Mixed Culture of Aerobic and Anaerobic Microorganisms Under Similar Condition

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Abstract

To clear the characteristics of EM culture, mixed culture of photosynthetic bacteria, lactic acid bacteria, yeast and N_2-fixing bacteria was tested in the same medium. *Rhodopseudomonas sp.*, *Lactrobacillus sp.*, *Saccharomyces cerevisiae* and *Azotobacter chroococcum* were able to live together in 5% molasses medium under a certain intensity of light. The microbic equilibrium was reached on the 36^{th} day after culture, At this time, the number of these 4 strains were 3×10^7, 5×10^6, 2×10^7 and 2×10^6 ml, respectively and pH of culture was 6.5, Although this microbic equilibrium was broken down by changing pH to 3, 5, 7 and 9 artificially, a new microbic equilibrium was recovered again only under lighting and when the pH of the culture was 6.5. However, pH decreased under dark and the microflora changed remarkably.

Introduction

The basic principle of EM preparation is the problem of mixed culture of various kinds of microorganisms. We can often meet the coexistance of yeast, lactic acid bacteria and zymogenic microorganisms in our common life but this was not studied adequately. Making the mixed culture condition for zymogenic and synthetic microorganisms, and then applying the culture of zymogenic-synthetic microorganisms, it can zymogenically decompose the organic materials and can be a good condition for plant growth. In addition, they can supply some deficient nutrients by themselves because they can coexist with synthetic microorganisms and play their roles, too. It seems that the photosynthetic bacteria can improve acidic soil because it synthesizes the saccharides from H^+ and CO_2 by photosynthesis. The lactic acid bacteria and yeast (Zymogenic type), and photosynthetic bacteria (synthetic type) were co-cultured but there is no report as yet on their culture process.

Yeast has the ability to assimilate glucose as a substrate and produce the pyruvic acid by the first metabolism of saccharide decomposed system. Pyruvic acid can be used as a substrate of aerobic acetic acid bacteria, microaerobic lactic acid bacteria and anaerobic butyric acid bacteria. In this way, if the lactic acid bacteria using the metabolite of yeast multiply, the formed lactic acid becomes the substrate of photosynthetic bacteria and they can be multiplied. Then yeast uses the saccharides formed by this photosynthetic bacteria as a substrate and can multiply repeatedly (Higa, 1994). This seems that there is a physiological ability by which these three strains are able to coexist.

The mixed culture of lactic acid bacteria, yeast and red non-sulfur photosynthetic bacteria was investigated in this study.

Materials and Methods

Strains:

Latic acid bacteria: *Lactobacillus sp*. Isolated from <Bokashi>, Japan.
Yeast: *Saccharomyces serevisiae* isolated from <Bokashi>, Japan.
Red non-sulfur photosynthetic bacteria: *Rhodobacter sp*. In our laboratory.

Media:

The molasses (5 percent) medium for *S. cerevisiae*, the molasses (5 percent) medium + $CaC0_3$ (1 percent) for *Lactobacillus sp.*, van Weaver's medium for *Rhodobacter sp*. and modified Burk's medium for *A. chroococcum* were used.

Each strain was used for mixed culture after incubation to the end of log-phase on each selective medium.

Experiment 1: $CaCO_3$ of 1 percent was added into the mixed culture of lactic acid bacteria and yeast in the molasses medium. The change in their numbers was tested according to incubation time. When the red non-sulfur bacteria were inoculated and cultured together, the change in their numbers was tested. The ester values of the culture after incubation were measured and compared for 30 days.

The number of strains was measured by the dilution plating technique and the ester value was considered as saponification value of organic acid produced by hydrolysis of sample.

Experiment 2: Using the procedure stated above, in the mixed culture of 3 strains, the change of their numbers and pH of the culture was measured according to incubation time.

Experiment 3: The changes of the number of strains and pH according to incubation time were measured in the culture by varying the pH of the mixed culture of lactic acid bacteria, yeast and photosynthetic bacteria, when reached equilibrium of microflora, to 3, 5, 7, 9 respectively.

