

PARTÍCULAS DE AEROSSÓIS COMO NÚCLEOS DE GELO

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PARTÍCULAS INORGÂNICAS

- Nucleação homogênea vs heterogênea
- Altitude vs latitude do nível de 0°C
- Desconhece-se o ΔG para a nucleação do gelo, no entanto,
- Mason acredita que ΔG seja da ordem de 20 ergs/cm² : água pura
- Neste valor gotículas de 5 μm transformam-se em gelo a -40°C
- E da ordem de 33 ergs/cm² para temperaturas de 0°C
- Esta teoria de Mason acredita que a nucl.homog. ocorra somente em extremos de supersaturação: 8x para -60°C e 20x para 0°C (Fletcher)
- Como ocorrem n. homog a -40°C e normal/e a -15°C, isto indica presença de núcleos de gelo.

■ Possíveis IN(ice nuclei) de origem inorgânica:

AgI (iodeto de prata)= artificial

Naturais: caolita (1 μm) em um intervalo de 0.1 a 4 μm .

Em geral: silicatos de alumínio

Origem em desertos (Saara: gde produtor)

Meteoritos e Argila

Orgânicos:

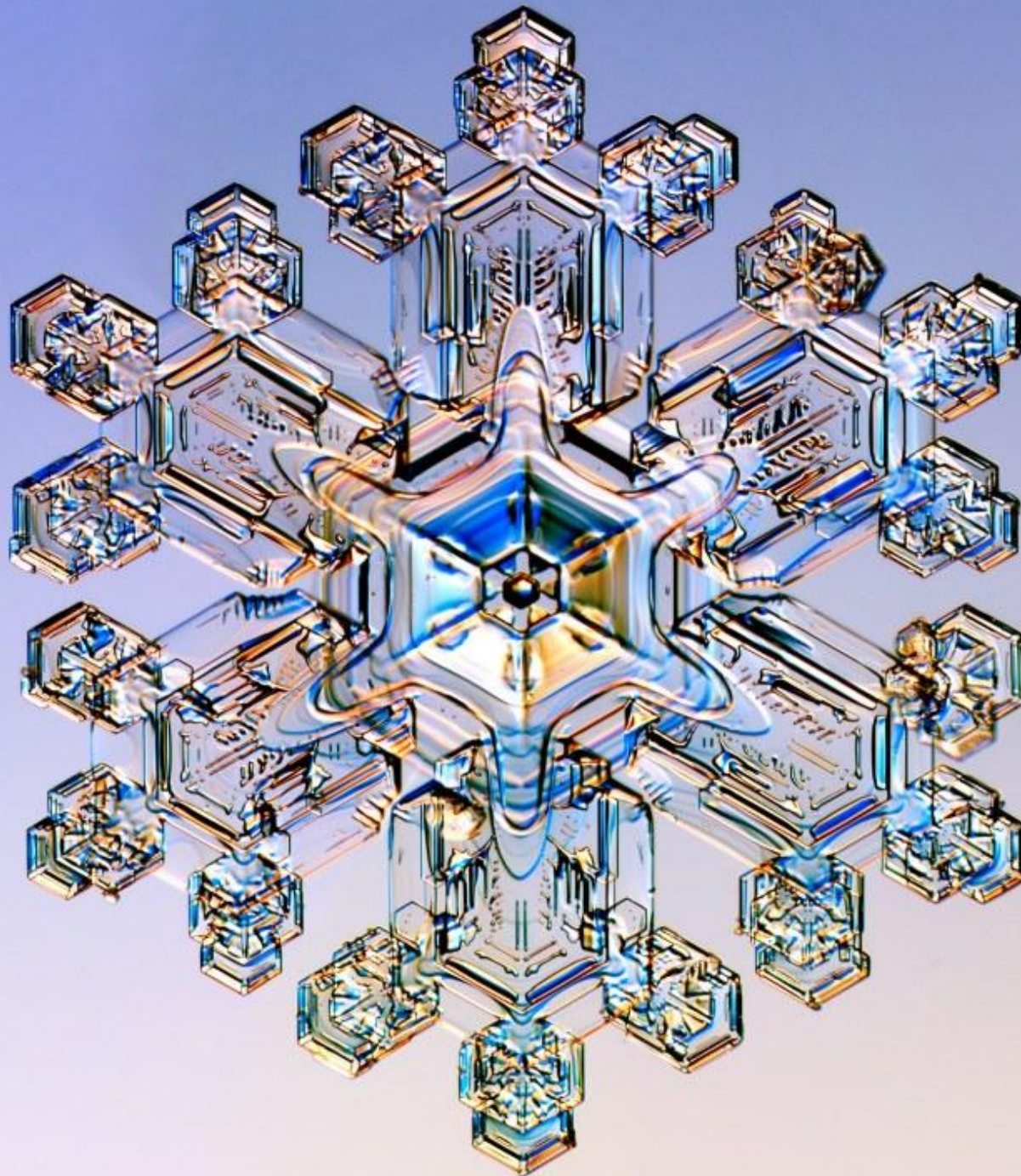
Testosterona e outros compostos orgânicos

Congelamento vs sublimação

Congela/o ocorre qdo há peq. Superesfria/o e gde supersaturação

Sublimação ocorre qdo há o oposto.

Substância	Temperatura de congelamento °C
Iodeto de prata	-4°C
Iodeto de chumbo (PbI ₂)	-6 °C
Vaterita	-7 °C
caolita	-9 °C
Cinzas vulcânicas	-13 °C
halositas	-13 °C
colesterol	-2 °C
testosterona	-2 °C
Bact. <i>P. syringae</i>	-2°C



Os mais belos
se formam em
Ar calmo e úmido

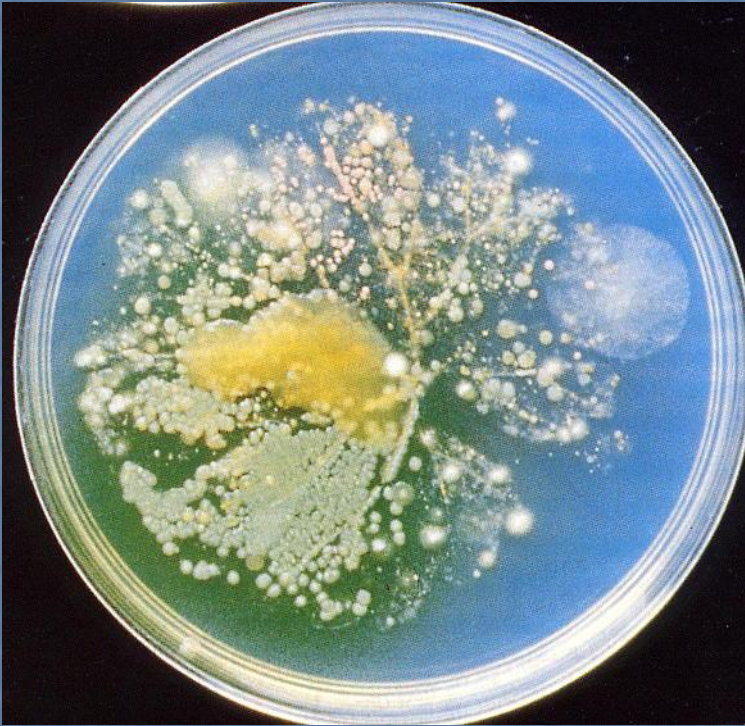
<http://bio-ice.forumotion.com/>
IN de origem biológica:

