Please assemble your MicroBattle Guidebook as follows:

- (1) Fold the pages in half to form your A5 Guidebook with images facing inwards.
- (2) Arrange the pages in numbered order (smaller page numbers increasing in sequence on the left and larger page numbers decreasing on the right.
- (3) Make sure the cover page and the back (this page) are flipped and appropriately placed on the back of your stack.
- (4) Staple the sheets together (preferably through the middle crease).

MicroBattle Project was funded by the National Biofilms Innovation Centre (NBIC) Public Engagement Grant (2020-2021). Game Development:

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# Find links to download files to print your own cards on:

Twitter: @project\_ub



# MicroBattle

## Guidebook

1st Edition

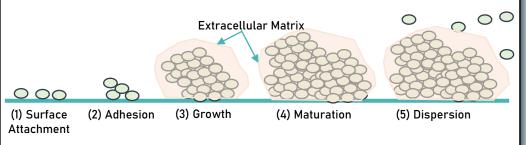
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## MicroBattle Introduction

MicroBattle ( $\mu B$ ) is a competitive card game with the aim to win battles of simulated microbial biofilm growth against your opponent. You can choose from many microbes (in the form of Microbe Cards) and many growth conditions (in the form of Battle Cards) and customize your path to victory.



Biofilms mark a dramatic change in the life cycle of many microorganisms and is categorized into distinct steps. (1) First, attachment to a surface. Biofilms require an initial substrate to localize (2) Second, is further adhesion to that surface and to other microbes. (3) Third, marks the growth stage where microbes in the biofilm will multiply and deposit an extracellular matrix. This matrix provides protection and further substrate for attachment. (4) Fourthly, the biofilm matures. This summarizes many metabolic changes for the inhabitants of this biofilm and marks the time when the biofilm is at its strongest. (5) Finally, microbes may disperse/leave the biofilm in search of new nutrients and to start the process again.

Whilst your microbes battle for biofilm supremacy please make sure to be courteous with your opponents!



Win a battle in a single turn.



## MicroBattle Rules

Aim of the Game: Take it in turns to reach 15 biofilm growth counters for your microbe before your opponent (2-4 players). A game has three "Battles" – with best of 3 Battles determining the winner.

Before the Game: Design a Battle Card Deck supporting the growth of a choice of 3 microbe cards that you have selected. There can only be a maximum of 3 of each kind of card and between 25 - 30 cards. See page 8 for help! Print or draw out your game board (page 7).

Before each Battle: From your roster of 3 microbe cards, select and place your first microbe into your microbe zone at same time as your opponent. Every Battle must start with a different microbe.

A Battle starts with 3 cards in the hand from a shuffled Battle Card Deck. Try not to show your opponent your cards.

A Turn starts by drawing a new card, then grow your microbe's biofilm or disrupt your opponent's microbe's growth using any number of battle cards.



At 10 biofilm growth counters your microbe cannot lose more than 2 counters as a result of a growth penalty battle card ( $\downarrow$ ) (the biofilm has matured and become more resistant).

At 15 biofilm growth counters, your microbe wins the round and is ready to disperse. These can be monitored on your game board.

A player also loses the battle if they have no more playable cards after their battle card deck is empty.

Resistances: None.



Upgrade4>

## Vibrio cholerae

V. cholerae is the causative agent of cholera; an infection of the small intestine resulting in heavy diarrhea dubbed "ricevater" due to appearance and consistency. The cholera toxin (CTX) interferes with the host's regulation of water and ions resulting in a cascade of fluid loss of up to 2 litres per hour.

#### Ability: RICE-WATER STOOL

Once during your turn, send one "Gastrointestinal Tract" card from any player's "Environment Battle Card Zone" to the respective waste zone and this microbe gains 3 biofilm growth counters.

## Vibrio cholerae Challenge

Remove the environment battle card "Gastrointestinal Tract" twice with the ability "RICE-WATER STOOL" during the same battle.