Experiment 4: On the mixed culture of above three strains after having reached equilibrium of microflora, the number of strains, pH, superoxide dismutase (SOD) activity, ester value, electronic conductivity (EC) and the content of the rest saccharides were measured.

Experiment 5: Incubating the mixed culture of 4 strains in molasses medium, the change of number of strains and pH according to incubation time were measured.

Results and Discussion

Experiment 1: Effect of $CaC0_3$ and photosynthetic bacteria on mixed culture of *Lactobacillus sp.* and *S. cerevisiae.*

As shown by Higa, (1994) there are some possibilities that *S. cerevisiae* and *Lactobacillus sp*. can co-exist in the mode of metabolism. But the optimum pH of *S. cerevisiae* range between 3 and 5, and that of *Lactobacillus sp*. between 5.5 and 6.5. Therefore it is important that the control of pH must be solved in the incubation of mixed culture of *S. cerevisiae* and *Lactobacillus sp.*

From this, the effect of addition of $CaCO_3$ of 1% as pH stabilizer and *Rhodobacter sp.* inoculated, together with, was tested.

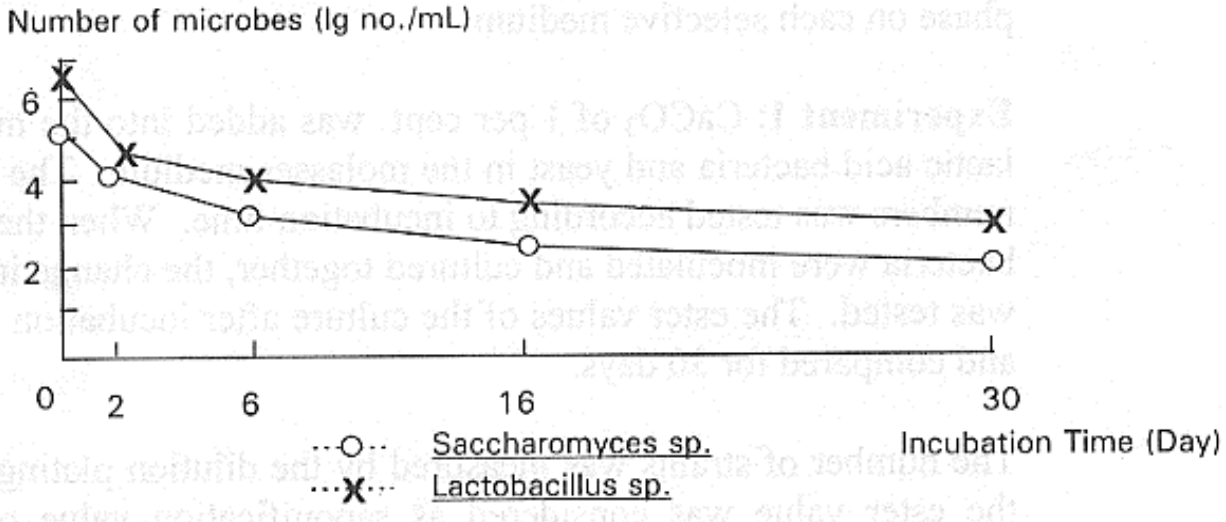

Fig. 1. Change of Number of Strains According to Incubation Time in Mixed Culture of *Lactobacillus sp.* and *S. cerevisiae.*