REINO MONERA E DOS FUNGOS

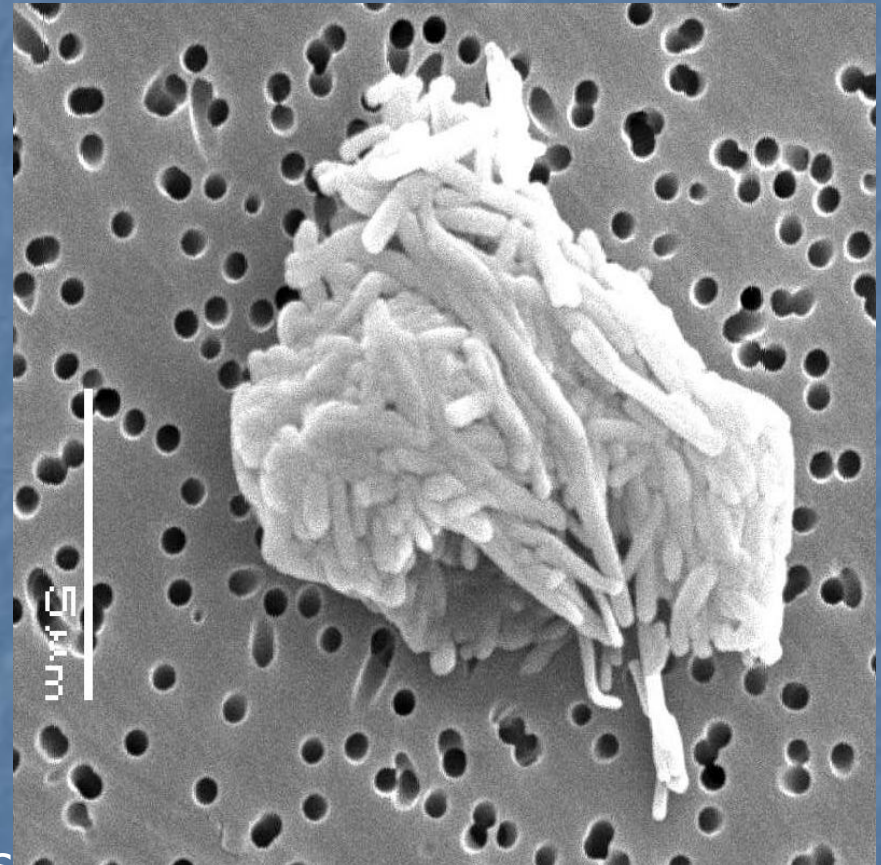
- Os seres vivos unicelulares:
 - - Bactérias (incluindo as cianofíceas)
 - - Fungos
 - - Protistas (incluindo algas unicelulares)
 - - Formas reprodutivas dos pluricelulares

BACTERIAS

TAMANHO DE $< 0.5 \mu\text{m}$ ATÉ $20 \mu\text{m}$ (BACILOS)



