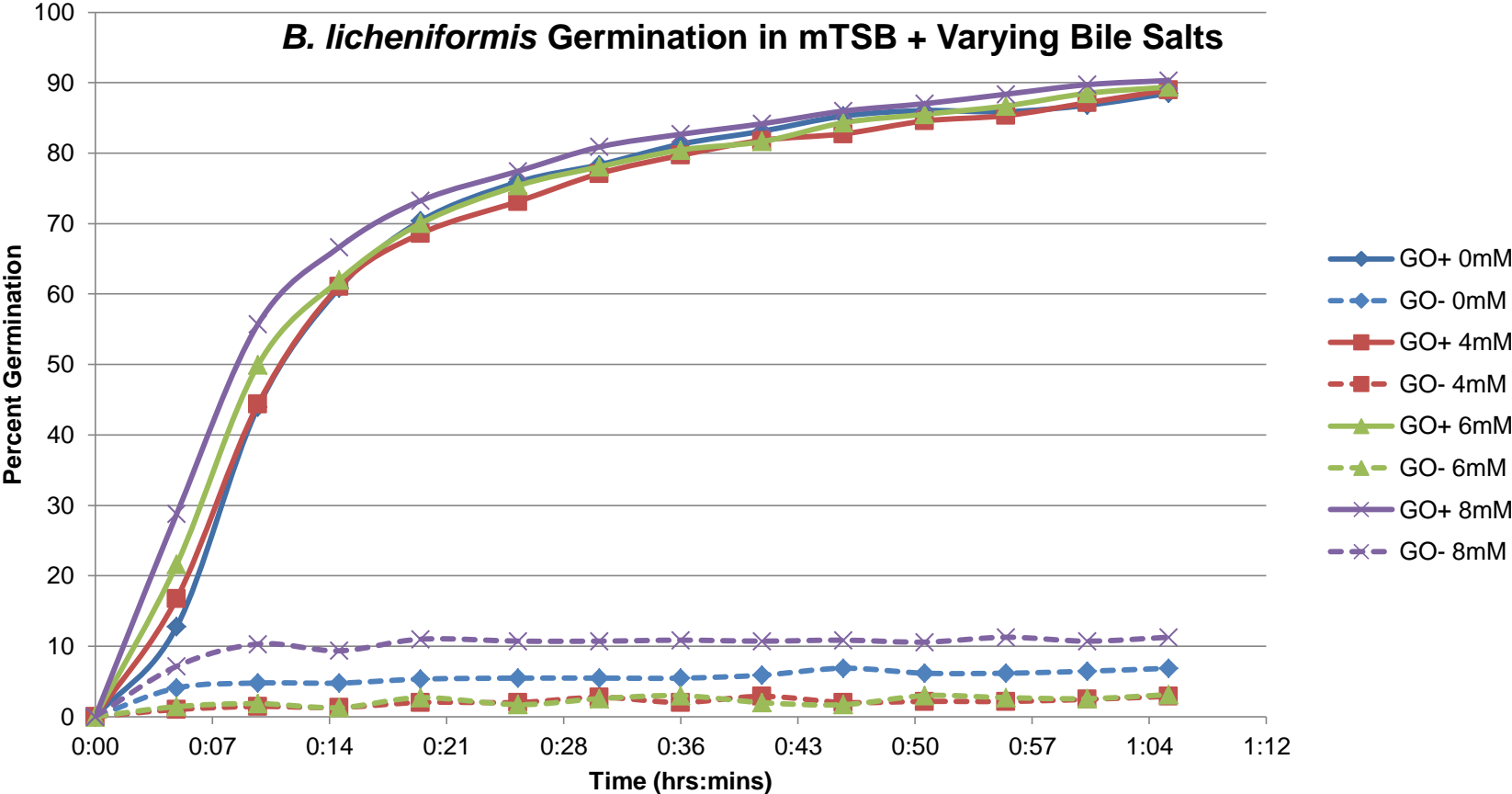




# GO Technology® *Bacillus licheniformis* in the Presence of Copper

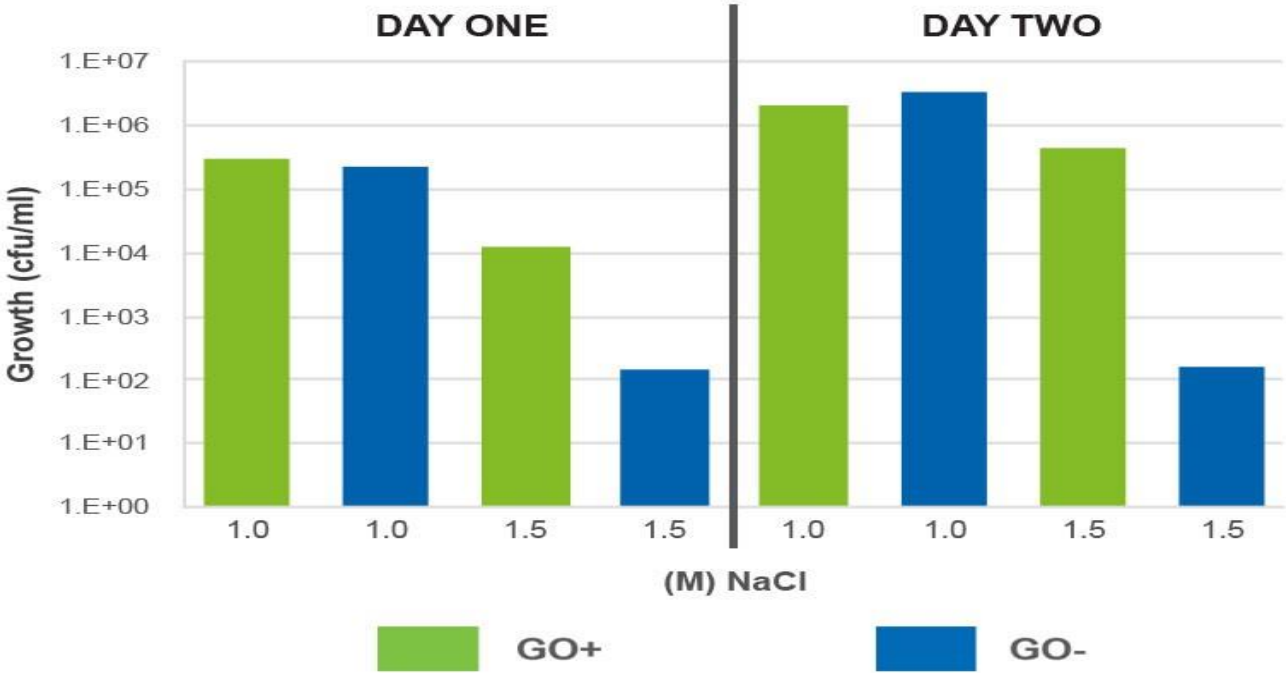


# GO Technology® Influences Germination