Supplementary Material

Experimental Method

Materials

1. Yeast Strain, SCE13
2. YPD (Yeast extract Peptone Dextrose)-Yeast growth media,
3. Salt, 1M NaCl
4. Agar Plates with 25ml YPD and 1M NaCl
5. Test tubes
6. 96-well Microtiter Plate

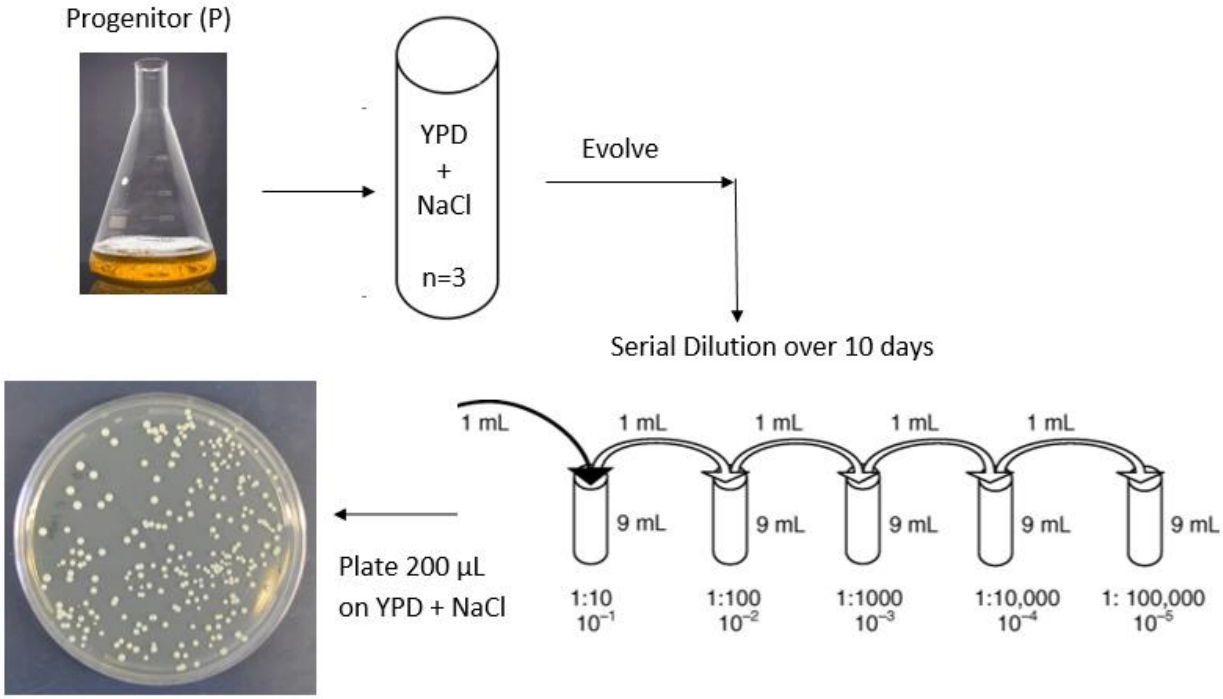


Figure 2. Flowchart of Experimental Method

Day 1- Setting up the Experiment

Recipe for 1 L YPD liquid (with 1M NaCl)

1) 10g Bacto Yeast Extract
2) 20g Bacto Peptone
3) 20g d-glucose
4) 1L water
5) 1M NaCl (58.44g)

Procedure

1. In a 2L beaker, add the ingredients to the beaker with continuous stirring
2. Autoclave the beaker
3. Once the YPD has cooled down, pipette 10ml into each of the 3 test tubes
4. Pipette 100µL of yeast strain, SCE13 into 3 test tubes, to create three populations named P1-T0, P2-T0 and P3-T0
5. Incubate at 30°C for two days.