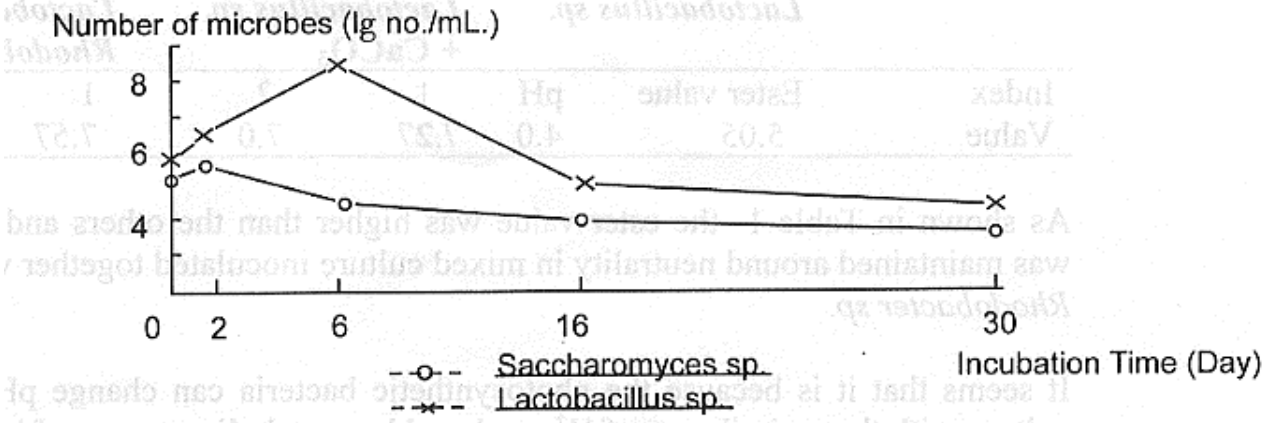

Fig. 2. Effect of $CaCO_3$ on Mixed Culture of *S. cerevisiae* and *Lactobacillus sp.*

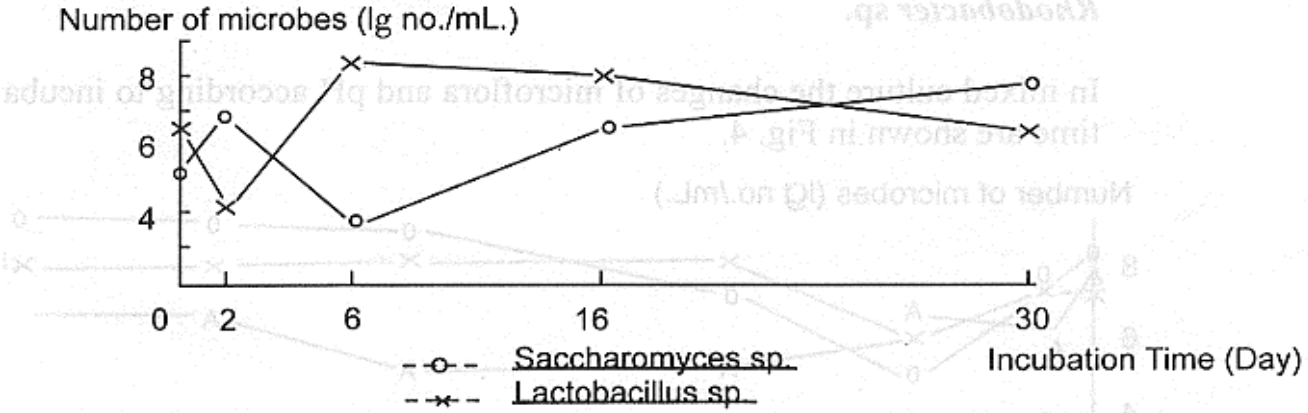

Fig. 3. Change of Number of Strains According to Incubation Time in Mixed culture with *Rhodobacter sp*. Together.

As shown in Fig. 1, the numbers of *S. cerevisiae* and *Lactobacillus sp*. decreased by 10^3, 10^4 cells/ml but not after that.

On the other hand, the growth of strains increased initially by the addition of $CaCO_3$ of 1 percent, but the number of strains decreased by 10^4 cells/ml on the 16th day after incubation (Fig. 2). Together with the inoculation of *Rhodobacter sp.,* the number of *Lactobacillus sp.* and *S. cerevisiae* not only increased but also kept to the levels of 10^6, 10^7 cells/ml respectively on the 30th day after incubation (Fig. 3). The pH and ester value of mixed culture on the 30th day after incubation are shown in Table 1.