Placa de Petri com colonias de bactérias

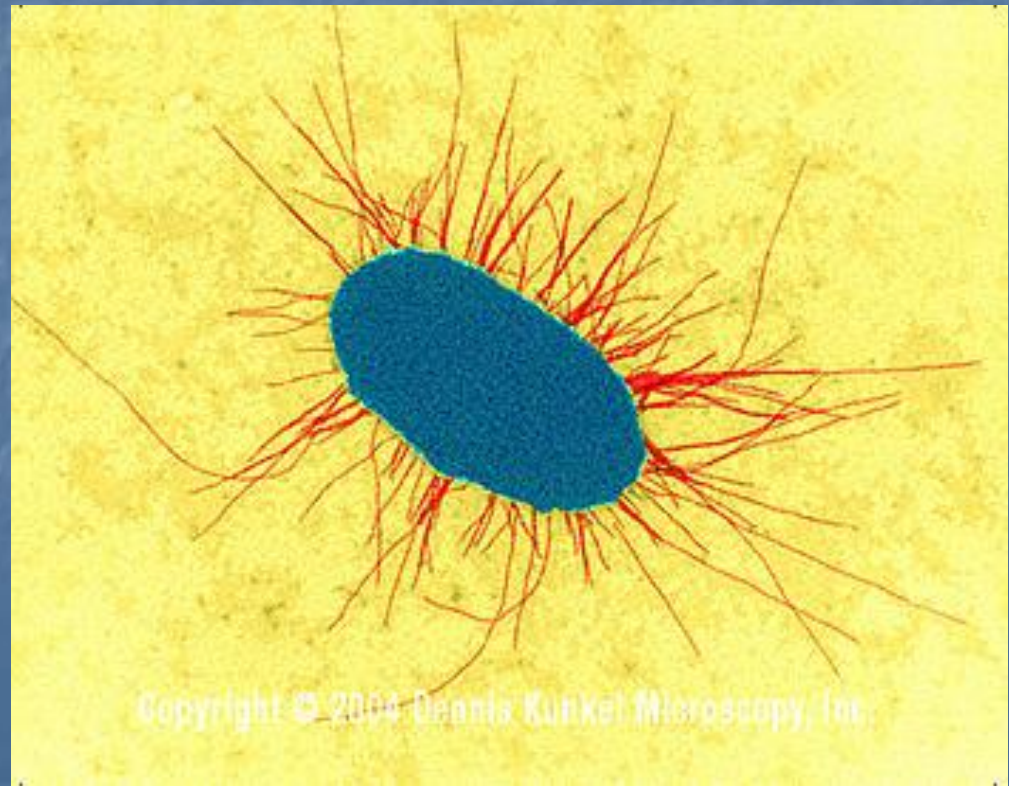


Bacterial aggregate sampled in air above barley



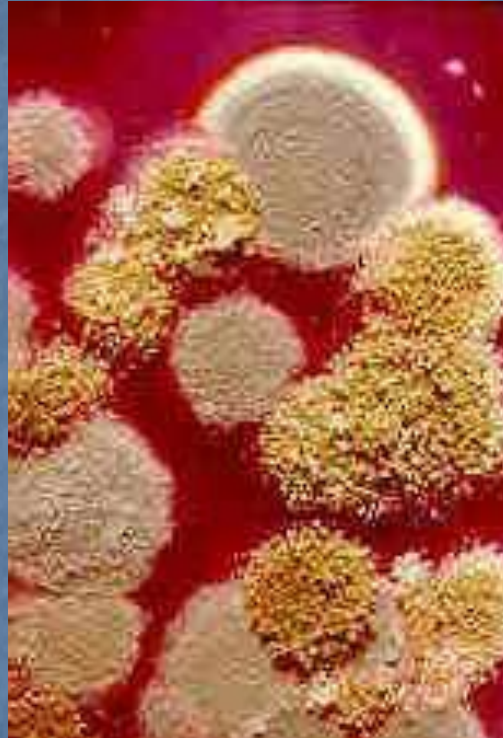
Pseudomonas syringae

Bacteria flagelada

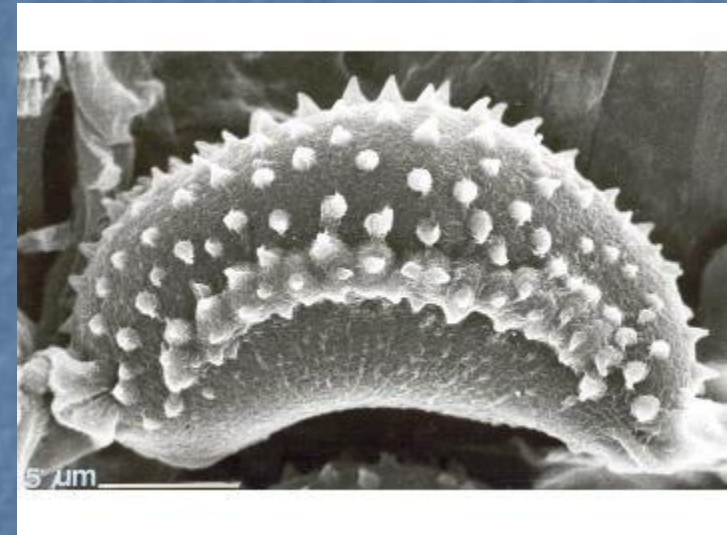


Fungos

Podem ser uni ou pluricelulares. As leveduras são unicelulares



Colonias de Penicillium

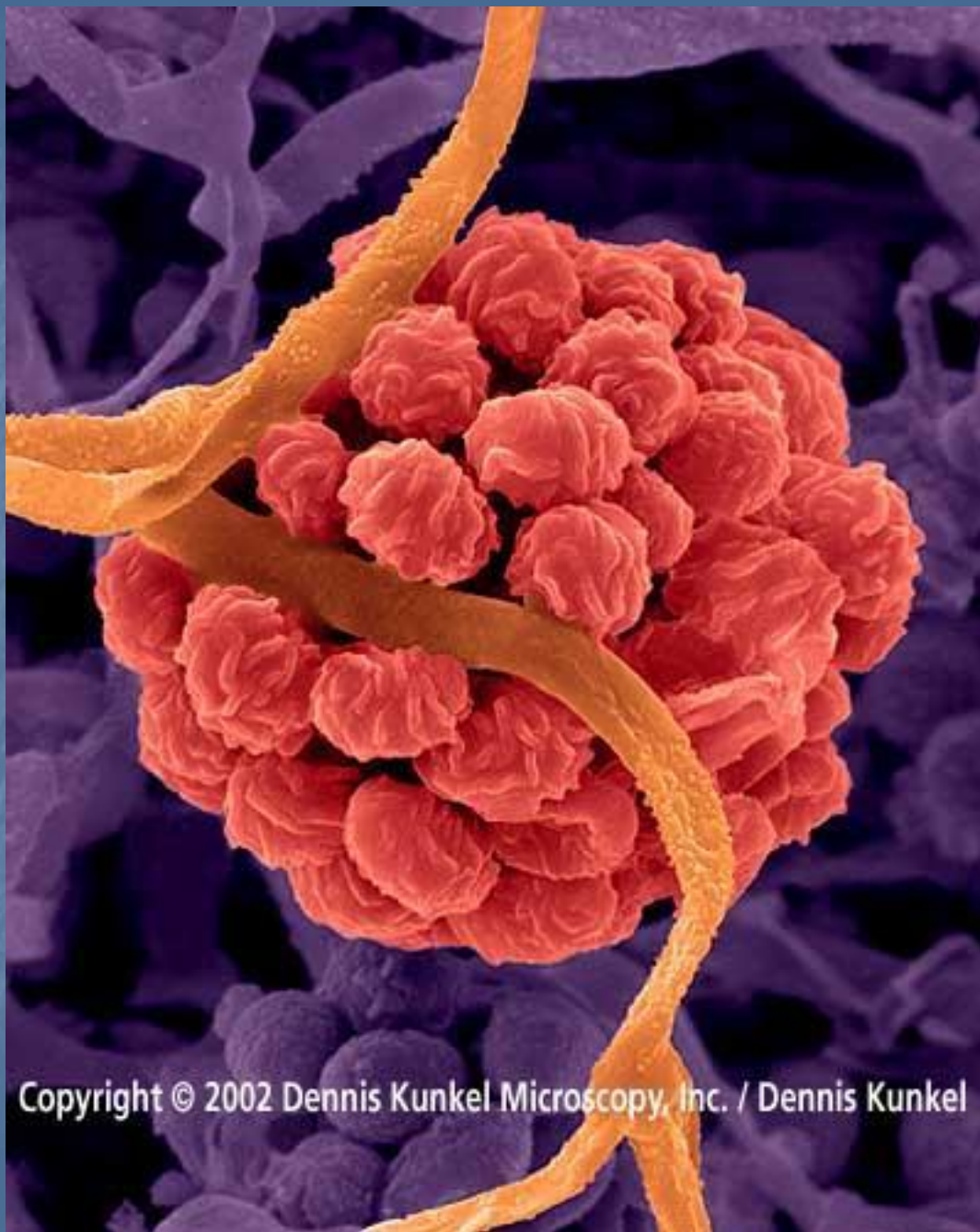


Uredinosporos de *H.vastatrix*

Esporos possuem tamanhos que variam de 20 a 100 μm.



Cogumelos: formas pluricelulares dos fungos



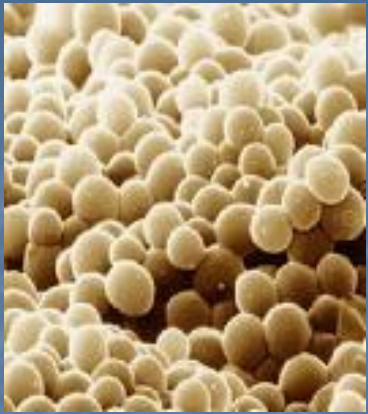
Leveduras

Algas

Algas unicelulares formam esporos de menos de 20 microns.



Algas azuis: cianofíceas



leveduras

=



=



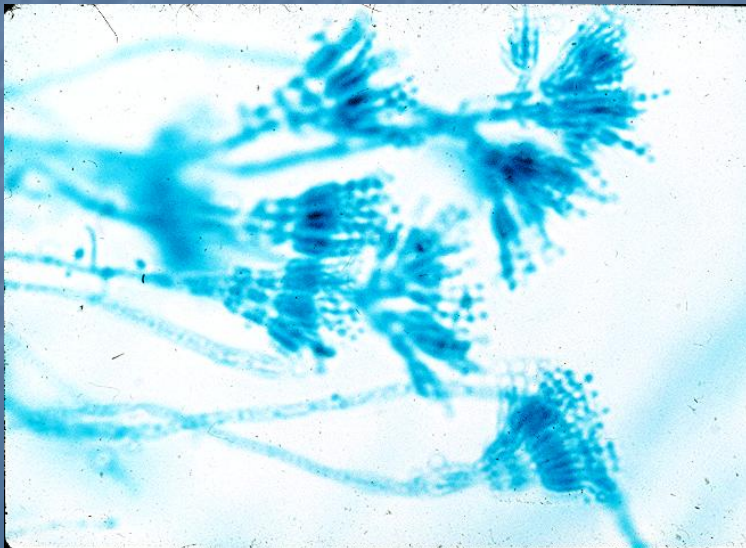
Cocci/bactérias





Algas azuis

quase=



Penicillium

=



Biological ice nucleation- active particles in the atmosphere

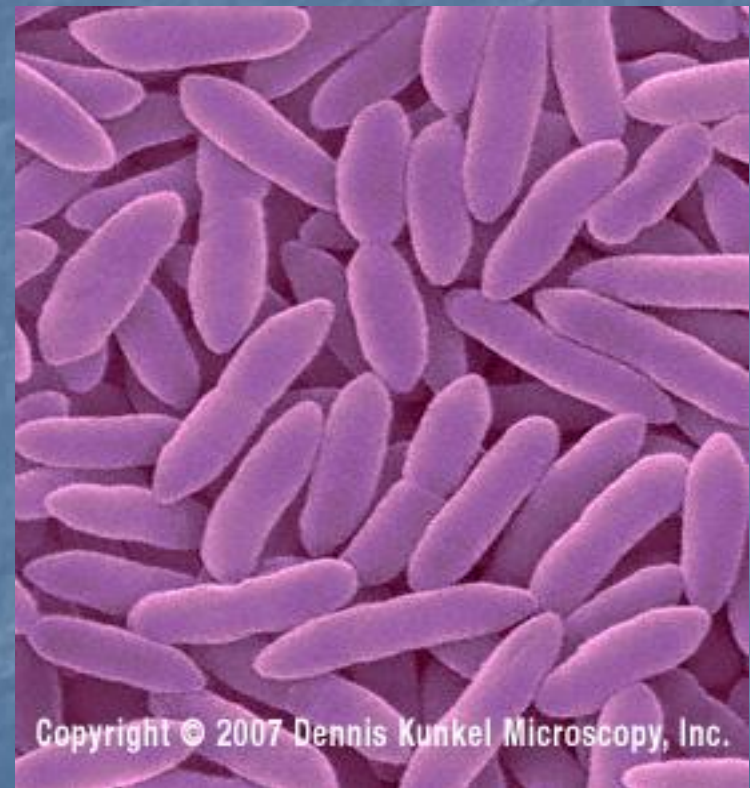
- **Origins**
- **Biology and ecology of INA organisms**
- **Detection and identification**

Histórico

- 1957 Soulage identifica agentes biológicos como nucleantes
- 1966 Parker encontra a vitamina B-12 como agente
- 1971-73 Schnell & Vali isola uma bactéria C-9 como um destes agentes por volta de -1°C.
- 1976 identificação da C-9 como *P.syringae v.syringae*, em folhedos e dentro do floema em diversos vegetais.
- 1973 Frodi encontra bactérias e esporos de fungos dentro de granizos. **Iodeto de Prata**
- 1975 Gregory calcula a velocidade terminal de muitos microrganismos.
- 1976 Rogers encontra fenóis e floroglucinol como agentes nucleantes entre -2°C e -7°C. Testosterona também é identificada como agente nesta faixa.
- 1978- Lindow identifica *Erwinia herbicola* como agente.
- 1983- Métodos de combate às bactérias nucleantes com antibióticos, controle biológico e modificação genética.
- 1986 Experimentos com *Escherichia coli* (*Bacillus subtilis*)
- 1986 Combate às bactérias geneticamente modificadas

Trabalho de Schnell & Vali

- Folheto das florestas de Wisconsin
- Presença exofoliar- bact. exógenas
- Presença Endofoliar- endógenas
- *E. herbicola* e *P.syringae* são encontradas em ambos.
- Problemas com agricultura- US \$ 1 bilhão em perdas nos EUA.
- Adaptação da bactéria para Invadir o hospedeiro



O que são núcleos de gelo ou núcleos biogênicos?!