in Presence of Bile Salts



# GO Technology® Enables Growth in Salt Stress (Two Days)

Growth of *B. licheniformis* from GO+ and GO- Spores in mTSB with Osmotic Stress

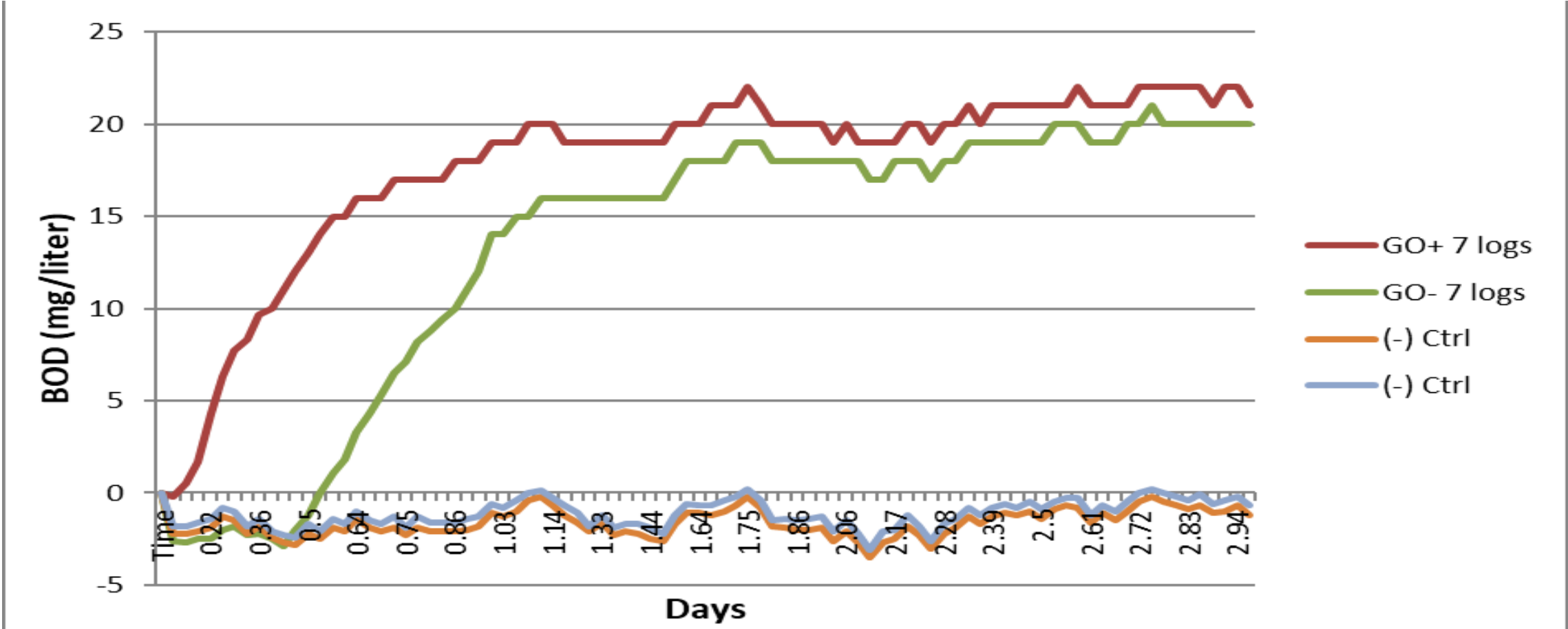


