Stimulation of Nisin Production from Whey by a Mixed Culture of *Lactococcus lactis* and *Saccharomyces cerevisiae*

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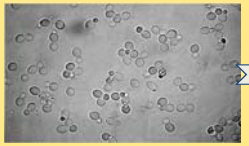
Introduction

Image 1. Lactococcus lactis

Image 2. Dairy products that utilizes *L.lactis*

L.lactis, a Gram-positive bacterium, grows on lactose or milk sugar and produces lactic acid as a byproduct. *L.lactis* also generates a non-toxic and antibacterial chemical called nisin which is often used as a natural food preservative. Therefore, a continuous growth of *L.lactis* is often desirable to increase the production of nisin. However, lactic acid accumulation lowers the pH of fermentation broth thus creates a non-sustainable environment for the growth of *L.lactis*.

This research project aims to create a stress-free growth environment for *L.lactis* via in-situ removal of lactic acid. *Saccharomyces cerevisiae* or generally known as Baker's yeast is used to consume lactic acid as a carbon source for metabolism activity.



- Does not consume lactose as a carbon source and thus will not inhibit the growth of *L.lactis*
- Capable of utilizing lactic acid as a carbon source
- Toxicologically safe and cheap

Image 3. *Saccharomyces cerevisiae*

Methodologies

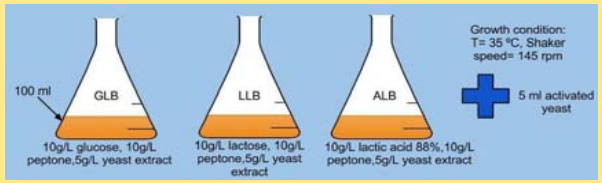


Image 4. Validating *S.cerevisiae* capability to consume lactic acid

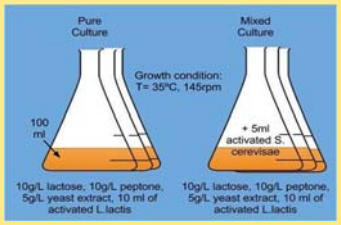


Image 5. Development of mixed culture

- For Image 4, samples were taken at 0, 24, and 45 hours of inoculation time and absorbance values at OD600 were measured to calculate the concentration of cells per milliliter solution.
- Samplings for each flask for Image 5 were taken at 0,2,4,6,8,10,22,24,50,and 96 hours of inoculation time. Each time, 5 ml of sample would be drawn from the flask and tested for pH. Next, 1ml of the sample was set aside for freezing and 4 ml of sample was centrifuged for 2 minutes at 800 rpm. The supernatant was collected and stored in the freezer.

• Measurement of result:

➢ pH test

➢ *L.lactis* and *S.cerevisiae* count

The counting chamber was wetted with sample and counting of *L.lactis* and *S.cerevisiae* was made under optical microscope at 40 times magnification. The formula used to calculation the concentration of *L.lactis* and *S.cerevisiae* per milliliter of solution was: $\text{Concentration} = \frac{\text{cell count}}{80} \times 400 \times \frac{10^4}{\text{ml}} \times \text{dilution factor}$

➢ Lactic acid analysis

First, 0.5 ml of standard solutions (0-25 µg/ml) were taken and placed into 10ml screw-capped test tubes. The step was repeated for sample solutions of 0 to 25 µg/ml of concentration. Next, 3ml of concentrated sulfuric acid (H_2SO_4) were carefully added into each of the test tube. Test tubes were capped tightly afterwards and shook rigorously to homogenize the mixture. Subsequently, the solutions were boiled for 15 minutes at 100 º C. The tubes were allowed to cool at room temperature for 15 minutes. Then, 50 µl of copper (II) sulfate (CuSO4) and 100 µl of p-phenyl phenol (pPP) were added into the tubes, respectively. The tubes were shaken frequently within 30 minutes of time allocated for chemical reaction to take place. After the solution turned from clear to dark blue in color, the tubes were boiled for 90 seconds and let cool at room temperature of 25 ºC.

Using spectrophotometer, absorbance values of lactic acid were determined at 570nm wavelength. Standard curve was generated from the absorbance values and concentrations of lactic acid in sample solutions were calculated.

