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How does CNB-2 act to its surroundings?

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Abstract. CNB-2 is a type of unharmful bacteria commonly found in water and dirt areas. It is sometimes used in biological experiments where it is given substances to see if the substance attracts it or not. There are two Typical experiments related to this, which is what will be discussed here.

Keywords: CNB-2, a-Keto Glutaric acid, Oxaloacetic acid, Pyruvic acid.

1. The Gradient Plate Experiment:

This experiment involves using lots of Petri dishes, chemotactic substances, and culture mediums. The aim of this experiment is to test which substance best attracts CNB-2. Before starting the experiment, one has to make sure that CNB-2 is stored correctly in a -80 degrees celsius cellular fridge as bacteria cannot live for long in a hot environment. The next step is to make 1 liter of solid and liquid culture medium (each 1 liter). Their ingredients are shown below:

Solid: Agar powder 15g/L, Yeast 5g/L, Sodium Chloride 10g/L, Tryptone 10g/L.

Liquid: Yeast 5g/L, Sodium Chloride 10g/L, Tryptone 10g/L.

Note: the solid culture medium will stay in a liquid state firstly, but after a period of time it will solidify and become jelly-like.

The aim of the culture medium is to activate the bacteria cells and let them start growing. When bacteria is stored in a low degree environment (such as a cellular fridge), it is in a “hibernation” state, meaning that it is not active and will not grow and develop. After the solid culture medium has been made, pour them into Petri dishes and wait for them to solidify and become jelly like. The next step is to take out a test tube of frozen CNB-2 bacterial fluid from a cellular fridge and hold it in both hands and wait for the frozen fluid to melt into liquid form. Then, using a pipette needle, carefully dip it into the CNB-2 bacterial fluid, making sure that only the needle tip touches the fluid. The next step is to take the pipette needle with the bacterial fluid and gently scrape it on the surface of the solid culture medium, letting the bacterial fluid spread and stick to the solid culture medium. After this, use tape to seal the petri dish and then put it into a Constant temperature Bacterial incubator until the next day.

Now that we have used up the solid culture medium, there is only the liquid one left. For the liquid culture medium, subpackage it into test tubes after it has been made.

Then, take a pipette needle and dip it into a test tube of CNB-2 bacterial fluid. Make sure that only the needle tip touches the fluid. After this, put the whole pipette needle into the test tube with the liquid culture medium and put the test tubes inside a constant temperature shaker until the next day.

A new type of culture medium(called MSB)has to be made on the second day(the one made before is called LB)The ingredients for it are listed below:

Solid: Agar powder 2.7g/L, Disodium Hydrogen Phosphate 1g/L, Dipotassium Hydrogen Phosphate 0.5g/L, Ammonium Chloride 1g/L, Magnesium Sulphate 0.03g/L.

Liquid: same ingredients, but no Agar powder.

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After the solid and liquid culture medium is made, subpackage the liquid culture medium and put it aside. Pour all the solid culture medium into petri dishes, and wait for them to dry and become jelly-like. After this is done, leave the petri dishes aside and take out the test tubes with liquid culture medium and pipette needles from yesterday(by this time the liquid culture medium inside the test tubes should appear yellowish and turbid. If not, then the steps from yesterday has to be repeated as the bacteria is not growing). Then, use a pipette to take 100 micro litre of the LB liquid culture medium with growing bacteria from yesterday and inject it into the MSB liquid culture medium made today. After this has been done, put the MSB liquid culture medium into a constant temperature Shaker and let the bacteria grow for six hours.