# Respirometer

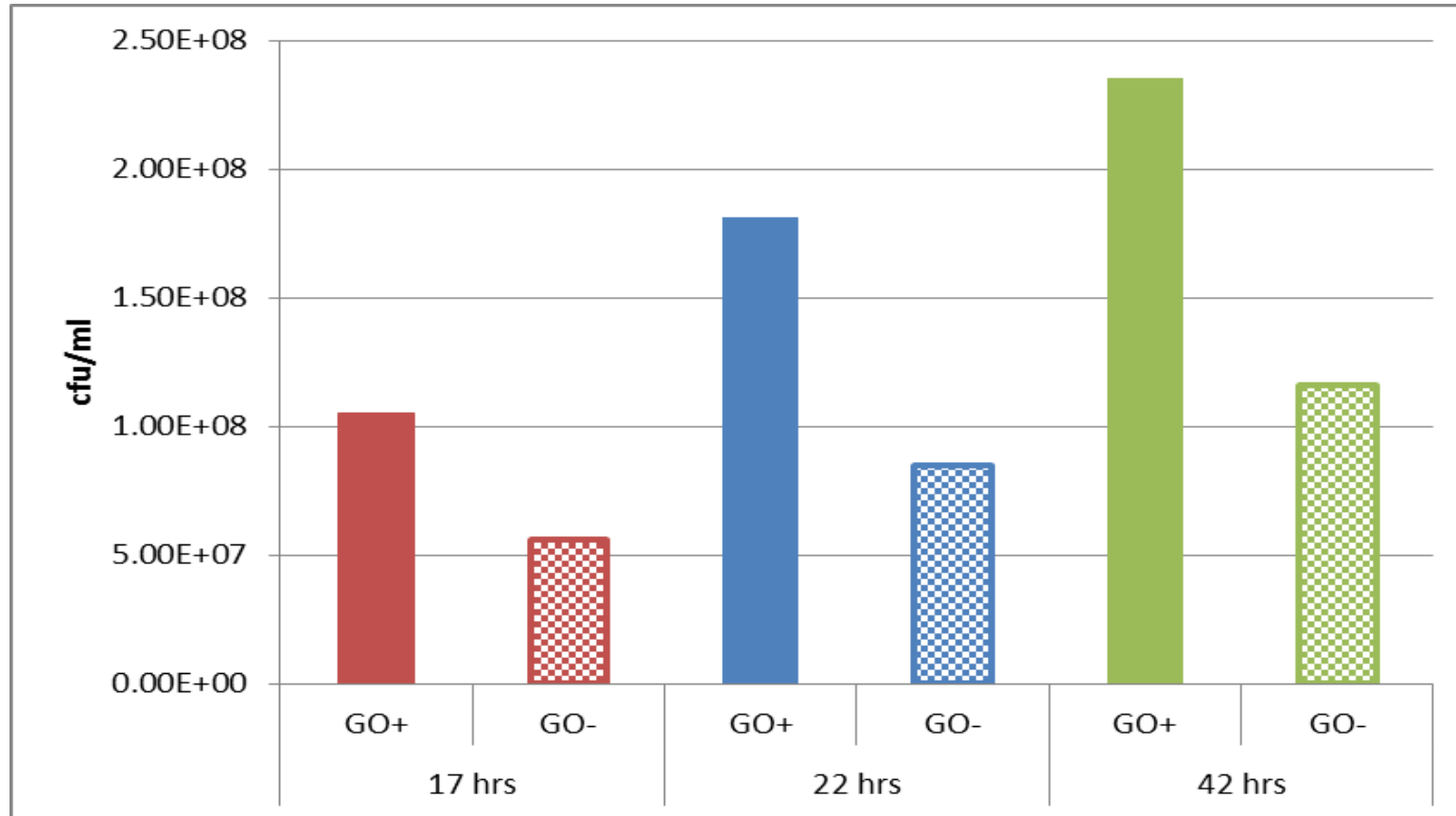
---



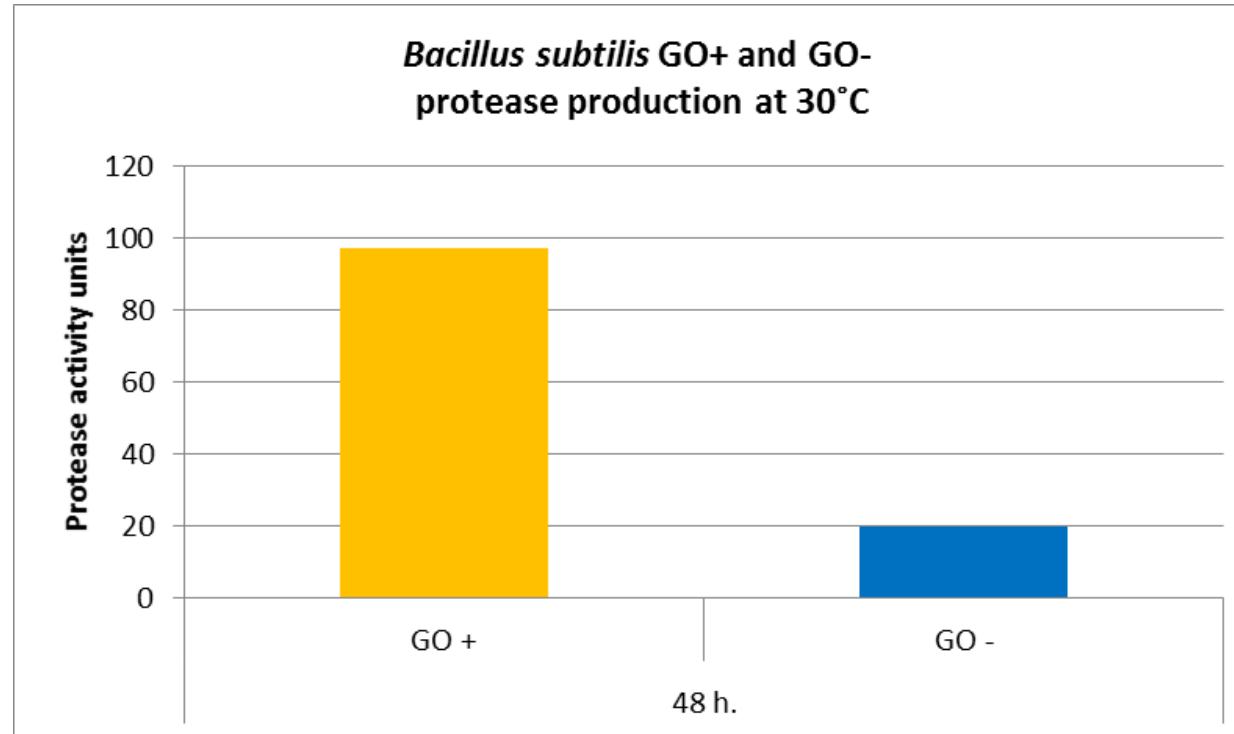
# Bacillus licheniformis BOD Reduction in Animal Feed with 4 mM Bile Salts