Table 1. pH and Ester Value of Mixed Cultures.

Plots	**S.cerevisiae+Lactobacillus sp.**		**S.cerevisiae+Lactobacillus sp. + $CaCO_3$**		**S.cerevisiae+Lactobacillus sp. + *Rhodobacter sp.***	
Index	Ester value	pH	Ester value	pH	Ester value	pH
Value	5.05	4.0	7.27	7.0	7.57	6.5

As shown in Table 1 the ester value was higher than the others and pH was maintained around neutrality in mixed culture inoculated together with *Rhodobacter sp.*

It seems that it is because the photosynthetic bacteria can change pH of culture with the assimilation of H^+ produced by metabolic process of lactic acid bacteria and yeast and supply the carbon source of culture by its photosynthetic action.

Experiment 2: Change of microflora and pH according to the incubation time in mixed culture of *Lactobacillus sp., S. cerevisiae* and *Rhodobacter* sp.

In mixed culture the changes of microflora and pH according to incubation time are shown in Fig. 4.

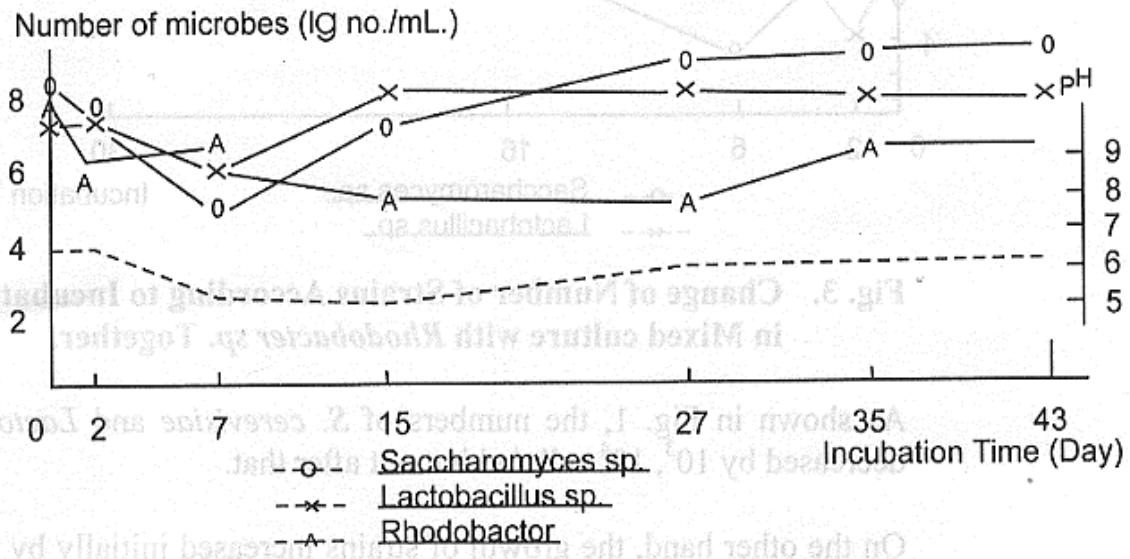

Fig. 4. Change of number of strains and pH in mixed culture of S. *cerevisiae, Lactobacillus sp.* and *Rhodobacter sp.*

As shown in Fig. 4. pH of culture did not decrease below 5 during the incubation time. But it decreased to pH 5 on the 7th day after incubation and maintained it up to 15 days. After that, pH increased with the increase of Rhodobacter sp. It is very interesting that the number of each strain and pH were kept in certain values and the microflora of mixed culture reached the dynamic equilibrium from the 35th day after incubation.