## Microbe Card Guide

Microbe Cards represent the microbes you have chosen to grow against your opponents. They have several important features illustrated below.

Environment Bonus Symbols: These relate to effects in Battle Cards and the environmental niche of the microbe.



Plant Host



Gastrointestinal Tract



en Extremophile



Soil



Full name of the microbe.

Resistances: "Immune System"

Ability: REUTERIN

Once during your turn, you can remove 1 biofilm growth counter from all opposing microbes.

An ability that is a biological feature of the microbe is written here. Use these to gain advantage in battle. This microbe is unaffected by all battle cards that contain the same listed words in the title, e.g., "Immune System - Phagocytosis".

Resistances: "Radiation".

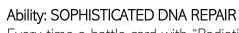






## Sulfolobus acidocaldarius

S acidocaldarius was first isolated from the thermal hot springs of the Yellowstone National Parks in the USA It thrives readily during acidic (low pH) conditions at around 65-90° C It is also able use  $CO_2$  as a carbon source and metabolises sulphur present in the hot spring. Unlike many other archaea, S acidocaldarius is also able to utilise a wide range of carbon sources



Every time a battle card with "Radiation" or "Extremophile" in its name is played, this microbe gains 1 biofilm growth counter.



## Sulfolobus acidocaldarius Challenge

Win a battle against another microbe with the "Extremophile" environment bonus.

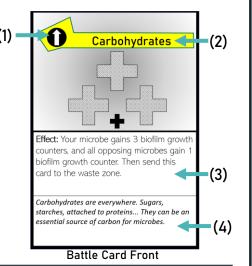
Illustrator



## **Battle Card Guide**

The front of the Battle Card has several features:

- A symbol identifying the battle card type.
- (2) A name.
- (3) An effect: The text here determines your action in the game.
- (4) A flavour text describing some biological or chemical aspects of the card.



Battle Cards have been grouped into 5 types: Growth Promoting, Growth Penalty, Environment, Gene Modification and General. They each have an identifying symbol shown below:



Growth Promoting: These go into the battle card zone. These cards increase biofilm growth counters.



Growth Penalty. These go into the battle card zone. These cards decrease biofilm growth counters.



Environment: These go into the environment battle card zone. They can only be removed by the effect of another battle card, microbe or replaced with a new environment battle card.



Gene Modification: These go into gene modification battle card zone. They can only be removed by the effect of another battle card, or microbe.



General: These cards go into the battle card zone.



elicolor

Streptomyces coelicolor

Streptomyces are considered antibiotic factories. They are actively used in the pharmaceutical sector to produce and discover new antibiotics. They have a complex lifecycle that govern the formation of long hyphal structures that end in a sporulation stage. The smell of earth can also be partially attributed to the volatile geosmin contained in the spores.

Ability: ANTIBIOTIC FACTORY

Whenever this microbe reaches 7 or more biofilm growth counters, add 1 battle card with "Antibiotics" in its name to your hand from your Battle Card Deck. Then, shuffle the deck.

## Streptomyces coelicolor Challenge

Win a battle in which you have used the growth penalty battle card "Antibiotics" at least twice.



## Game Board Guide

The Game Board can be printed (links on twitter @project ub) or you can draw your own on a A4 piece of paper according to the image below.

It is divided into different zones for different microbe and battle cards.

A panel of biofilm growth counters is provided for the players to keep track of their growths and losses using a token/coin/die.

The rules can also be found on the board on the bottom left.



Before the Game: Design a Battle Card Deck supporting the growth of a choice of 3 microbe cards that you have selected. There can only be a maximum of 3 of each kind of card and between 25 - 30 cards. See page 8 for help! Print or draw out your game board (page 7).

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A Battle starts with 3 cards in the hand from a shuffled Battle Card Deck

A Turn starts by drawing a new card, then grow your microbe's biofiln



Each Battle must end with a different one of your microbe cards

At 10 biofilm growth counters your microbe cannot lose more than 2 counters as a result of a growth penalty battle card (1) (the biofilm has

At 15 biofilm growth counters, your microbe wins the round and is ready to disperse. These can be monitored on your game board

A player also loses the battle if they have no more playable cards after their battle card deck is empty.