# *Bacillus licheniformis* BOD Test Growth in Animal Feed with 4 mM Bile Salts



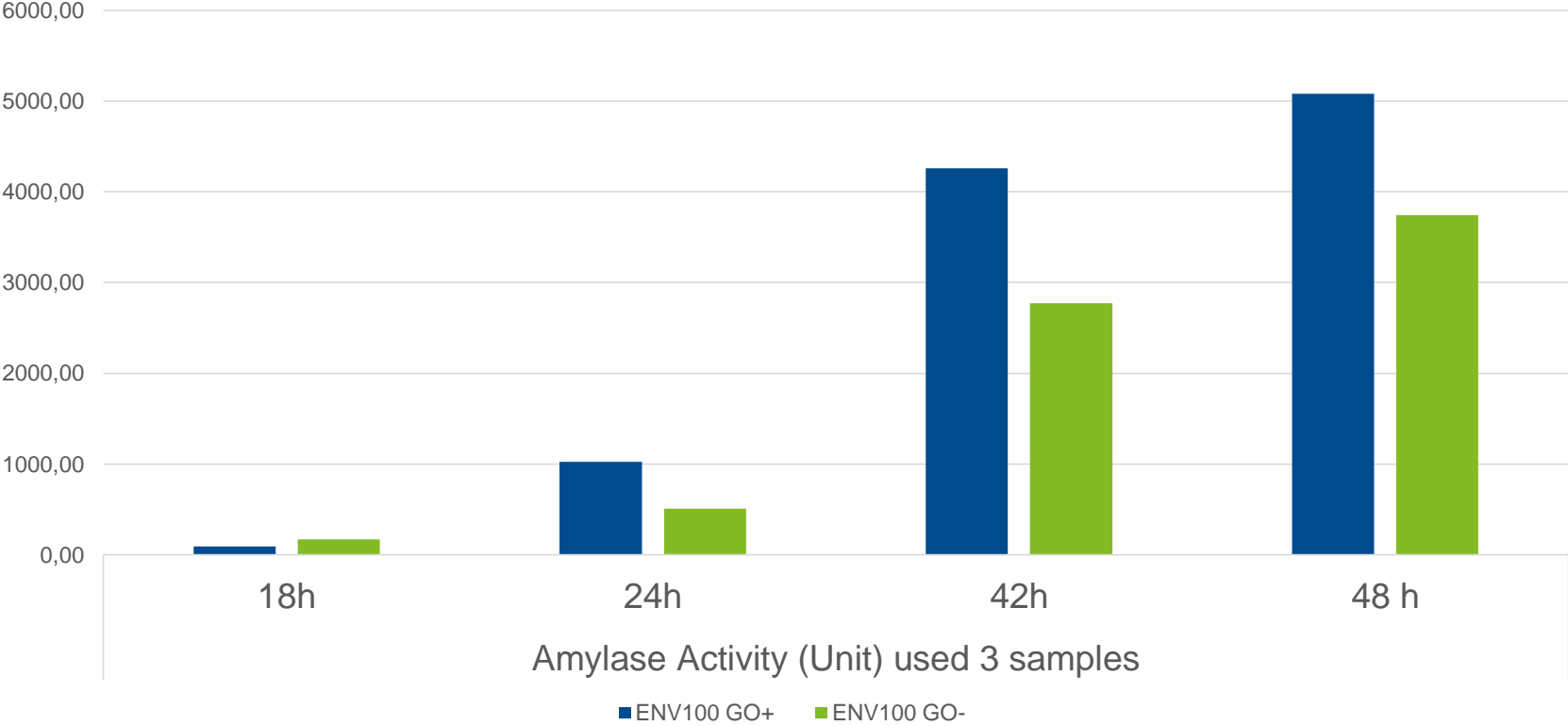
# GO Technology® Increases Metabolism as Measured by Improved Enzyme Action



Sample		Protease activity units	OD at 650 nm	
48 h.	New Culture	GO +	97	0.583
		GO -	20	0.131