Experiment 3: Effect of pH on dynamic equilibrium of microflora in mixed culture of *Lactobacillus sp., S. cerevisiae* and *Rhodobacter sp.*

Changing pH of mixed culture reached the dynamic equilibrium to pH 3, 5, 7 and 9 artificially, the number of strains and pH according to the incubation time are shown in Figs. 5, 6, 7 and 8.

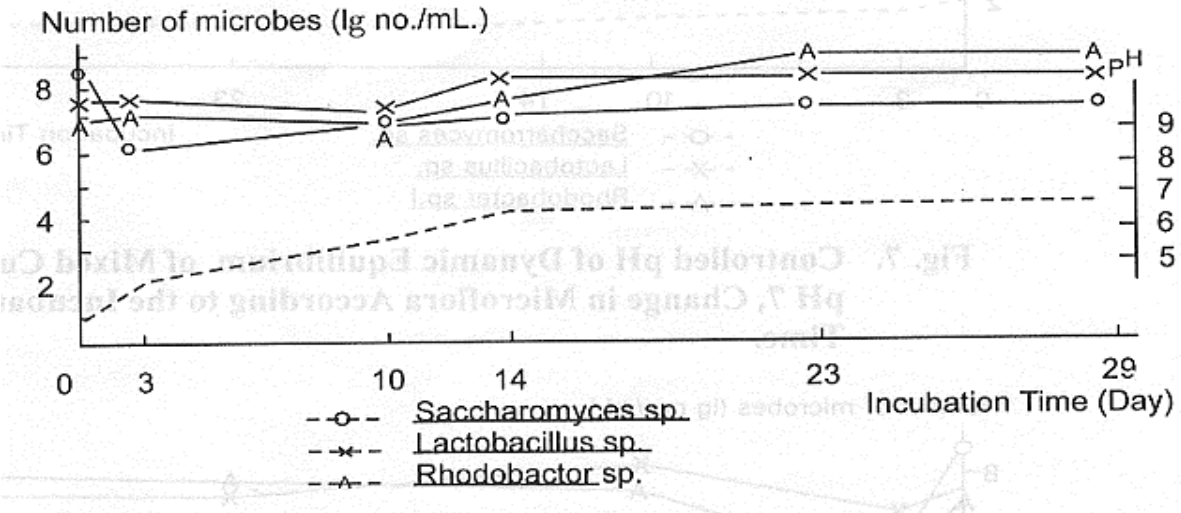

Fig. 5. Change in Microflora According to Incubation Time when pH of the Dynamic Equilibrium of Mixed Culture was Controlled to pH 3.