Load your microbe into this space for it to grow. Microbes stay in this zone unless moved by other means.

#### Gene Modification **Environment Battle** Card Zone

Place up to 1 "Gene Modification" Battle Card at a time here to affect your

They stay active in this zone until your microbe leaves its 'Microbe Zone", They can only be removed by the effect of battle card, or



## Card Zone Place "Environment" Battle Cards here. It can only be replaced by a emoved by another effect

Battle Card Zone

This Zone needs to be free to

use Growth Promoting, Growth Penalty or General

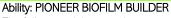
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Resistances: None.



## Streptococcus gordonii

S gordonii is a pioneer biofilm builder of dental surfaces. As a consequence, it provides a surface substrate for many other microbes to adhere and colonise as well. Under rare circumstances, S gordonii can infective endocarditis cause (infection of heart and artery) when dislodged into the blood stream



The following abilities apply:

- During your turn, you can swap this microbe with any other of your microbe cards retaining any biofilm growth counters gained.
- Once per turn, you can send 1 card from your hand to the waste-zone and this microbe gains 2 biofilm growth counters.

# Streptococcus gordonii Challenge

Win 2 battles in the same game after using the ability "PIONEER BIOFILM BUILDER" to swap into other microbes.





## **Battle Card Deck Creation Guide**

Generally, your battle cards should compliment the growth and strengths of the three microbes you choose. There can only be a maximum of 3 of each kind of card and between 25-30 cards.

To start, we recommend a deck size of 27 cards, of which ~50% are Growth Promoting Battle Cards and the rest are spread between the other battle card types.

You can create your own deck using the "Custom MicroBattle Deck Template" and dragging appropriate card images into the desired positions. Below is an example deck recipe and other premade deck recipes are available.

Title: HUMAN GUT SYMBIONTS					
Growth Promoting Battle Cards	Growth Penalty Battle Cards				
<ul> <li>3 x Muc2 – Mucin</li> <li>3 x Prebiotics</li> <li>3 x Dietary Polyphenols</li> <li>3 x Human Milk Oligosaccharides</li> <li>1 x Adhesion Protein</li> </ul>	<ul> <li>3 x Immune System – Innate Immunity</li> <li>3 x Immune System - Phagocytosis</li> </ul>				
Environment Battle Cards	Gene Modification Battle Cards				
> 3 x Gastrointestinal Tract	> 1x Gene Modification - Resistance				
General Battle Cards	Microbe Cards				
> 1 x Niche Occupation	Bifidobacterium breve				
<ul><li>1 x Chemotaxis</li><li>1 x Community Biofilm</li></ul>	Lactobacillus casei				
> 1 x Electrochemical Communication	Limosilactobacillus reuteri				

Resistances: None.





## Staphylococcus aureus

S aureus is a commensal bacterium colonising many host environments, but it is also an opportunist pathogen that can cause devastating infections. The biofilms are highly resistant to stressors by developing antibiotic resistance and production of staphyloxanthin, a golden pigment, providing protection from the immune system

Ability: ACQUIRED RESISTANCE When this microbe loses biofilm growth counters from a "Growth Penalty Battle Card" (\psi), it gains resistance to cards with that exact same name for the rest of the battle.

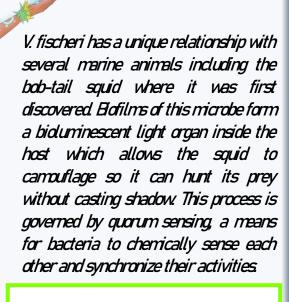
## Staphylococcus aureus Challenge

Gain resistance to 3 different growth penalty battle cards during the same battle.



Resistances: None.

## Aliivibrio fischeri



Ability: BIOLUMINISCENT SQUID Every time this microbe's biofilm growth counters go to 7 or higher, draw 1 card.