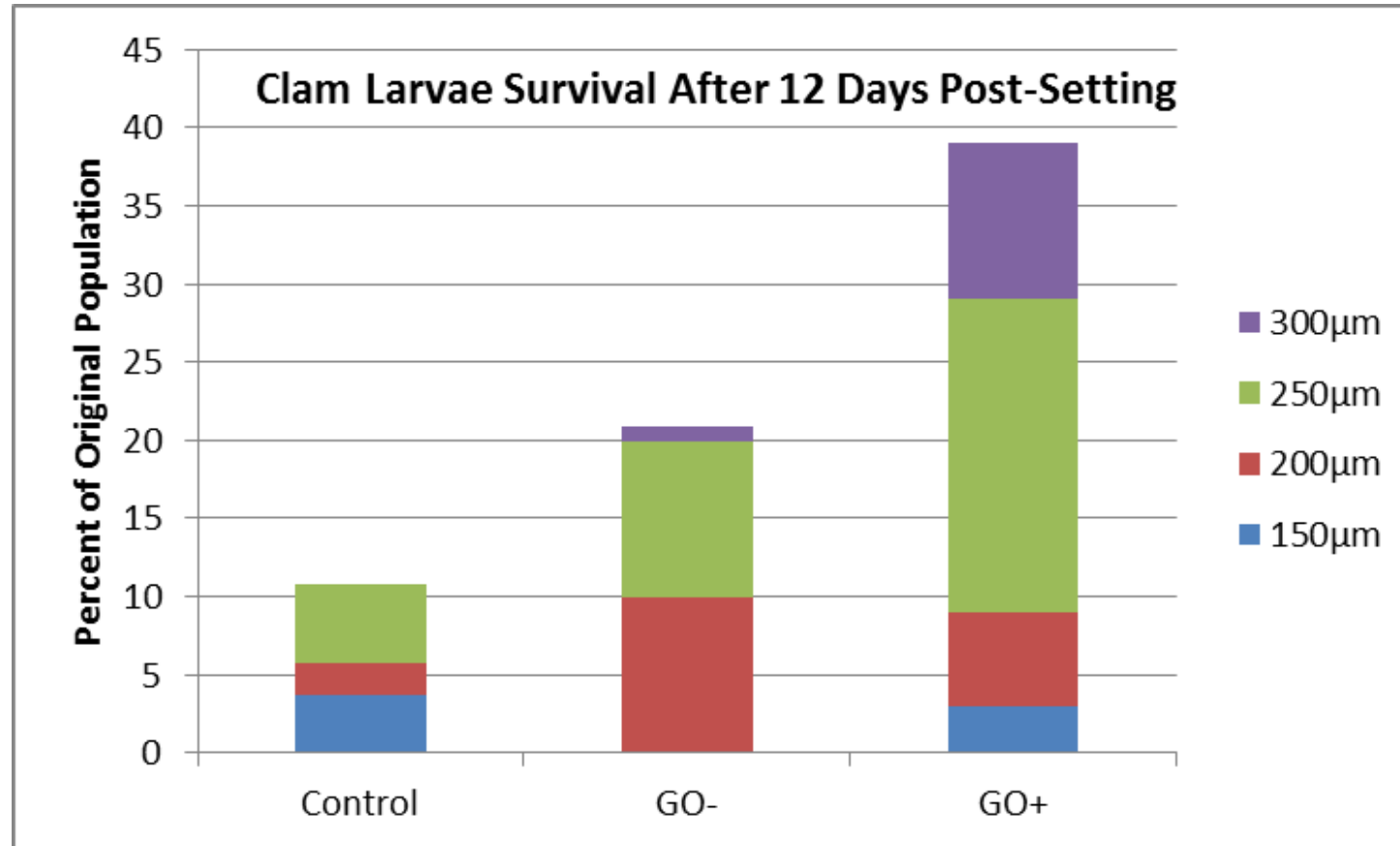
# GO Technology® Increases Amylase Activity

Amylase Activity of *Bacillus licheniformis*  
GO+ vs. GO- (3 Replications)