After six hours of growing, use a pipette and take a small amount of MSB liquid culture medium(the one with CNB-2) and inject it into a 1.5 mL EP tube. Then, put the EP tube into a centrifuge and let it spin for two minutes 5000 rpm. After the centrifugation has finished, take out the EP tube and a small reddish dot will appear sticking at the bottom of the tube. This reddish dot is concentrated bacteria. Next, pour the remaining liquid in the EP tube into a trashcan. The reddish dot will stick to the bottom of the tube, so don't worry about it being washed away by the liquid culture medium. Use a pipette and take 1 mL of MSB liquid culture medium and inject it into the EP tube, then use the pipette to scrape the concentrated ball of bacteria until it disintegrates and dissolves in the liquid culture medium. Then, suck all of the liquid culture medium inside the EP tube into a pipette needle, then inject it back in the EP tube. Repeat this step about 4-5 times, then put it back in a centrifuge, 5000 rpm 2 minutes. After centrifugation, take out the EP tube and repeat the steps before.

For this whole centrifugation and bacterial washing process, repeat 3 times. The aim of these processes is to wash the bacteria. Because the bacteria was originally growing in LB liquid culture medium, it has to wash off the culture medium that is sticking on its surface, as future experiments has to be strict and rigid in order to have an accurate result, so substances that are not related to the experiment has to be cleaned off. After the bacterial washing process has finished, use a pipette and take 0.5 micro litre of the concentrated CNB-2 bacterial dot and inject it into MSB solid culture medium Petri dishes and put them inside a constant temperature incubator and let the bacteria grow for about 9 hours.

Now that all the preparation has been done, we can finally start the experiment. After 9 hours(about one night) of waiting, all the bacteria should be well-grown. Take out a test tube of MSB liquid culture medium with growing bacteria inside it, and, using a pipette, transfer 100 mL from it into another test tube filled with MSB liquid culture medium and put it into a constant temperature shaker for about 5-6 hours. This is the last step of preparation. Next, eight different types of chemotactic solid culture medium has to be made. A chemotactic culture medium is basically a culture medium that contains chemotactic substances inside it. Here we use MSB solid culture medium as its base. There are eight types of chemotactic substances that will be tested, so we have to make at least eight petri dishes of MSB solid culture medium(ingredients are listed above if more is needed). The eight types of chemotactic substances are:

(1) Citric acid

(2) Malic acid

(3) Succinic acid

(4) Empty Agar

(5) Fumaric acid

(6) Pyruvic acid

(7) Oxaloacetic acid

(8) a-Keto Glutaric acid

After the eight chemotactic substances are prepared, take eight 50mL test tubes and pour 30mL of MSB solid culture medium in each one. Then, inject 10mL of chemotactic substance into the test tube, and gently shake it to let the culture medium and the chemotactic substance mix together. Repeat this step for eight times, making sure that each chemotactic substance only takes up one test tube. Next, pour all of the chemotactic culture medium into Petri dishes and wait for them to solidify and become jelly-like(each test tube of chemotactic culture medium corresponds to only one Petri dish). After the culture medium has solidified, take out a puncher and punch holes inside the chemotactic culture medium. Do not throw away the culture medium inside the puncher(the culture medium will appear as a small cylinder after punching). Each Petri dish should have at least five holes in it. After all the Petri dishes have been punched, take 32 Petri dishes and, using a permanent marker, draw a cross on the back of each Petri dish. Then, take out four cylinders of chemotactic culture medium(4 cylinders from each type, which makes it 32 cylinders in all) and using tweezers, put one cylinder of culture medium into a Petri dish(only one cylinder per Petri dish). Next, take out some MSB solid culture medium and pour it inside each Petri dish. If the solid culture medium has solidified, put it inside a microwave and heat it until it becomes liquid again. Leave the 32 Petri dishes aside to let the MSB culture medium solidify. The most important step comes next. After the MSB culture medium has solidified, take out the test tube of CNB-2 bacterial fluid from before and, using a pipette, take 10 micro litre of the bacterial fluid and inject it into the 32 Petri dishes. Make sure that the point of injection is on the line of the cross on the back of the Petri dish, and that the point of injection has to be at least 2cm away from the chemotactic culture medium cylinder. Repeat this step for each Petri dish, and when finished, put all of them inside a constant temperature incubator for two whole days and wait for the experiment results.