## Aliivibrio fischeri Challenge

In the same battle draw 2 cards using the A. fischeri's ability "BIOLUMINISCENT SQUID" and win the battle with at least 2 cards in the hand.

Resistances: None.





## Salmonella enterica

Infection by Salmonella is a common cause of food paisaning responsible for many deaths every year. Symptoms can vary from mild to severe, with some strains resulting in Typhoid fever from a systemic infection through entry to the lymphatic system



Once during your turn, send one environment battle card with "Gastrointestinal Tract" or "Infection" in its name from any "Environment Battle Zone" to the Waste Zone. Then this microbe gains 2 biofilm growth counters.

## Salmonella enterica Challenge

Win a battle with "Gene Modification - Resistance" in your Gene Modification zone.





Resistances: "Immune System".

## Bifidobacterium breve

Several species of Effidobacterium are well known for the colonisation of the infant gut. They have uniquely coevolved with mammals to partake in the breast-milk diet and protect the infant from debilitating gastrointestinal diseases



On this microbe's first turn, play one environment battle card with "Gastrointestinal Tract" in its name directly from your battle card deck. Then shuffle the deck.



## Bifidobacterium breve Challenge

Win a battle (reaching 15 biofilm growth counters) after using the battle card "Human Milk Oligosaccharides" and reducing the opponent's biofilm growth counters to 5 or less. Resistances: "Radiation".

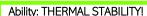






## Pyrococcus furiosus

The optimal environment for P. furiosus is around 100°C. In order for this archaeon to survive these harsh temperatures its proteins are extremely stable, and many have been dedicated to the repair of DNA It can even repair damage from gamma radiation...



Two following two effects apply:

- This card is not affected by "Gene Modification" Battle Cards.
- During every start of your turn, this microbe gains 2 biofilm growth counters when an Environment Battle Card with "Extremophile" in its name is in your zone.

# Wi

## *Pyrococcus furiosus* Challenge

Win a battle in 3 turns.

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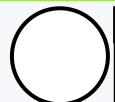


Resistances: None.

## Bradyrhizobium japonicum

B japonicum is part the group of nitrogen fixing bacteria and forms a symbiotic relationship with soybeans. The plant provides nourishment and a protected root nodule structure. In return B japonicum harvests atmospheric nitrogen and converts it into ammonia which is essential for





## *Bradyrhizobium japonicum* Challenge

Win a battle while an environment battle card with "Plant Host" in its name is in your zone.

Resistances: None.





## Pseudomonas syringae



P. syringae is one of the most common plant pathogens. It uses biofilm as key step in its plant colonisation strategy. It is also responsible for frost damage in plants by producing Ice Nucleation-Active proteins. These lower the freezing temperature of water causing frost damage inside the plant and releasing nutrients to the bacteria outside.

#### Ability: ICE NUCLEATION-ACTIVE

Once during your turn, send one battle card with "Plant Host" in its name from card from any player's "Environment Battle Card Zone" to the respective waste zone and this microbe gains 3 biofilm growth counters.

## *Pseudomonas syringae* Challenge

Win a battle (reaching 15 biofilm growth counters) after using the ability "ICE NUCLEATION- ACTIVE".

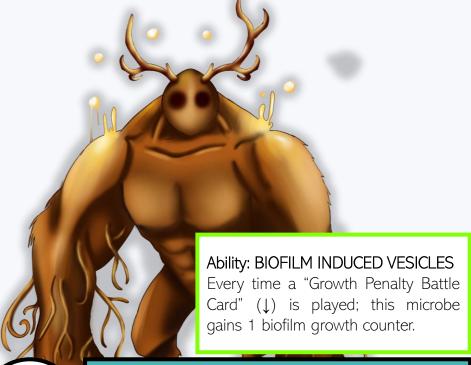
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Resistances: "Antibiotics". "Bacteriophage"

## Candida albicans

C. albicans is a commensal fungus that is common in the mouth and digestive tract. It also can present as an opportunistic pathogen during times of a weakened immune system or the local population of microbes has been significantly disturbed. All forms of life produce vesicles (a membrane bubble filled with a diverse possible cargo) and C. albicans may use these to protect itself from attack in its biofilm state.