Bactérias e esporos de fungos, bem como diversas outros materiais de origem biológica podem atuar como nucleadores de gelo, i.e., catalisam a formação do gelo à temperaturas mais elevadas que seria esperado.

Propriedades das membranas (proteína beta-helicoide) podem ser semelhante à forma do cristal de gelo (hexagonal) como no caso da *P.syringae* variedade *syringae*.

Neste caso 1 gene é o responsável pela formação desta estrutura na membrana.

(Expression of carboxymethylcellulase on the surface of Escherichia coli using Pseudomonas syringae ice nucleation protein, JUNG H.-C.; PARK J.-H.; PARK S.-H.; LEBEAULT J.-M.; PAN J.-G.;
Enzyme and microbial technology 1998, vol. 22, n.5, pp. 348-354)

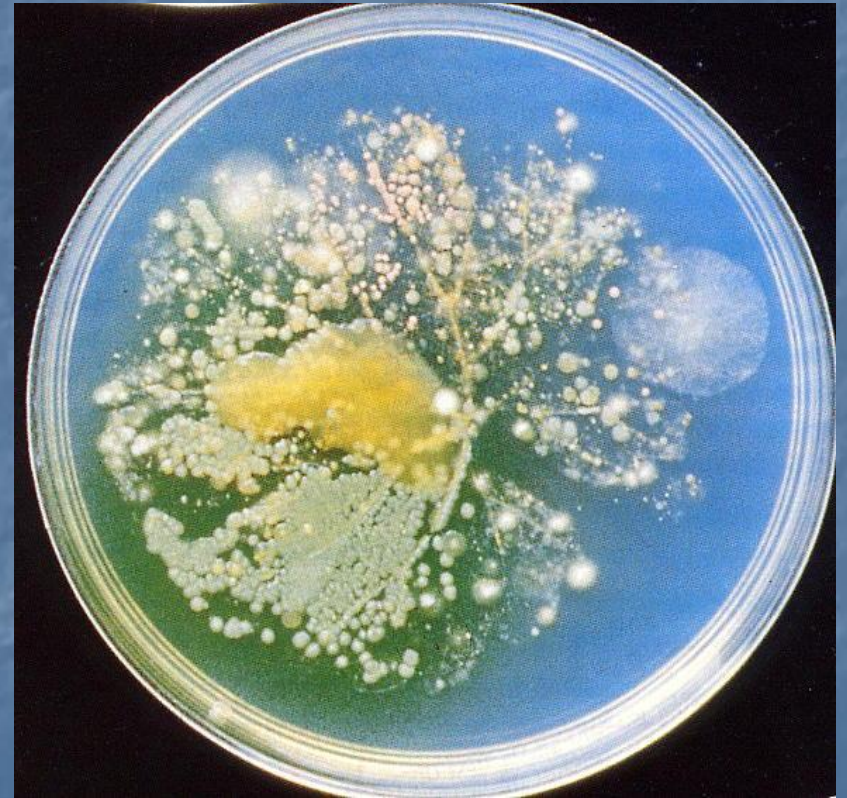
INA organisms and tissues

	max T_{ina} °C
Bacteria 	-1°
Fungi (free-living) 	-5°
Fungi (lichen symbiont)	-2°
Algae 	-5°
Pollen 	-5°
Other plant tissues	-2°
Insect haemolymph proteins	-5°

Habitats for proliferation of INA organisms

focus on Bacteria

Aerial plant surfaces: the most well-known habitat of INA bacteria

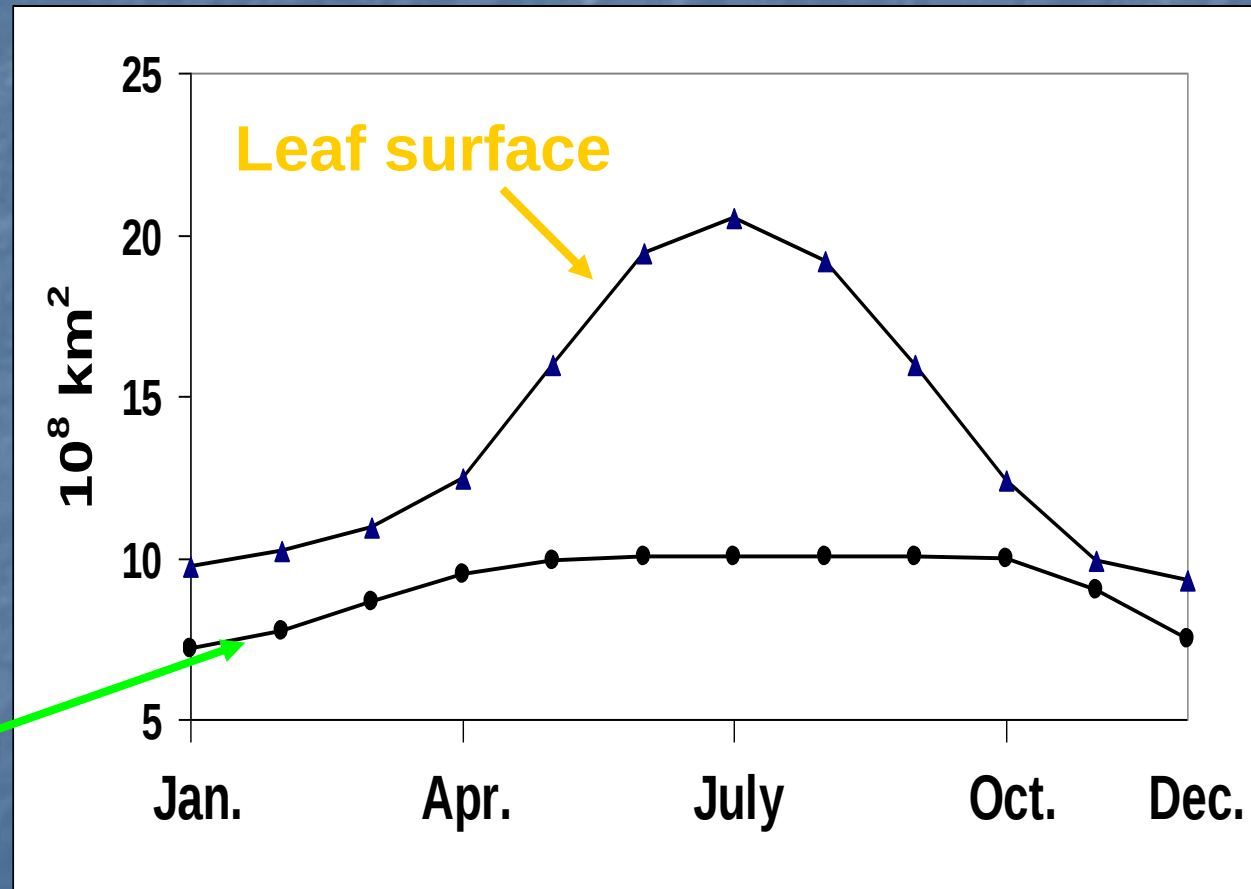


Habitats for proliferation of INA organisms

focus on Bacteria

Aerial plant surfaces: Total leaf surface on Earth

Land surface covered by vegetation



Total leaf area (10^6 km^2) by ecosystem

Boreal forest	144
Tropical rain forest	136
Temperate forest	95
Savanna	60
Cultivated land	56
Tropical seasonal forest	35
Woodland & shrubland	34
Temperate grassland	32
Desert & semi-desert scrub	18
Tundra & alpine	16
Swamp & marsh	14
Extreme desert (rock, ice, sand)	1

