# The Anti-bacterial Power of Agave

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## What is Agave?

- · A kind of Monocotyledon that is distributed in Latin America
- · Categorized into Asparagales , Agavaceae
- · Its form is similar to Aloe because they have evolved in parallel evolution.
- \* As a side note, Aloe is categorized into Asparagales, Asphodelaceae
- \* It is said that Agavaceae and Asphodelaceae are closely related.



#### Preliminary Experiment

- 1. Investigate the pH and viscosity of the Agave and Aloe. →Agave pH: around 6 (weak acidity), viscosity: low Aloe pH: around 4.5 (acidity), viscosity: high
- 2. Put the Bacillus into the Microtubes and centrifuge them at 1 6000 rpm for 15 seconds and 2 6000 rpm for 30 seconds. (1) and (2) are applied to the agar medium in 500 $\mu$ l. Culture them in the incubator at around 25°C and 80% humidity for 2 days.
- $\rightarrow$ They grew all over the medium. (We determined them to be culturable bacteria.)



\* E.coli was determined to be culturable bacteria because Figure 3: (left)Bacillus<sup>①</sup>, (right)Bacillus<sup>②</sup> it was already incubated.



- · Agave hasn't been studied much, and there is little literature on this.
- $\rightarrow$  The goal is **to reveal their properties** even a little.
- · Aloe is known to have antibacterial effects against Bacillus .
- → Are there similar anti-bacterial effects on the closely related Agave?

### **Experiment #1 Bacillus**

- 1. Put the supernatant of the Bacillus solution into a Microtube and centrifuge them at 1 6000 rpm for 15 seconds and 2 6000 rpm for 30 seconds.
- 2. Smear ①,②,③[1/10 dilution of ①],④[1/50 dilution], and ⑤[1/100 dilution] all over the agar medium. And put a paper disc soaked with the Agave's leaf juice and the epidermis in the center of the Petri dish. \* Do the same for Aloe.
- 3. Culture them in the incubator at around  $25^{\circ}C$  for 1 to 2 days.

### Experiment #2 E.coli

- 1. Using JM109 bacterial solution, make ①[undiluted solution], ②[1/10 dilution of (1), (3)[1/50 dilution], and (4)[1/100 dilution] without centrifugation.
- 2. Smear (1), (2), (3), and (4) all over the LB medium and put the same as in experiment #1.
- 3. Culture them in the incubator at around  $25^{\circ}$ C for 1 to 2 days.

## Result

Table 1 : Reaction to Bacillus and E.coli									
Bacillus		1	2	31/10 dilution	④1/50 dilution	⑤ <b>1/100</b> dilution			
Agave	epidermis	-	—	-					
	leaf juice	Δ	_	+	Δ	Δ			
Aloe	mesophyll	-							
	leaf juice	<b>※</b> ±	_	-	_	_			

E.COII		(I)	31/10 dilution	41/50 dilution	(51/100 dilution		
Agave	epidermis	-	-				
	leaf juice	-	_	+	+		
Aloe	mesophyll	-					
	leaf juice	-	_	_	-		
$+$ : Positive $\wedge$ : difficult to conclude							

'- : Negative

/ : No data

- 1. Formation of inhibition circles for Bacillus and E.coli in leaf juice
- ightarrowThere is an anti-bacterial effect on leaf juice.
- 2. No formation of inhibition circle on epidermis.
- $\rightarrow$ There is no anti-bacterial effect on epidermis.
- 3. No inhibition circle was observed for aloe
- ightarrow No anti-bacterial species exist.
- $^{\ast}$  In Bacillus 4 and 5 , the bacteria were not uniform and it was not possible to determine the presence or absence of inhibition circle.
- \* There was a sample in which an inhibition circle was not formed for Bacillus ①.





Figure 4: Agave leaf juice Bacillus 3 1/10 dilution



Agave leaf juice

E.coli 3 1/50 dilution



Figure 6: Agave leaf juice E.coli 4 1/100 dilution

### Consideration

- 1. Anti-bacterial effect on the leaf juice of Agave
- $\rightarrow$ It may be able to inhibit bacterial erosion from the world. 2. No inhibition circle on the epidermis of Agave
- $\rightarrow$ The epidermis doesn't play a role against bacteria
- 3. Aloe sometimes didn't have a inhibition circle
- →Aloe may have varying in anti-bacterial effect depending on species and growing environment.

### **Future Outlook**

- 1. Identify the substance that is the source of the antibacterial power.
- 2. Discover which organ makes the substance (tracking the stream of the substance).
- 3. Research how antibacterial power differs depending on temperature.
- 4. Discover relationships between growing environment and antibacterial power.
- 5. Verification of differences in antibacterial power between different species of Aloe. 6. Quantifying the antibacterial powers.
- 7. Discover whether the Agave have the antibacterial power against the other bacteria.

#### References

- ・エバーグリーン植物図鑑 <u>https://love-evergreen.com/zukan/plant/5591</u> (2021.10.26)
- ・最近の植物系統分類学の研究手法 千葉大学 園芸学部 國分尚 http://www.kayokai.net/kayo/25/25\_8-10.pdf (2021.11.14)

Figure 2: Agave used in the experiment