After two whole days of waiting, the bacteria should be grown. Take the 32 Petri dishes out of the constant temperature incubator, and look at the results. Let's analyse the results in a more scientific way, rather than just describing what's on the Petri dishes. Below shown is an example of a scientific way to look at the results.

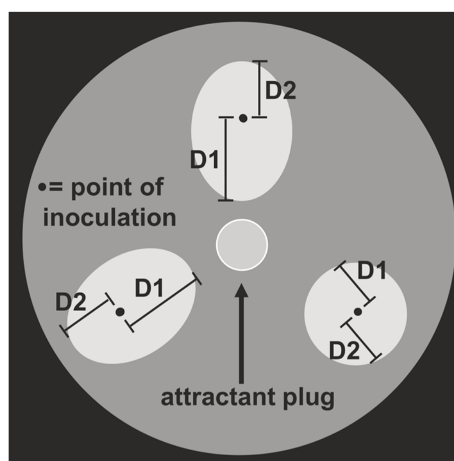

Figure 1. Experiment Result

Attractant plug=chemotactic culture medium cylinder.

D1=distance from injection to the farthest point where the bacteria have grown.

D2=distance from injection to the farthest point where the bacteria grows the opposite direction to the chemotactic culture medium cylinder.

Some sample experiment result pictures are shown below.

RI is the response index (RI=D1÷D1+D2).

The D1, RI, and D2 of CNB-2 will also be shown as an example.

Note: delta 20 is just an another type of bacteria that does not have the genes for moving. It is just used as a comparison bacteria. The D1, D2, and RI will not be shown for it.