## Candida albicans Challenge

Win a battle (reaching 15 biofilm growth counters) after a growth penalty battle card is played (triggering the ability "BIOFILM INDUCED VESICLES").

Resistances: None.









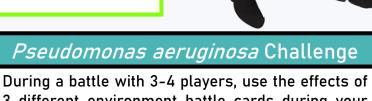




P. aeruginosa is an opportunistic pathogen that is known to be able to colonise many different natural and artificial surfaces. The biofilms it develops are extremely resistant. Particularly babies, elderly, people with particular genetic disorders such as cystic fibrosis and those with a weakened immune systemare vulnerable to infection



During your turn, your microbe has access to all opponent's active environment battle card effects.





3 different environment battle cards during your







Resistances: None.

## Clostridium botulinum

C botulinum is a food borne pathogen and also present in soil or aquatic environments. It is most well-known for the production of botulinum toxin causing potent muscle paralytic, which has been repurposed in plastic surgery. It also can enter a resistant vegetative state known as endospore.

### Ability: ENDOSPORE

At the start of your turn, you can apply the following effect: This microbe cannot gain biofilm growth counters for the rest of your turn and cannot lose biofilm growth counters until the start of your next turn.



Use 3 growth penalty battle cards during your turn and win the same battle.

Resistances: None.



## Prochlorococcus marinum

Photosynthesis describes process of converting light to chemical energy with often oxygen being a by-product.

P. marinum are the smallest photosynthetic organisms on the planet, but by occupying warm oceans end up supplying Earth with the greatest contribution of oxygen.



Once during your turn, during local daytime, this microbe gains 2 biofilm growth counters.





Resistances: "Antibiotics".

"Bacteriophage"

## Cryptosporidium parvum

Oyptosporidiosis is caused by ingestion of water contaminated with mammalian parasite C parvum and is typically self-limiting in people, but can be dangerous for those who have weakened immune systems. It uses large glycoproteins similar to those in human mucus to prevent destruction and attach to the host small intestine or respiratory tract.

Ability: RESISTANT OOCYTE

This microbe's biofilm growth counters cannot be reduced while biofilm growth counters are 5 or less.

## Cryptosporidium parvum Challenge

Win a battle while an environment battle card with "Gastrointestinal Tract" in its name is in your zone.

Resistances:

"Antibiotics".

"Bacteriophage"



Yassine Sey

## Phytophthora infestans

P. infestans is one of the most destructive plant pathogens on Earth. It is classified as an comycete (or water mold) and mainly infects potato plants. Hence, it is most recognised for causing over 1 million people to die of starvation during the Great Irish Famine in the 1840s. To this day it still costs billions of pounds in damages to potatoes world-wide.



#### Ability: BLIGHT

Once per battle, send all "Plant Host" environment cards in all environment zones to the waste zone. This microbe gains 2 biofilm growth counters for each card sent to the waste zone by this ability. Then search your battle card deck for a Gene Modification Battle Card and add it to your hand. Shuffle the deck.



Exchange your "Gene-Modification" battle card three times during a battle through the ability "BLIGHT".





Resistances: None.

## Geobacter sulfereducens

Geobacter species are well known for their ability to use metals as part of their respiration (means to get chemical energy). G sulfureducens forms nanowires to conduct and produce electricity. They are actively explored as living batteries and generators, as well as bioremediation (recovery) of environments that have been contaminated by radioactive metals such as uranium



Once during your turn, you can shuffle one card in your hand into your deck and draw 1 new card.

## Geobacter sulfereducens Challenge

Win a battle using the effect of the battle card "Bioremediation".

Resistances: None.