Habitats for proliferation of INA organisms

focus on Bacteria

Aerial plant surfaces:

home to ca. 10^{24} – 10^{26} of the total planetary population of 10^{30} bacteria

Bacterial population sizes and ice nucleation activity vary due to:

- plant species
- environmental conditions
- bacterial strain

Habitats for proliferation of INA organisms

Leaf surface

Critical environmental parameters

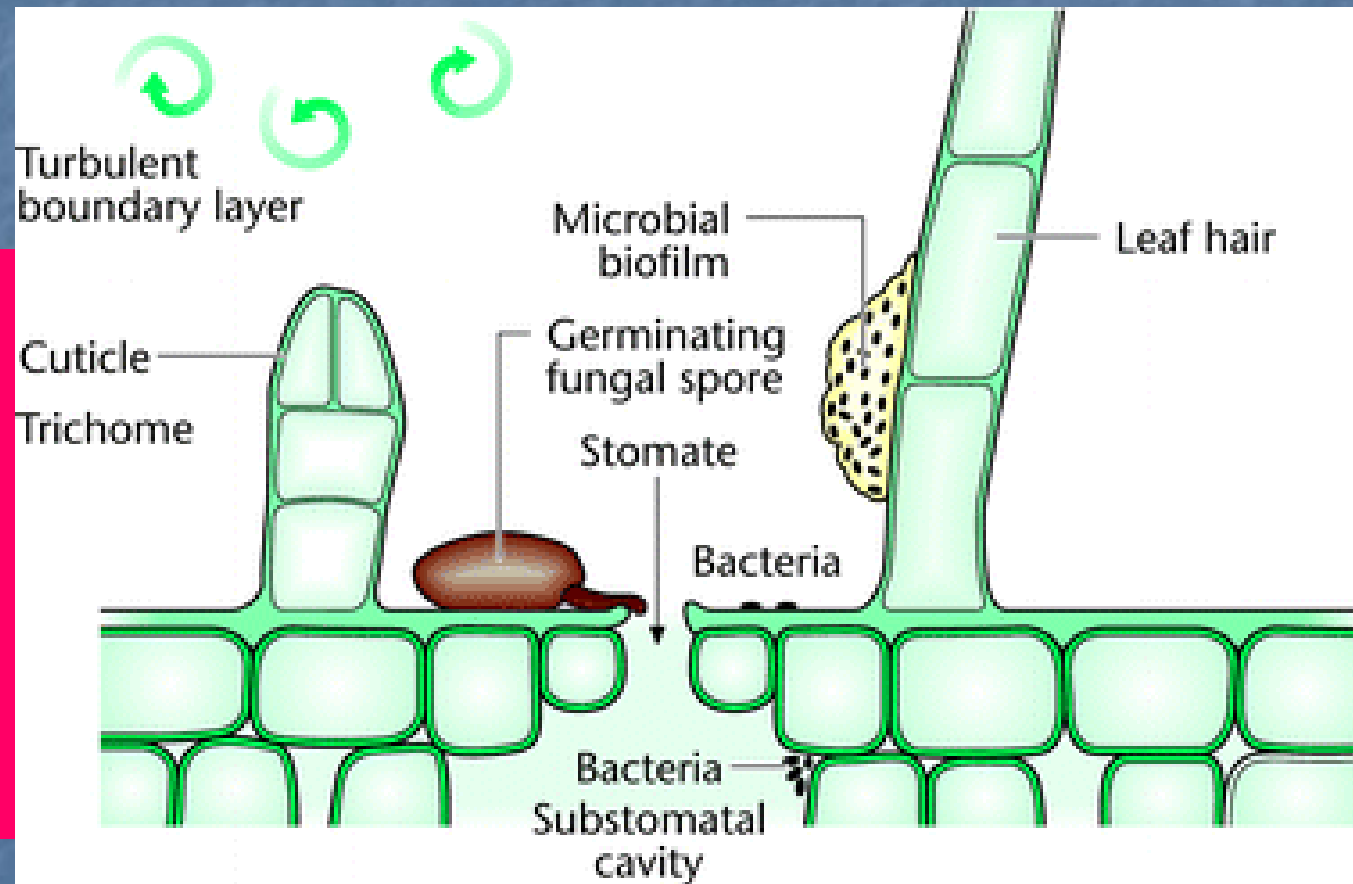
UV

RH, free water

[O₂], [CO₂]

T°C

nutrients (volatiles?)



(diagram drawn to scale; bacterial cells are 1 µm long)

Physical properties of micro-organisms

- Density, mass
- Charge
- Aerodynamic diameter of microbial associated particles

Compare to data used for modeling of pollen dispersal:

weight/grain: 250 to 350 ng ($2.5 - 3.5 \times 10^{-7}$ g)

diameter: 50 to 90 μ m (pollens)

density: 1.25 to 1.45 g/cm³

settling velocity: 20 – 32 cm/s

number per plant: 0.5 to 6×10^6

duration of shed: 4 to 6 days

(Westgate *et al* / Integrating the biological and physical components of maize pollen dispersal. http://www.aphis.usda.gov/brs/confine_workshop/westgate_present.pdf)

Physical properties of micro-organisms

Density, mass

Bacteria (single cells)

-mass: 10^{-16} to 10^{-12} g (Physics Factbook: <http://hypertextbook.com/facts/2003/LouisSiu.shtml>)

dry matter: 12 - 23 % (Bakken, 1983)

-bouyant density, bacteria from soil : 1.2 g/cm^3 (Bakken, 1985)

-pure cultures from soil: $1.03 - 1.09 \text{ g/cm}^3$ (Bakken, 1983)

-bacteria directly from leaves: 10% of cells remained in supernatant after 1hr centrifugation at $60k \times g$ (Morris, unpublished)

Fungi

pure cultures from soil: 1.08 to 1.11 g/cm^3

18-25% dry matter (Bakken, 1983)

INA bacteria

Pseudomonas syringae ♣

P. fluorescens

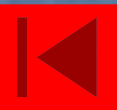
P. viridiflava ♣

Pantoea agglomerans (*Erwinia herbicola*) ♣

Xanthomonas campestris pv. *translucens* ♣

Gram – negative
non spore-forming

♣ epiphytic, plant pathogens



INA fungi

Free-living

Fusarium acuminatum

F. avenaceum

F. oxysporum

F. tricinctum

soil-borne, vascular plant pathogens
limited amount of air-borne spores

(cell-bound and cell-free ice nuclei)

Lichen-symbionts

Lichens and axenic cultures of fungal symbiont, divers species.

Most active species were collected from rocks (vs epiphytic and soil lichens).



INA algae

Algae isolated from Antarctic soils. (Some have also been reported in biotic crusts in deserts.)

<i>Prasiola crispa</i>	-5°	terrestrial
<i>Myrmecia irregularis</i>	-6°	(no info)
<i>Chlorella minutissima</i>	-6°	unicellular; widespread in soils, aquatic (fresh and marine); marine industry and pharmaceutical uses.
<i>Scotiellopsis</i> sp.	-7.5°	unicellular
<i>Bumilleria</i> sp	-8°	filamentous
<i>Elliptochloris subsphaerica</i>	-9°	closely related to <i>Chlorella</i>
<i>Pseudophormidium</i> sp.	-9°	filamentous; cyanobacterium?