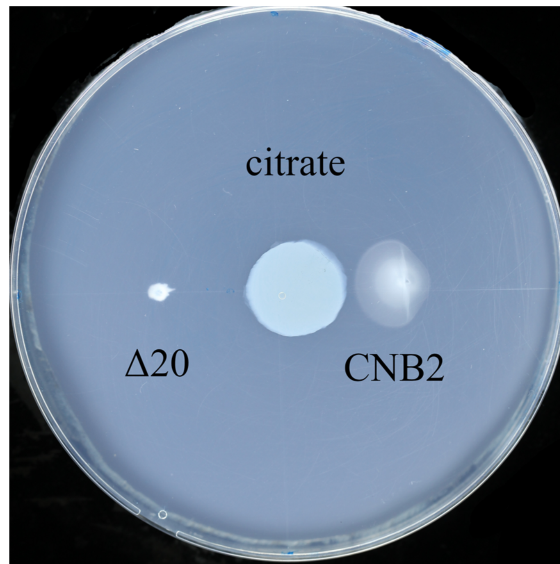

Figure 2. Citric acid
D1=7.74mm; D2=3.75mm; RI=0.68

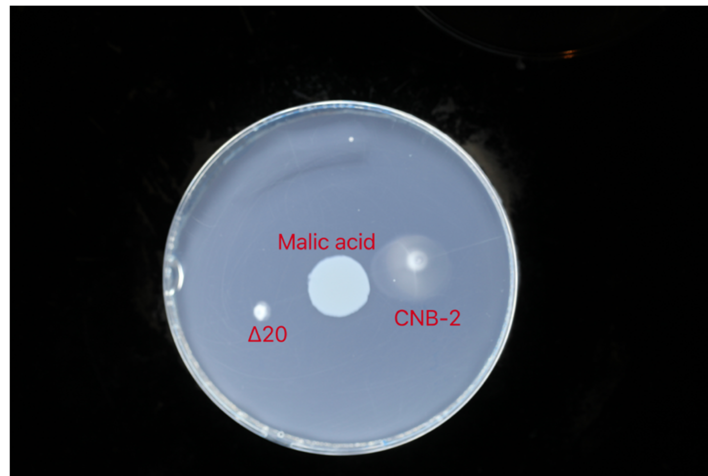

Figure 3. Malic acid
D1=8.91mm; D2=6.16mm; RI=0.54

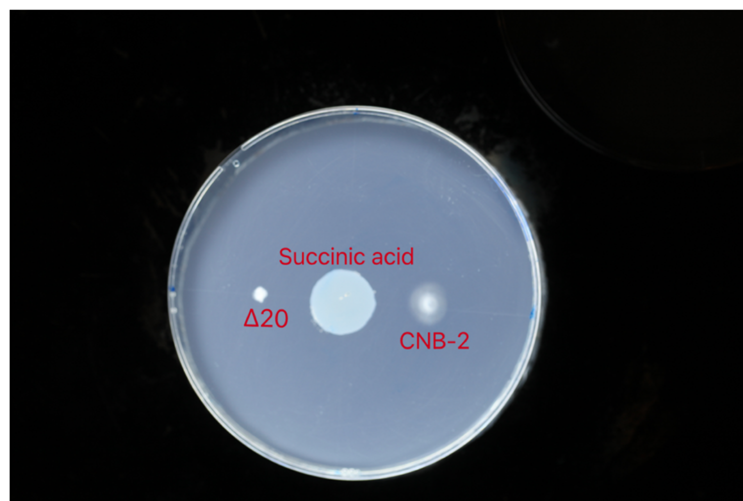

Figure 4. Succinic acid:
D1=5.06mm; D2=4.65mm; RI=0.51

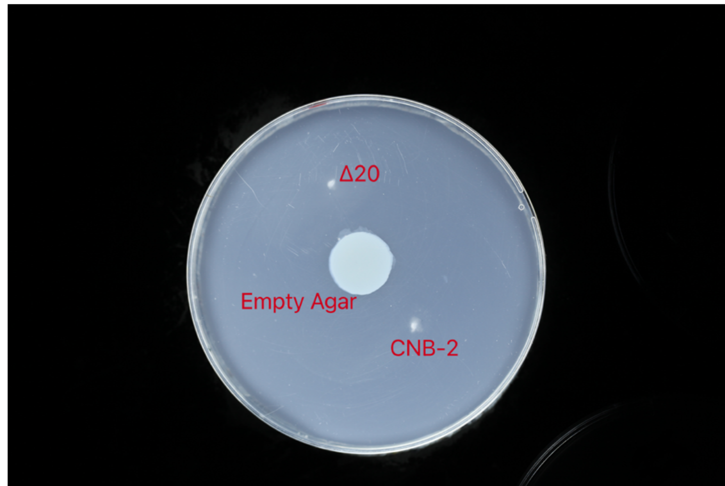

Figure 5. Empty Agar:
D1=approximately no growth, negligible. D2=approximately no growth, negligible. RI=unavailable because no D1 and D2.