Results and Discussion

***S.cerevisiae* growth in medium**

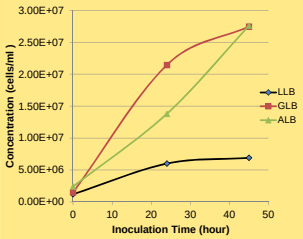


Figure 1. Inoculation of *S.cerevisiae* 3 media

***S.cerevisiae* growth in Mixed Culture**

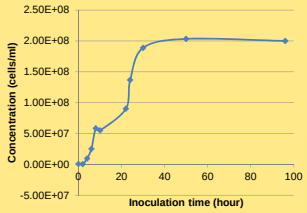


Figure 2. Inoculation of *S.cerevisiae* in mixed culture

Figure 1 indicated that similarly to glucose, lactic acid supported the growth of *S.cerevisiae*. However, the growth rate for *S.cerevisiae* in ALB was notably lower than in GLB within the first 30 hours of inoculation. This could be caused by *S.cerevisiae* utilizing a different metabolic pathway for lactic acid. It ccould also be seen that lactose did not promote the growth of *S.cerevisiae* based on the low concentration of cells per milliliter of solution.

The result validated the prospect of mixed culture of *L.lactis* and *S.cerevisiae* in maintaining an acceptable level of pH of the broth. As expected, *S.cerevisiae* did grow well in the mixed culture where lactic acid was continuously produced by *L.lactis*. Based on Figure 2, the highest metabolism activity or the log phase occurred between hour 2 and hour 30 of inoculation time.

pH of fermentation broth

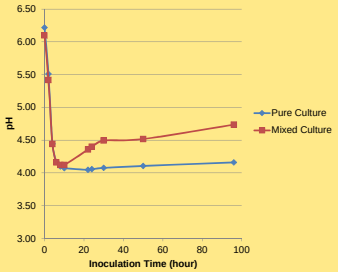


Figure 3. pH comparison in pure and mixed culture

Lactic acid presence in fermentation broth

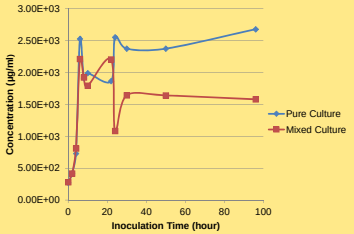


Figure 4. Comparison of lactic acid concentration in pure and mixed culture

Both pH and lactic acid analysis illustrated the acidity of pure and mixed culture. When compared, there was a congruency between both analysis results. For the first 10 hours, there was an acute drop of pH due to high accumulation of lactic acid. There were several justifications that can be made: 1) growth rate of *L.lactis* was higher than *S.cerevisiae*, 2) as a result, lactic acid would be in excess and the limited amount of *S.cerevisiae* restrained it from immediate consumption of the surplus, 3) increasing the initial amount of activated *S.cerevisiae* would be important to rectify the drastic pH drop by refraining the lactic acid to accumulate at a high level.

After 10 hours, the acidity in mixed culture decreased gradually and became stagnant after 30 hours. Meanwhile, acidity in pure culture remained low at pH 4 and stagnant after 10 hours. As described previously, *S.cerevisiae* consumed lactic acid in a moderate pace and the initial concentration was small and these conditions did not allow acute rise in pH. Also, *S.cerevisiae* growth curve indicated that it achieved stationary phase or growth limiting phase after 30 hours. There was depletion in nutrient and space as the experiment was conducted in flasks where no new medium wass added and samplings caused the volume of broth to decline over time.

Conclusion

A common method to neutralize a solution is using a synthetic chemical. As utilization of synthetic chemicals can potentially be hazardous for human consumption, there is a need to explore the potential of natural non-toxic neutralizer such application of *S.cerevisiae* on in situ removal of lactic acid. *S.cerevisiae* shows a high prospective to maintain the pH of *L.lactis* fermentation broth in ensuring a continuous growth of *L.lactis*. It is suggested that more research on the ratio of biomass in the mixed culture be conducted to increase the efficiency of *S.cerevisiae* in the mixed culture. Among the application of mixed culture is it can be used on cheese whey, a cheese-making industrial waste that contains traces of lactose.

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