INA plant tissues

Pollen

Grasses (*Dactylus glomerata*)

Pine (*Pinus sylvestris*)

Oak (*Quercus rubra*)

(-8 to -11°C, contact freezing)

Birch (*Betula alba*)

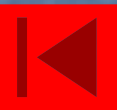
(-5°C, contact freezing)

Other tissues

Lobelia telekii flowers (-4.5°C)

Winter rye mesophyll cells (1/10⁵ cells active at -7°C)

Woody tissue of *Prunus* spp. (-2°C)

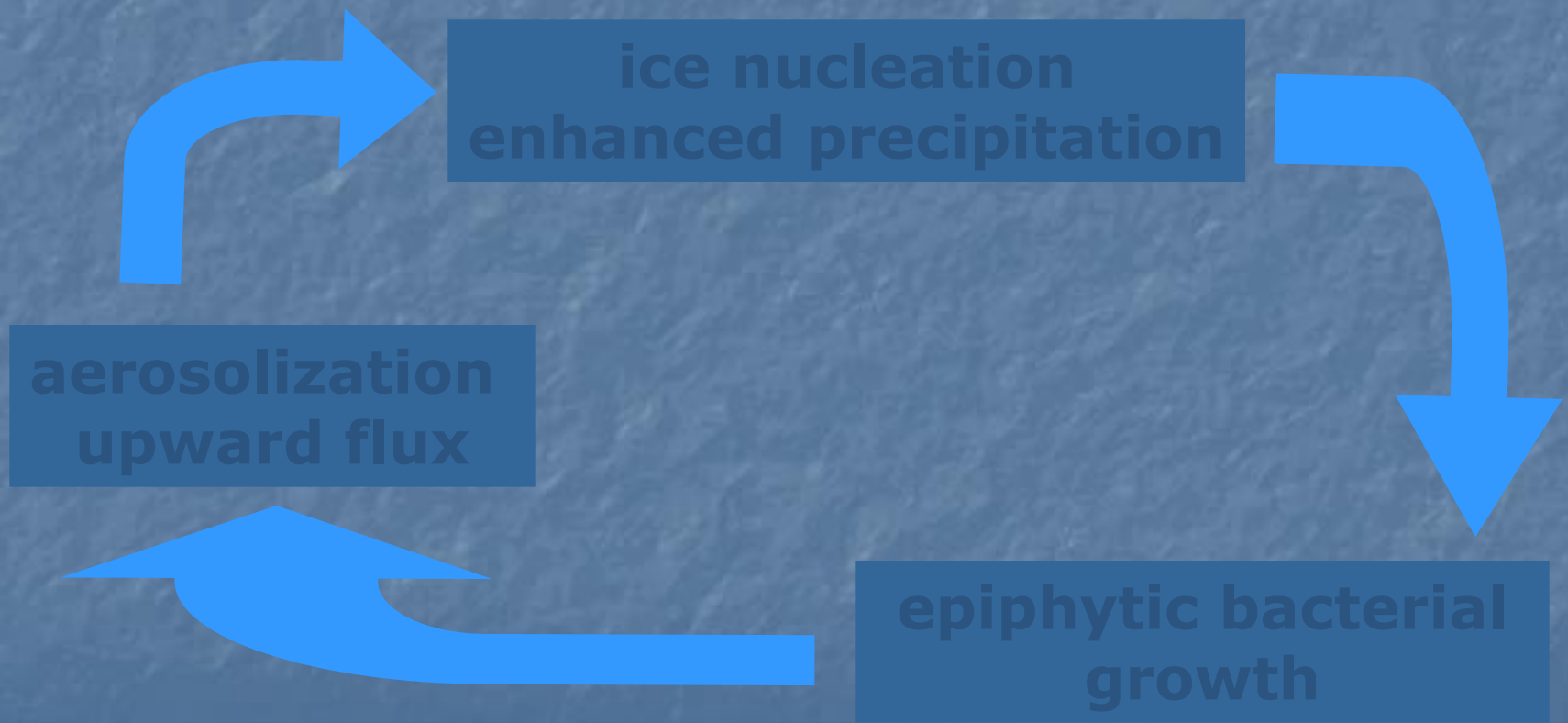




Seed for 400 hectares treated, planted, *P. syringae* moved in within 3 weeks. From where?

Bioprecipitation concept

Sands et al, 1982, 1985



P. syringae in aerosols

Net upward flux (dry) ca. 10^5 bacteria /m²/h

(Lindemann et al, 1982)

Dry deposition ca. 2×10^3 bacteria /m²/h

(Lindemann & Upper, 1985)

Diurnal cycles

O fenômeno da geada e as nuvens frias

■ 1. Formação da Geada

- Perda radiativa, ausência de nuvens, solo descoberto.
- Geada branca x geada negra: congelamento intracelular
- Papel dos agentes nucleadores.
- Presença protetora de açúcares e sais

2. Formação de nuvens

- Parcela de ar com a presença de CCN e IN
- Mudança de estado para líquido e gelo.
- Liberação de calor latente
- Formação de diferentes cristais de gelo: neve, graupel, granizo.
- Modificação de nuvens: nuvens quentes vs nuvens frias

Dissertação: núcleos de gelo em *Coffea arabica* L.

- Objetivo: Encontrar possíveis fungos e bactérias em folhas de café que sejam agentes nucleadores de gelo.

Metodologia

Os dados e a metodologia foram divididos em:

2.1 *Coffea arabica* amostragem de folhas

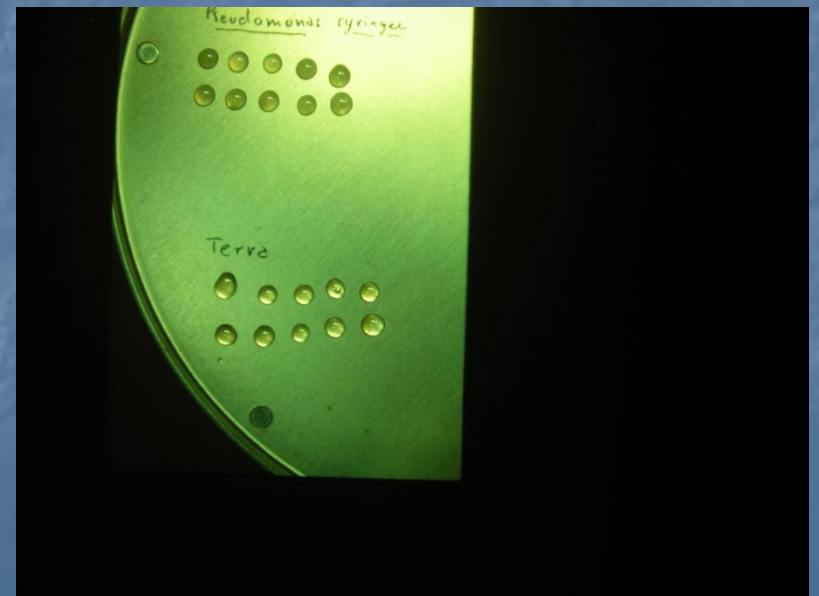
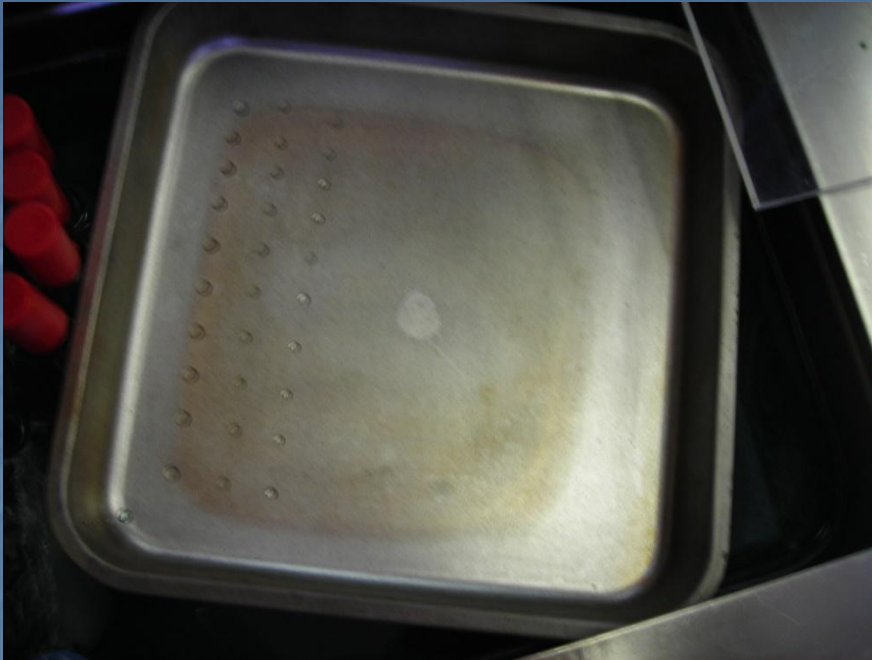
Folhas maduras e caídas (folhedos) coletados na fazenda *Nossa Senhora* em Pinhal, 800 m de altitude cerca de 150 km de São Paulo Capital

As folhas foram lavadas em hipocloreto de sódio 25% por 15 minutos e depois cortadas em pedaços de 1 cm² e colocados em batata-dextrose-agar (BDA) durante 3 ou 4 dias, a 27°C. BDA usa 15 g of Agar Difco and 15 g of dextrose. Depois todos fungos e bactérias foram isolados.