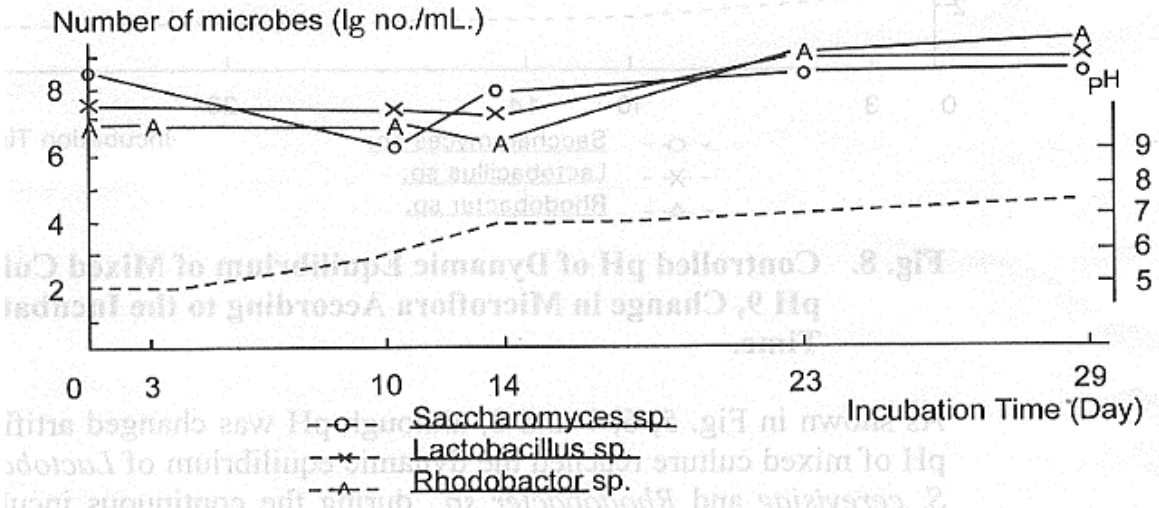

Fig. 6. Controlled pH of Dynamic Equilibrium of Mixed Culture to pH 5, Change in Microflora According to the Incubation Time.

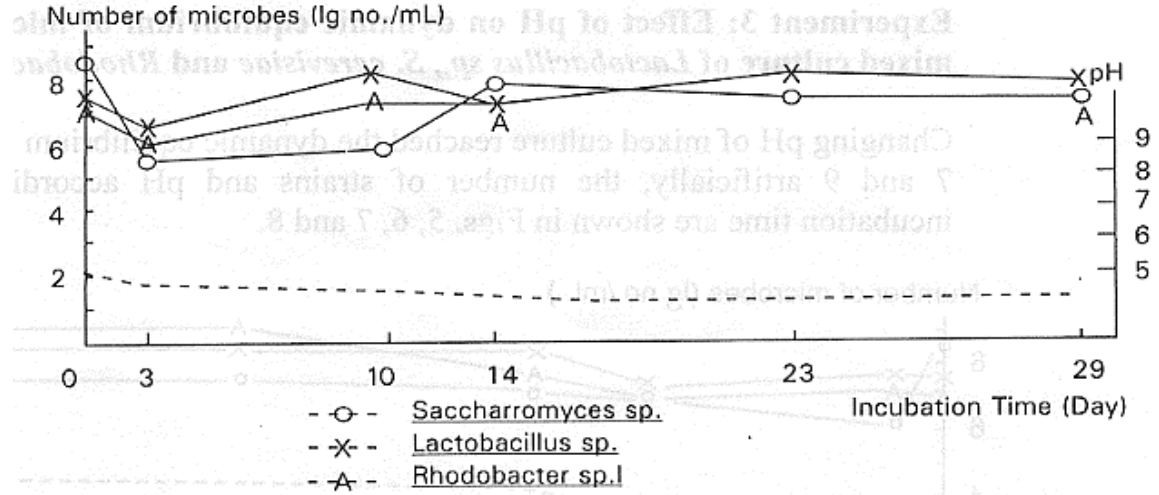

Fig. 7. Controlled pH of Dynamic Equilibrium of Mixed Culture to pH 7, Change in Microflora According to the Incubation Time.