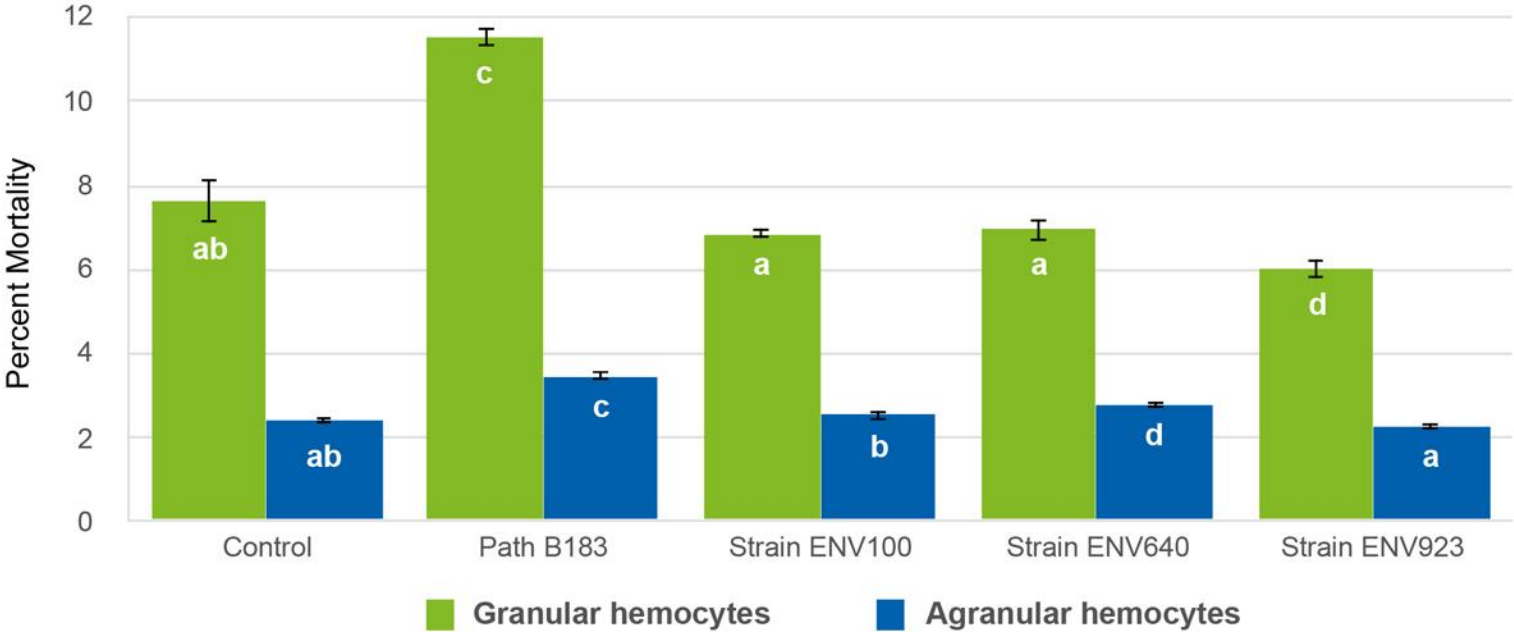


# GO Technology® Performance In Mollusc (Clam) Trials

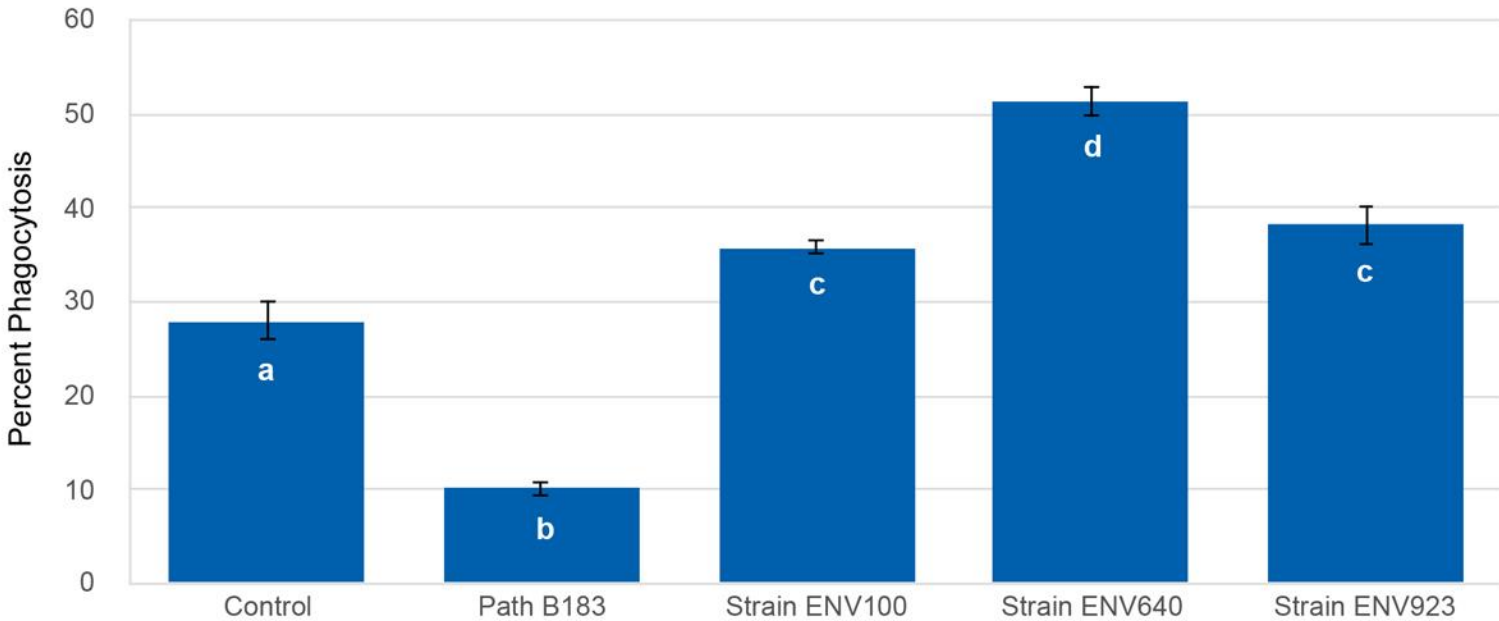


# Hemocyte Immune Function

Viability for granular and agranular hemocytes