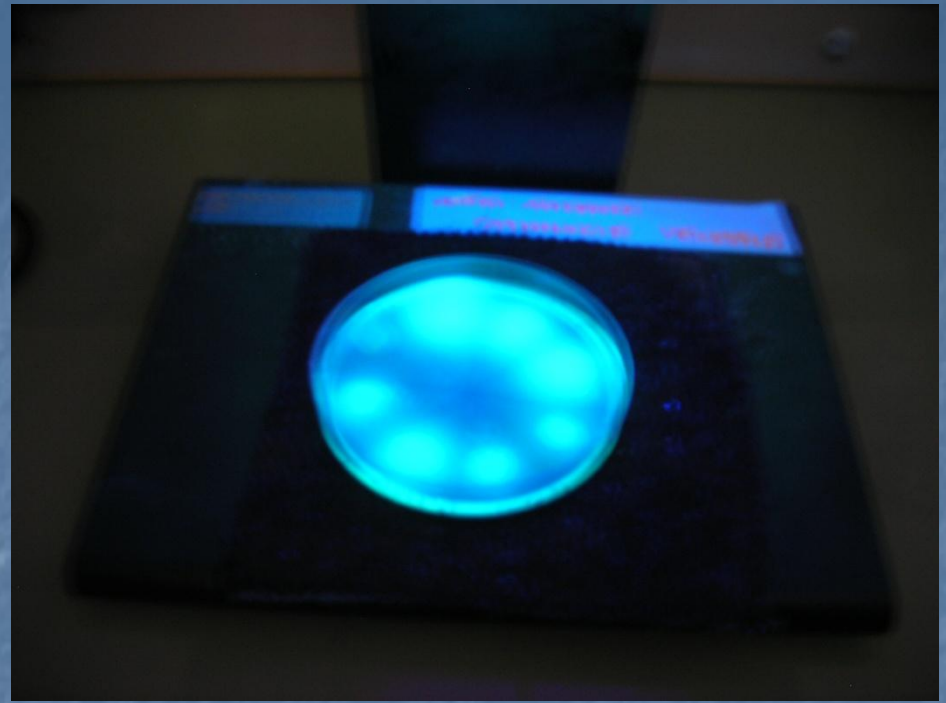
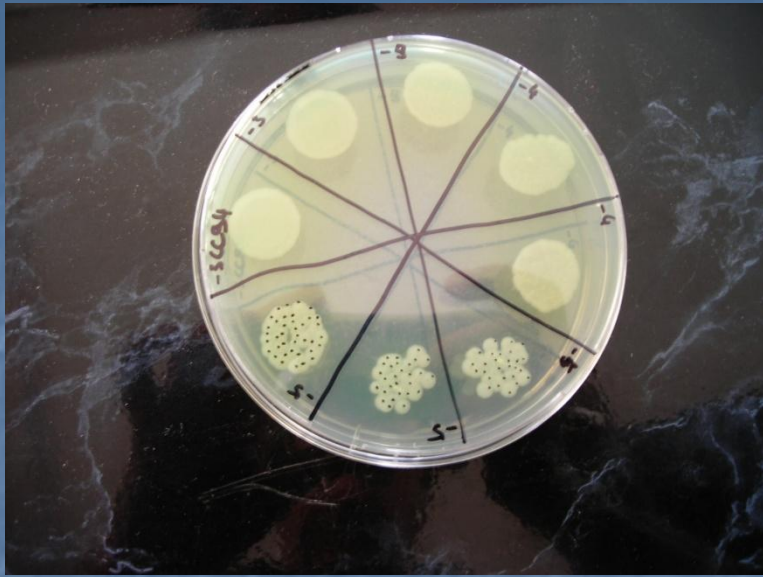
2.2 Testes de congelamento

Os testes utilizaram um lyophilizador (Schnittzeichnungen, Delta 1-A, type 336 Osteröde/harz) para congelar gotículas de solução salina com uma suspensão de fungos e bactérias, para encontrar os INA+, na forma:

- 30 gotículas de 20 µl. Dois testes foram feitos
- Curva de distribuição de ptos de congelamento para cada bactéria e fungo
- Salina 0.85%, NaCl saturado, glicose 0.48 M e água bi-destilada como branco

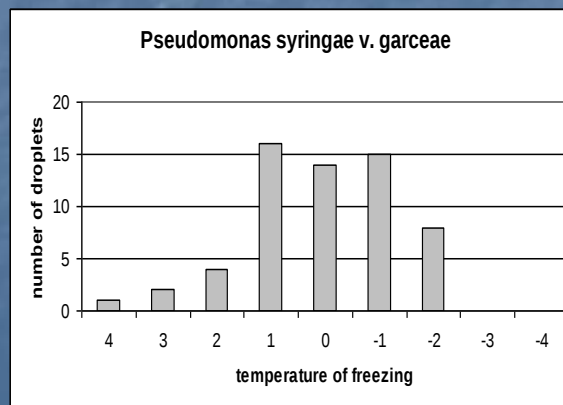
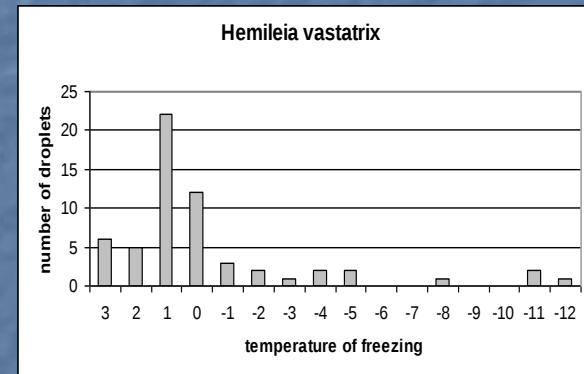
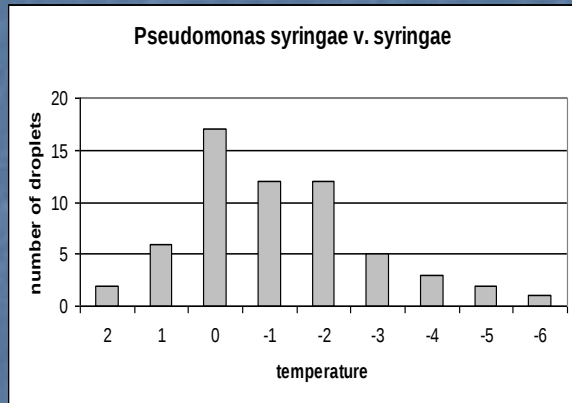