Day 3- Performing First Serial Dilution

1. In three sterilized test tubes, pipette 10mL of liquid YPD from the stock prepared previously
2. Label each of the test tubes as P1-T1, P2-T1 and P3-T1
3. Pipette 100µL of solution from P1-T0, P2-T0 and P3-T0 and add to P1-T1, P2-T1 and P3-T1 respectively
4. Incubate the tubes at 30°C for two days.
5. Place the test tubes P1-T0, P2-T0 and P3-T0 in the freezer

Day 4 to 12 - Continue with serial dilutions

Serial dilutions were performed for 10 days.

Day 14 – Plating on Agar Plates

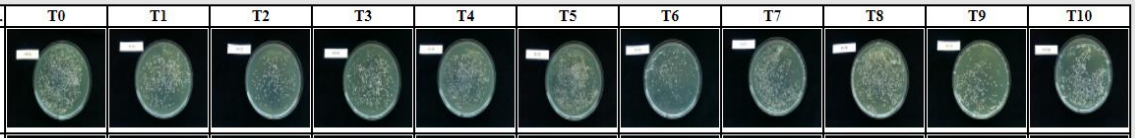
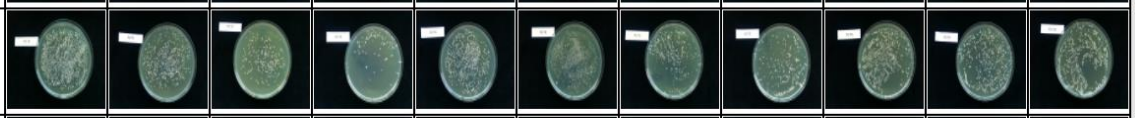
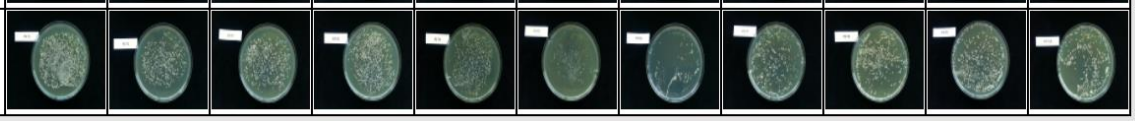
Recipe for 1 L YPD solid (with 1M NaCl)

1) 10g Bacto Yeast Extract
2) 20g Bacto Peptone
3) 20g d-glucose
4) 1L water
5) 1M NaCl (58.44g)
6) 20g agar

Procedure

1. In a 2L beaker, add the ingredients to the beaker with continuous stirring
2. Autoclave the beaker
3. Once the YPD has cooled down, pour 25ml YPD into agar plates. 33 agar plates were made.
4. On the agar plates containing YPD and 1M NaCl, 200µL of 10^5 dilutions were plated from each serial dilution tubes (T0-T10) for all populations (P1, P2, P3). See Table 1.

Table 1. Yeast NaCl Agar Plates for 6 Populations across 10 serial dilution tranfers

Population/Transfer No.	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
P1											
P2											
P3											

Making 10^5 dilutions

1. In a 96 well microtiter plate, pipette 200µL of solution from all 33 test tubes. This was the standard plate.
2. In another microtiter plate (dilution plate), pipette 90 µL of sterile water into wells 1-4
3. In the 5th well, pipette 180 µL of sterile water
4. From the standard plate, pipette 10 µL from the first well and pipette it into well 1 of the dilution plate.
5. From this well, take 10 µL and pipette into well 2.
6. Repeat the previous step, each time taking 10 µL from one well and releasing it into the next well
7. From well 5, take 200 µL of the solution and pipette it on to an agar plate
8. Using cell spreader, spread the solution evenly on the agar plate
9. Repeat the above steps for all 33 test tubes.
10. These plates were incubated at 30°C for two days

Day 16 – Photographing the agar plates and measuring optical density

All the plates were photographed. 5 images were taken for each plate for all the populations from T0, T1, T5 and T10.

The optical density of the of all 33 test tubes was also measured using Spectronic™ 200 Spectrophotometer.