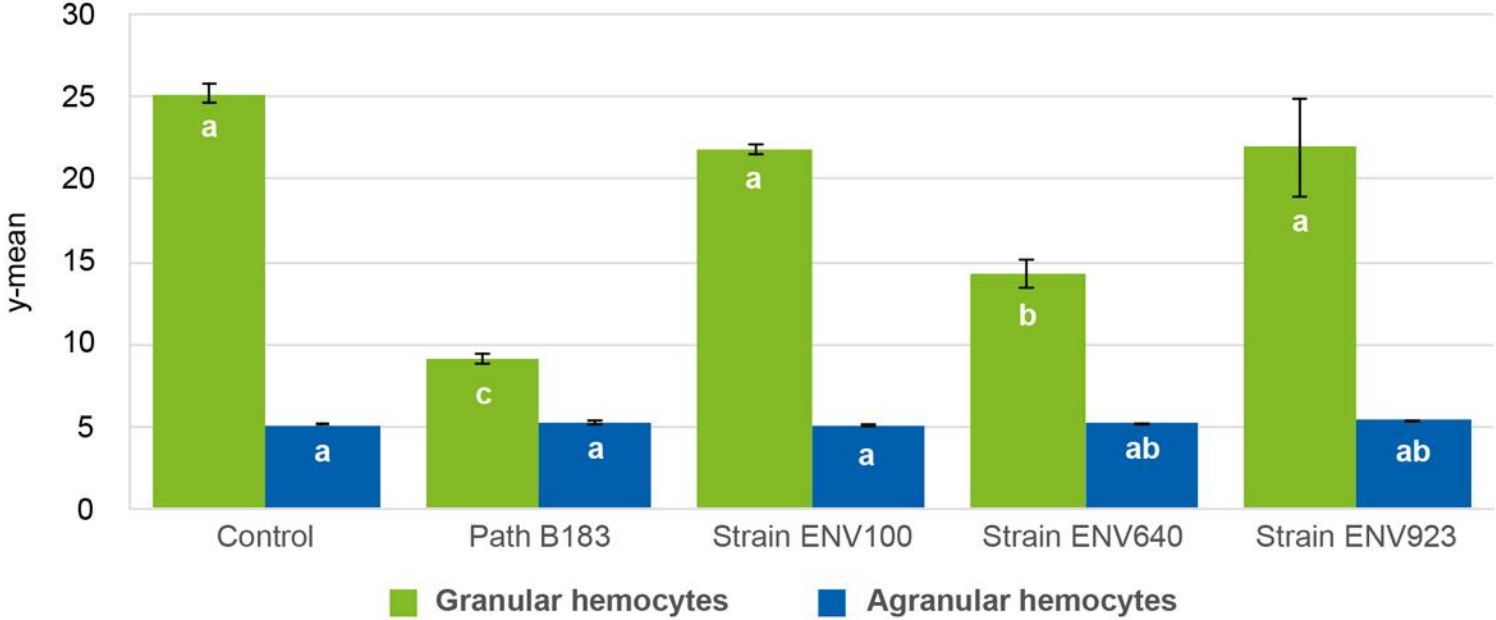


# Percentage of Highly Phagocytic Hemocytes\*



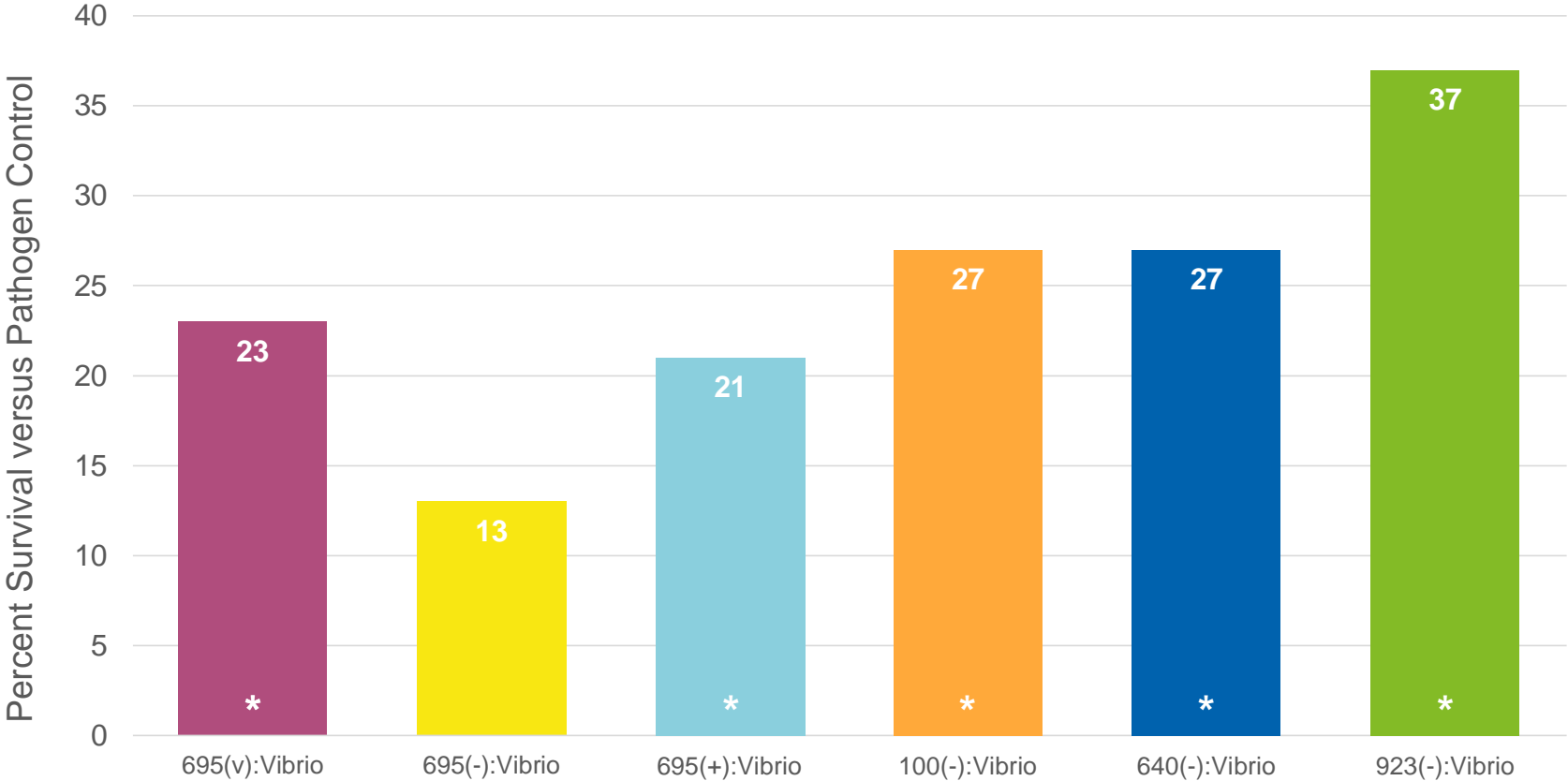
\*Granular hemocytes that engulfed three or more fluorescent beads.