Testes em Avignon acima e à direita
No I.Biologico

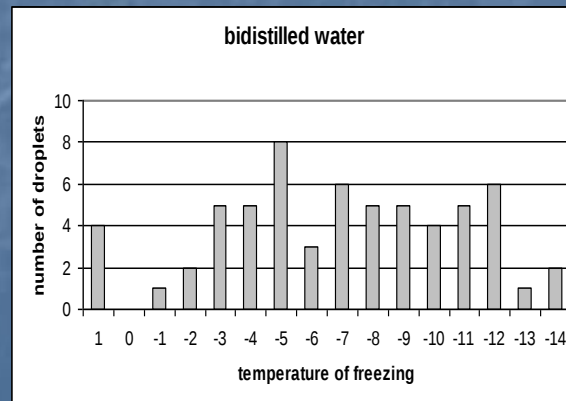
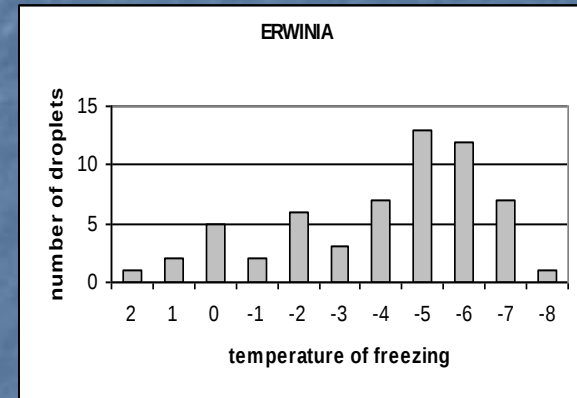
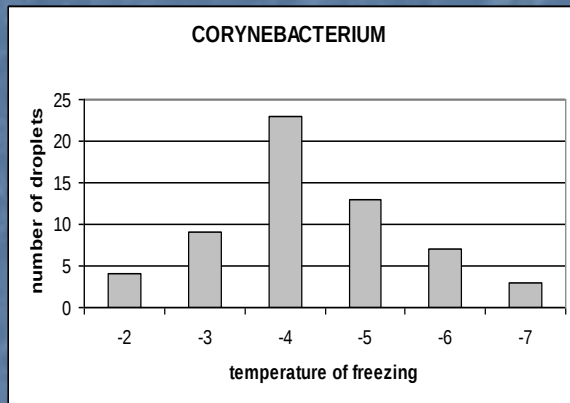


RESULTADOS

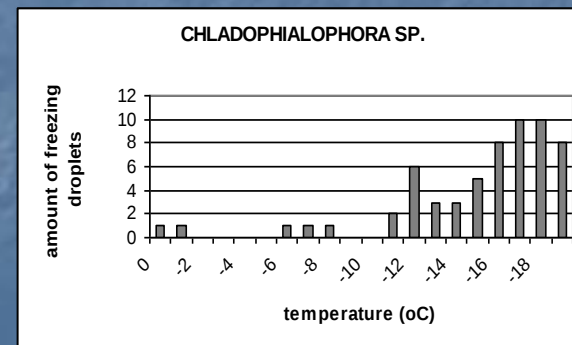
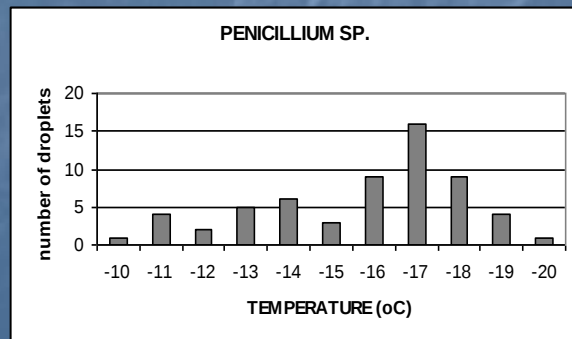
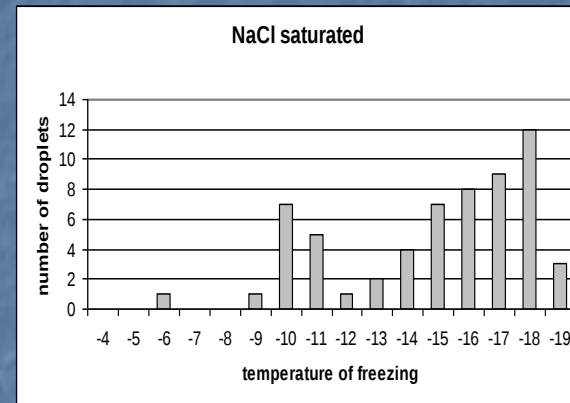
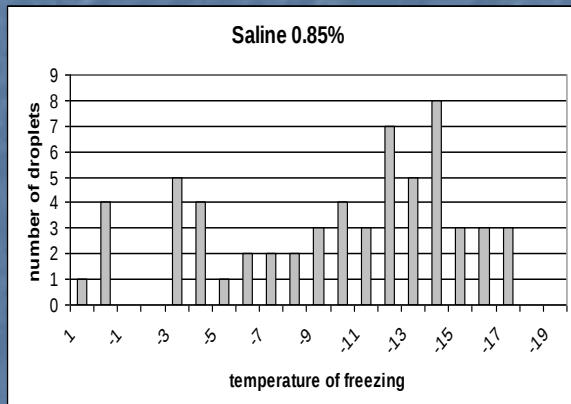
GRUPO A: INA+



GRUPO B : INA NEUTROS OU + E O BRANCO



GRUPO C : INA-



CONCLUSÕES

a) truly ice nuclei activity or INA+ which includes both *Pseudomonas* and uredinospores of *H.vastatrix*, with freezing point mean between 0°C and -1°C;

b) partially ice nuclei activity (INA0), with freezing point mean around -3°C and -6°C, including a group of non-identified bacteria (F1, F2, F6 and F7), *Erwinia herbicola* and *Corynebacterium*;

c) non-ice nuclei activity (INA-), with freezing point below -7°C, around bi-distilled water freezing point mean, which includes all other fungi and bacteria. These group includes bacteria (F8 to F13) and fungi (*Mucor sp.*, *Cephalosporium sp.*, *Streptomyces sp.*, *Chladophyarola sp.*, *Melanospora sp.* and, *Penicillium sp.*) with freezing point mean around NaCl saturated , from -11 C to -14 C. On the other hand, *Chlamidomyces*, saline solution 0.85% and glucose 0.48 M present freezing points around -9 C.

HÅRIS/ ANGEL HAIR ICE



Apresentação em Praga

2.1 Input data

The data were obtained as it follows in Table 1.

Table 1. INA with different temperatures for *Fusarium* spores and *P.syringae* pv. *garceae* suspension droplets showing freezing points at different concentrations, used as preliminary input per cubic meter.

Temperature (°C)	<i>Fusarium</i> with low conc.	<i>Fusarium</i> with mean conc	<i>Fusarium</i> with high conc.	<i>P.syringae</i> with low conc.
0	0	0	0	6
-1	0	0	0	12
-2	5	50	500	12
-3	10	100	1000	84
-4	10	100	1000	72
-5	5	50	500	62
-6	0	0	0	54
-7	0	0	0	
total	30	300	3000	300

The air concentrations of spores and bacteria were developed according to Gonçalves et al. (2008) and Gonçalves et al. (2009). For spores, the samplings were performed indoors and outdoors of different homes scattered through the São Paulo City. The obtained concentrations were from 300 spores per cubic meter up to 36000 spores. Around 10% were viable spores

3.Results

Preliminarily numerical modeling results are shown in **Table 2**, using BRAMS with different fungi and bacteria concentrations as explained in Section 2.2.

Table 2. Total water integrated, with different hydrometeors, on column and rainfall amount at the surface using different IN concentrations. All concentrations in g per kg of air. Yellow color shows the highest values.

	BRAMS default	<i>Fusarium with low conc.</i>	<i>Fusarium with mean conc.</i>	<i>Fusarium with high conc.</i>	<i>P.syringae with low conc.</i>
Pristine	0.067	0.299	0.781	0.786	0.364
snow	0.175	0.012	0	0	0.005
aggregates	0.834	0.701	0.924	0.829	0.658
graupel	0.210	0.171	0.070	0.055	0.162
Hail	0.795	1.123	1.223	1.168	1.123
Cloud	1.857	1.856	1.865	1.856	1.860
Rain	2.989	3.079	3.128	3.031	3.064
vertint_conden	12.61	13.35	13.51	13.64	13.32

Papel do *Bacillus subtilis* nas curvas de Köhler