Yassine Se

## Methanobrevibacter smithii

M smithii is the main archaea in the human gastrointestinal tract. Very few microbes can produce methane by harvesting carbon and hydrogen from their environment, and so far, all methanogens have been classified as archaea. In the gut, M smithii is able to utilise many by-products of other microbes as its energy source.





## *Methanobrevibacter smithii* Challenge

Win a multi-battle with 3 or 4 players.



Resistances: "Radiation".

## Halobacterium salinarum

As a halophile, H salinarum can thrive in high salt concentrations. The bright pink/red colour in some salt lakes and in the Dead Sea is partially due to archaea such as H salinarum that produce a pigment to protect from UV radiation. It is unable to metabolise many extracellular carbohydrates but produces all sugars itself through the gluconeogenesis process.

This also makes Halobacteria candidates for survival on extra-terrestrial planets, like Mars

#### Ability: ANCIENT DNA

Two following two effects apply:

- This microbe only gains a maximum of 1 biofilm growth counters from "Growth Promoting" (†) Battle Cards.
- Once during your turn, this microbe gains 2 biofilm growth counters.

## Halobacterium salinarum Challenge

Win a battle without using any growth promoting battle cards.

Resistances: None.



Upgrade4>

## Mediterraneibacter gnavus

M gnavus is a commensal bacterium often found occupying the human colon As a result, it has evolved strategies to harvest sugars from host carbohydrate sources, such as from the mucus layer that is covering the intestinal tract. Ability: MUCIN FORRAGER Battle Cards with "Muc2" in the name give +1 biofilm growth counters to this microbe.

## Mediterraneibacter gnavus Challenge

Use the growth promoting battle card "Muc2 – Mucin" three times during the same battle.



Resistances: "Radiation".

## Ignicoccus hospitalis

L hospitalis is a hyperthermophilic archaea found at hydrothermal vents (80°C) and uses sulfur as its main energy source. It also serves host to super small Nanoarchaeum equitans, but their relationship is not fully understood



# *Ignicoccus hospitalis* Challenge

During a battle, gain 8 biofilm growth counters through the ability "SYMBIONT or PARASITE".

Resistances: "Immune System".



## Limosilactobacillus reuteri



L reuteri is a beneficial bacteria and true symbiont of vertebrates, typically colonising (occupying) the small intestine. It has been shown to be able to combat a wide variety of harmful gut bacteria with the production of the broad-spectrum anti-microbial Reuterin

## Ability: REUTERIN

Once during your turn, you can remove 1 biofilm growth counter from all opposing microbes.

## Limosilactobacillus reuteri Challenge

Win a multi-battle with 3 or 4 players using Limosilactobacillus reuteri.



Resistances: "Immune System".

Lactobacillus casei,

L casei has been observed in the fermentation of dairy products, and vegetation (e.g., green dives). It is also often shown as a probiotic bacterium in many mammals with several health benefits attributed to its colonisation of the gastrointestinal tract (gut) including regulation and communication with the host immune systemand nervous system

#### Ability: IMMUNOREGULATOR

Every time this microbe reaches 7 or more Biofilm Growth Counters; search your "Battle Card Deck" for 1 card with "Immune System" in its name and add it to your hand. Then shuffle your deck.



Use 3 "Immune System" growth penalty battle cards during the same battle.

Resistances: "Protozoa". "Immune System".





## Legionella pneumophila



L pneumophila enjoys a symbiotic relationship in a protist biofilm even when internalised by the larger microbes. Those features make it equally able at surviving macrophages of the immune system when transmitted through consumption or inhalation of contaminated water. Growing in the respiratory tract it can cause severe damage resulting in Legionnaire's disease.

#### Ability: AMEBOID HOST

On this microbe's first turn coming into play, add one Battle Card with "Protozoa" in its name to your hand from your Battle Card Deck and shuffle the deck.

Whenever a Battle Card with "Protozoa" in its name is played, this microbe gains 1 biofilm growth

## Legionella pneumophila Challenge

Win a battle (reaching 15 biofilm growth counters) after using the ability "AMEBOID HOST".

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