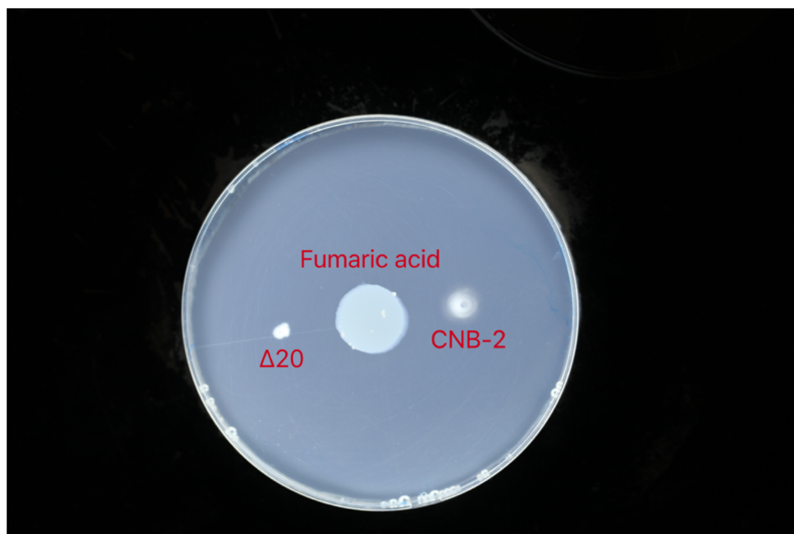

Figure 6. Fumaric acid
D1=3.80mm; D2=3.62mm; RI=0.50

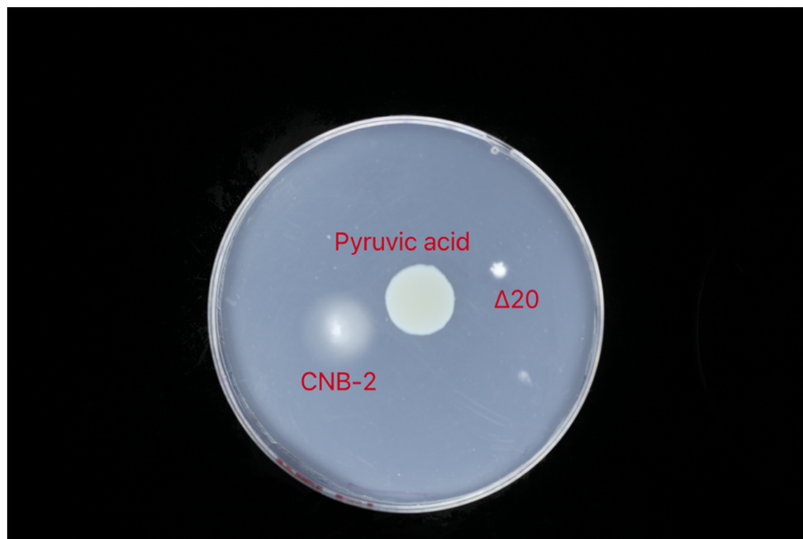

Figure 7. Pyruvic acid:
D1=7.95mm; D2=7.59mm; RI=0.51

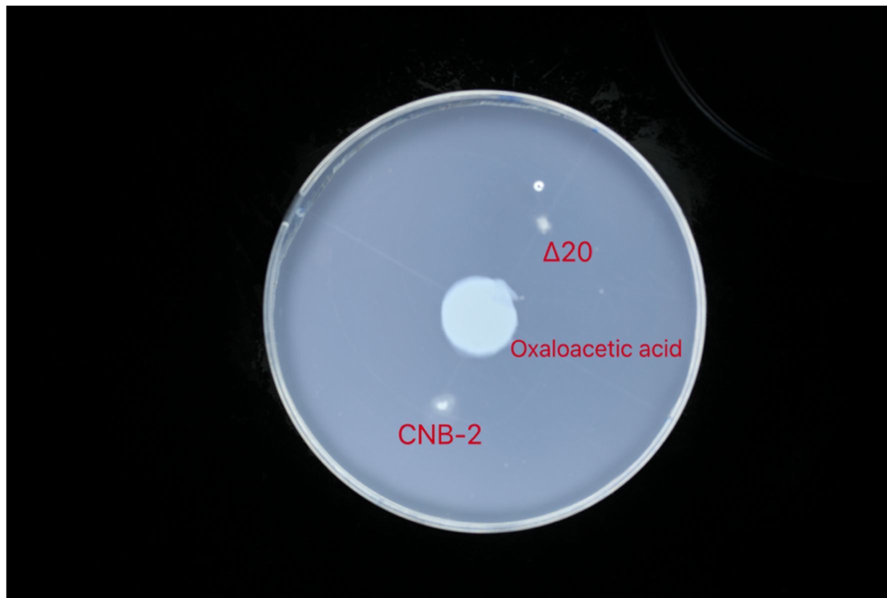

Figure 8. Oxaloacetic acid:
D1=negligible; D2=negligible; RI=unavailable

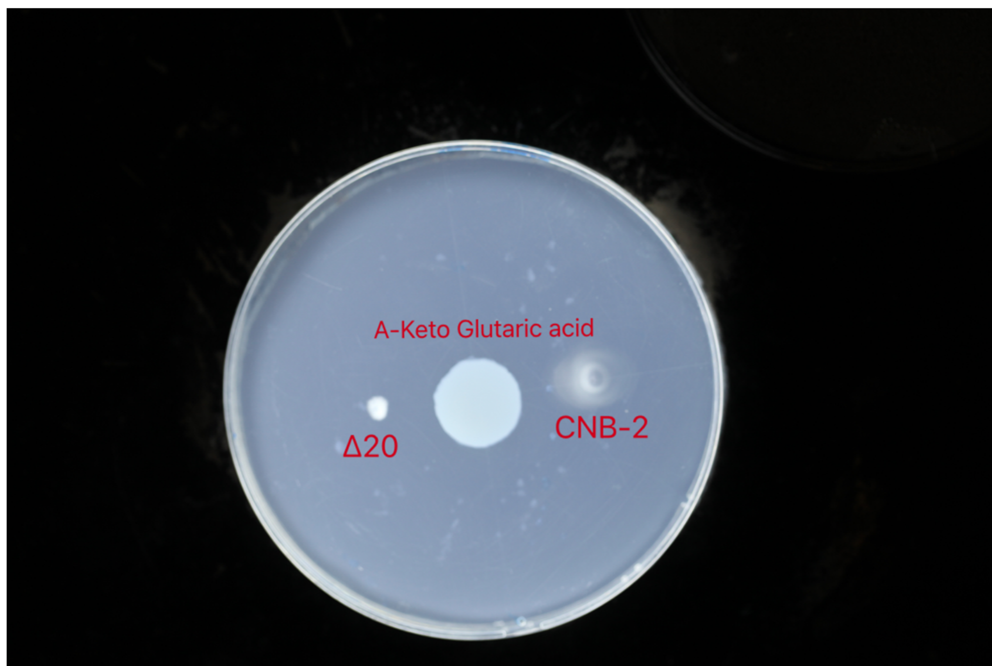

Figure 9. A-Keto Glutaric acid:
D1=5.27mm; D2=5.35mm; RI=0.50

Through the D1 and D2 of each chemotactic substance, we can see that CNB-2 tends to grow more when the chemotactic substances are Citric acid, Malic acid, Pyruvic acid, and A-Keto Glutaric acid.

This is now the end of the first experiment- The Gradient Plate Experiment. Below will be the second experiment-The Capillary Experiment.

2. The Capillary Experiment:

The aim of this experiment is the same as the first one, but only one type of chemotactic substance will be used, which is citric acid. Before the actual experiment starts, there is still a little bit of preparation that has to be done.

The first step is to make some more of the LB solid culture medium and MSB liquid culture medium. The ingredients are listed below:

LB solid: Agar powder 15g/L, Yeast 5g/L, Sodium Chloride 10g/L, Tryptone 10g/L.

MSB liquid: Disodium Hydrogen Phosphate 1g/L, Dipotassium Hydrogen Phosphate 0.5g/L, Ammonium Chloride 1g/L, Magnesium Sulphate 0.03g/L.

Like before, after the solid culture medium has been made, pour it into Petri dishes and leave it aside until it solidifies.

While waiting, take out 100 1.5mL EP tubes and pour all of the MSB liquid culture medium into each one. This is the preparation for the diluting of the experiment.

After this has been done, the LB solid culture medium should have already solidified. The next step is to draw 5 parallel lines in the back of each Petri dish. The 5 lines should divide the Petri dish into six equally-spaced columns.

This is then the end of all preparation. We can now start the experiment.