# Reactive Oxygen Species Release



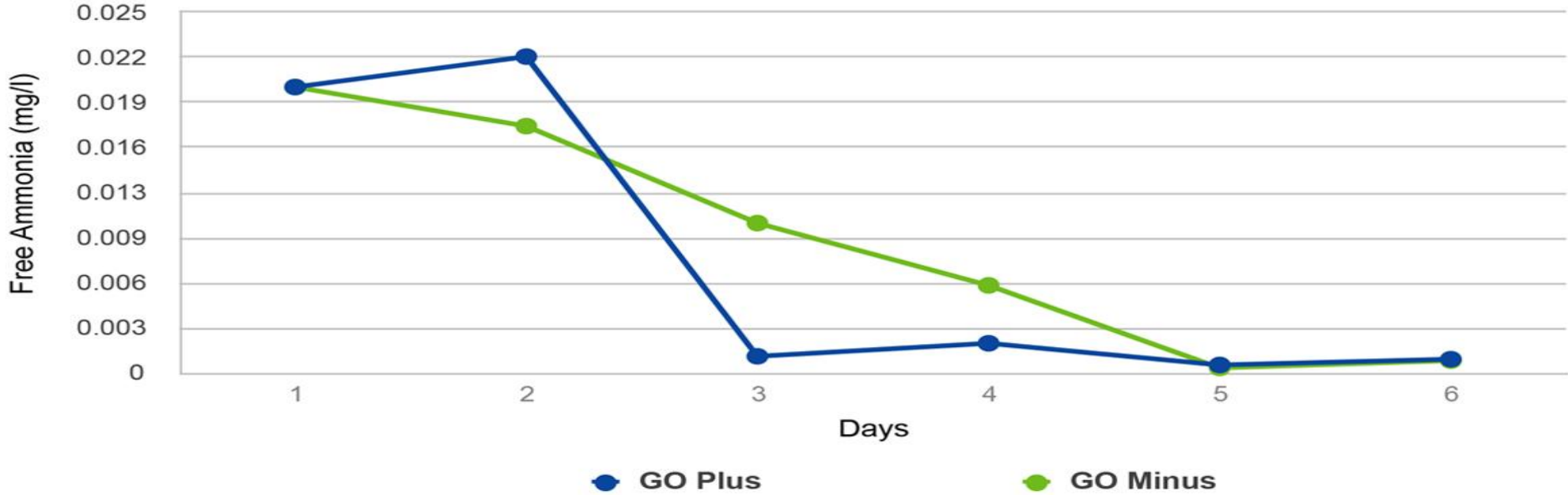
\*Note: Unstimulated

# Survival of Oyster Larvae Treated with Probiotic Formulations (URI)

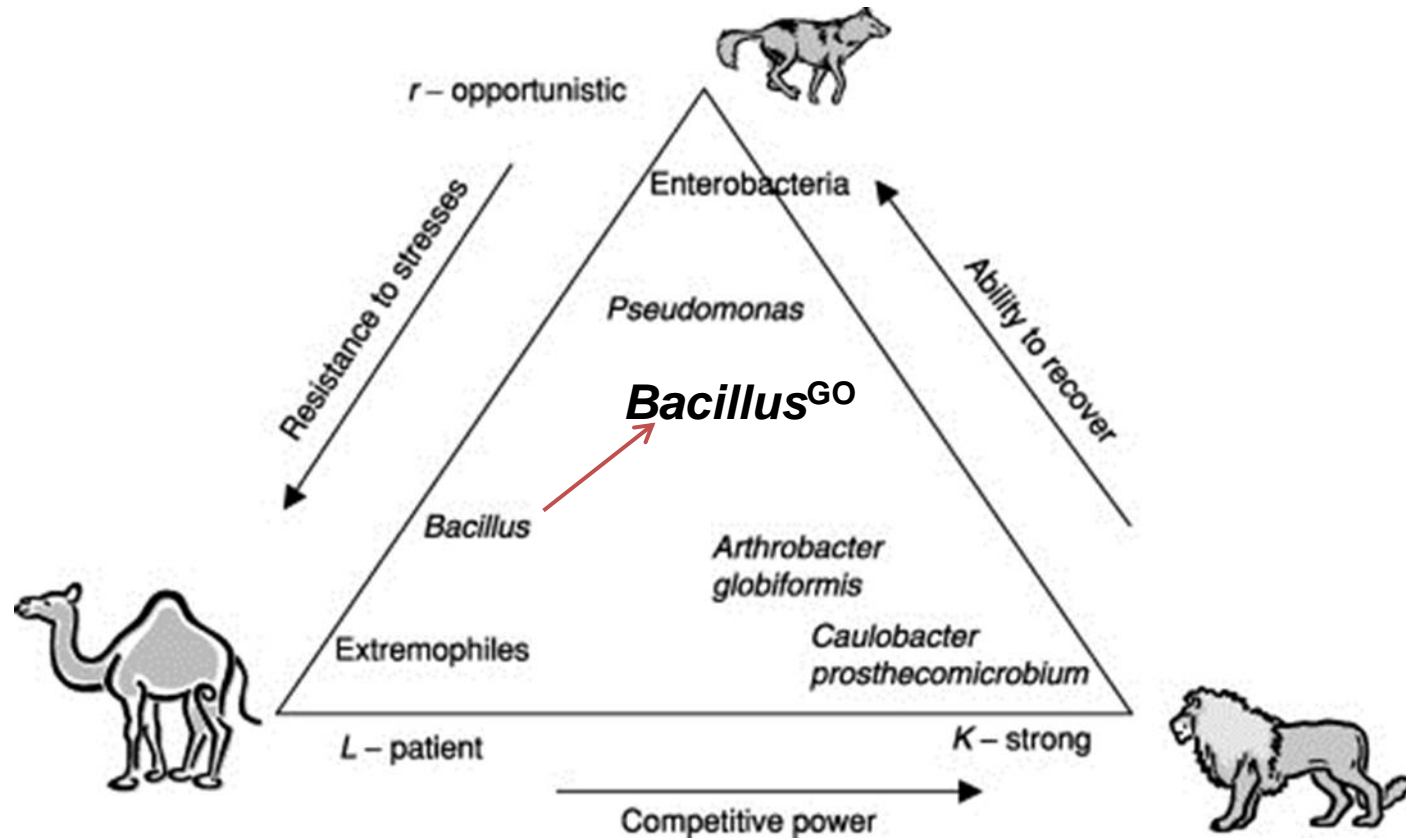


\*P <0.05

# Variation of Free Ammonia of Treatments



# Germination Optimization Results in a Stronger More Competitive *Bacillus*



# GO Technology® More Than Germination Speed

---

- ↑ Germination down to pH3
- ↑ Germination in Temperature down to 10°C
- ↑ Germination in Salt up to 1.5M
- ↑ Germination in Cu 200ppm & Al 1ppm
- ↑ Germination in Bile Salts up to 8mM
- ↑ Growth in all Above Stress Conditions
- ↑ Metabolism in Bile Salt
- ↑ Production of Enzymes (Protease & Amylase)
- ↑ Growth and Survival in Animal Trials



- Applicable across *Bacillus* species
- All spore forming bacteria covered in patent
- Ready to use stable spores
- Increased speed of germination
- Increased breadth of germination
- Increased metabolism and performance
- Greater consistency in performance
- Proprietary technology



*Thank You*

Confidential

November 16, 2018