The first step is to prepare at least 5 sterile medical needles and a test tube of CNB-2 bacterial fluid. Then, prepare a test tube of citric acid(the chemotactic substance in this experiment), and, using the needles, suck 0.1mL of the citric acid into each one(each needle has to contain a different concentration of the chemotactic substance. In this experiment we use 5 concentration levels: 0, 0.3, 1, 3, 10. The unit is in mM). Then, using a pipette and pipette needle, suck 0.1 micro litre of the CNB-2 bacterial fluid. Take off the pipette needle from the pipette, then insert the pipette needle into the medical needle with citric acid. Repeat this on all five medical needles. After this has been done, leave all the needles aside for 1 hour to let the bacteria react with the citric acid. After one hour, take off the pipette needle and throw it away. Do not throw away the medical needle and the liquid inside. Next, take out the 100 EP tubes filled with MSB liquid culture medium from before, and inject the liquid inside the medical tube into the EP tube. Each medical needle corresponds to only one EP tube, because there are five different concentration level, so they cannot get mixed up. The next step is diluting. Take the EP tube with the injected liquid inside, and using a pipette, transfer ⅕ of the liquid inside the tube into another EP tube. Then, transfer ⅕ of the liquid inside the second EP tube into a third EP tube. Mark the first EP tube as 1, the second as 2, and the third as 3. The first EP tube is the original high-concentration bacterial fluid. The second and third are more dilute ones that contain less bacteria. This process has to be repeated with each concentration level. The diluting process helps us to see and count more clearly after the results are out, because the original bacterial fluid contains too much bacteria, which will appear as a blob of mess after it grows up. After the diluting process has finished, take out the Petri dishes with the 5 parallel lines on the back. Next, take out EP tube 2 and 3 of any concentration level, then using a pipette, take a little bit of the liquid inside EP tube 2, and inject it into the surface of the LB solid culture medium inside the Petri dish. Note: the injection place has to be between the lines, inside a column. For each EP tube(2,3), it has to take up exactly 3 columns. This is why 5 lines has to be drawn to make 6 columns in all. Repeat the same process for EP tube 3. The exception here is EP tube 1(the original bacterial fluid), it can take up a whole Petri dish(6 columns). Repeat all of these steps for the other four concentration levels. Each level of concentration has to take up at least 2-3 Petri dishes. After this is finished, put all of the Petri dishes inside a constant temperature incubator for 10 hours to let the bacteria grow.

After 10 hours, there should be white little dots covering the surface of the solid culture medium. The way to look at the results is to count all the white little dots. These white dots are colonies of bacteria. Below is an analysis of the results.

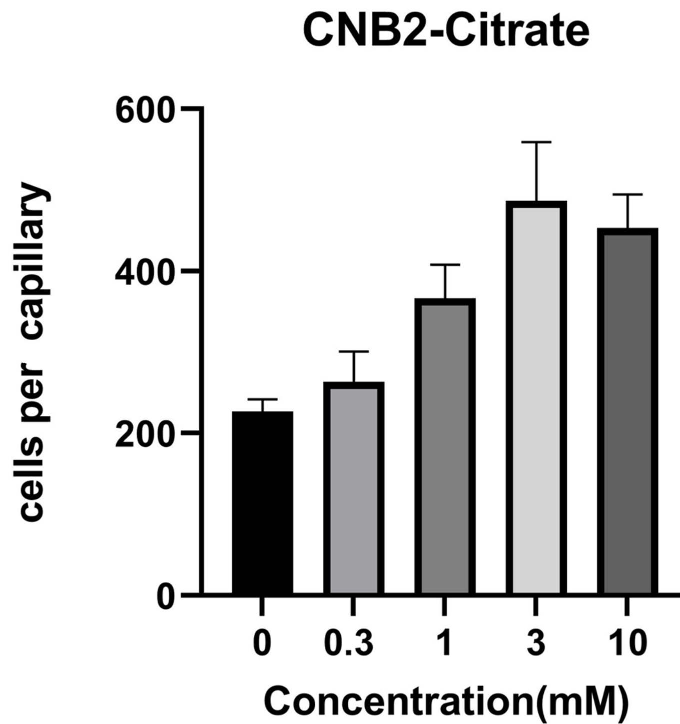

Figure 10. CNB2-Citrate

The data now proves that CNB-2 grows the best under a concentration of 3mM.

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