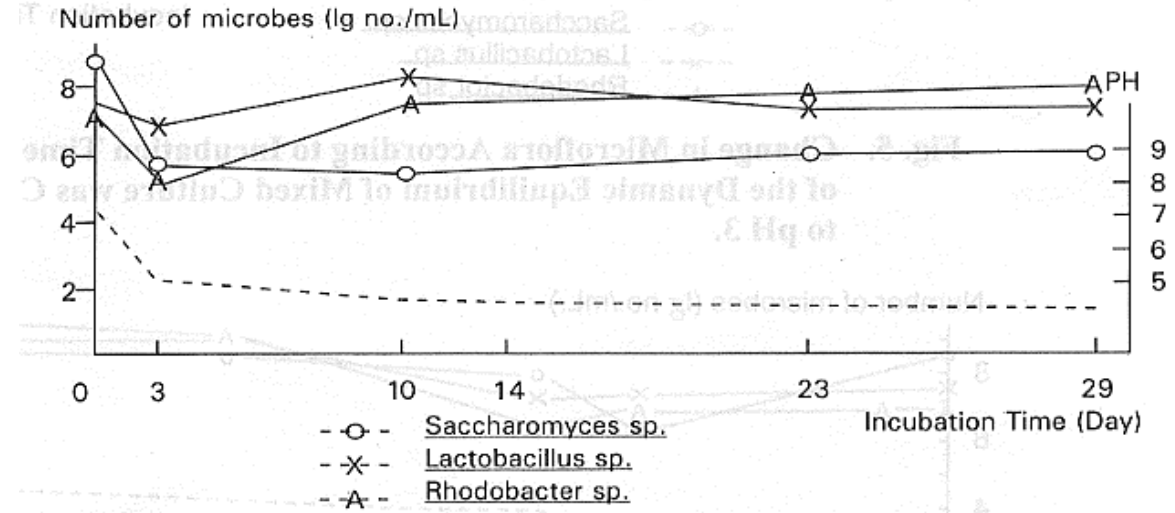

Fig. 8. Controlled pH of Dynamic Equilibrium of Mixed Culture to pH 9, Change in Microflora According to the Incubation Time.

As shown in Fig. 5, 6, 7 and 8, although pH was changed artificially, the pH of mixed culture reached the dynamic equilibrium of *Lactobacillus sp., S. cerevisiae* and *Rhodobacter sp.,* during the continuous incubation. It approaches on the 14th day after incubation and maintained the value thereafter.

The numbers of each strain reached specific values from the 23rd day after changing pH, and dynamic equilibrium appeared again. This means that there is a close co-existance relationship among these three strains and their mixed culture is kept in a state of dynamic equilibrium against the external pH changes.

It is interesting that even changing pH of dynamic equilibrium to any value and after having reached the equilibrium through any process, the last dynamic equilibrium are almost same, that is, the numbers of *Rhodobacter sp*. and *Lactobacillus sp.* are almost same, but the number of *S. cerevisiae* is smaller. Moreover there are some differences between the numbers of strains in the equilibrium reached in the dynamic equilibrium before changing pH. That is, it seems that the number of strains in equilibrium is dependent on the detailed incubating process. But the pH of mixed culture in the last dynamic equilibrium is the value, 6.5 independently on the detailed incubating process.

The content of saccharide in the last dynamic equilibrium is 3.0-3.4 percent not almost different from the initial value, 3.0 percent. And leaving the mixed culture in the last dynamic equilibrium in dark, pH is decreased to pH 4.0 and the content of saccharide, 2.4 percent after one week. This seems that the photosynthetic bacteria plays an important role for the supply of carbon source and the pH stability in mixed culture of these strains.

Experiment 4: Some characters of mixed culture of *Lactobacillus sp., S. cerevisiae* and *Rhodobacter sp.*

In the mixed culture *of Lactobacillus sp., S. cerevisiae* and *Rhodobacter sp.* when reached dynamic equilibrium, the number of strains, pH, electronic conductivity (EC), ester value, SOD activity and content of residual saccharide were measured (Table 2).

Table 2. Some Characters of Mixed Culture *of Lactobacillus sp., S. cerevisiae* and *Rhodobacter sp*. Reached Dynamic Equilibrium.

Index / Sample	Lacto-bacillus sp.	Rhodo-bacter sp.	S. cere visiae	E.C (s/m)	Ester Value	SOD Activity (unit/ml)	pH	Content of residual saccha-ride (%)
Mixed Culture	$1x10^8$	$5x10^7$	$8x10^8$	0.507	7.57	0.50	6.5	3.3
EM-4	$5x10^8$	$1x10^7$	$1x10^7$	0.446	2.24	0.17	3.6	1

Experiment 5: Change of microflora in mixed culture *of Lactobacillus sp., S. cerevisiae, Rhodobacter sp*. and *A. chroococcum* depending on incubation time.

The mixed culture inoculated *Lactobacillus sp., S. cerevisiae, Rhodobacter sp.* and *A. chroococcum* was cultured by stationary incubation in molasses medium under the condition as mentioned in Experiment 2.

The changes of microflora with incubation time were measured (Fig. 9).

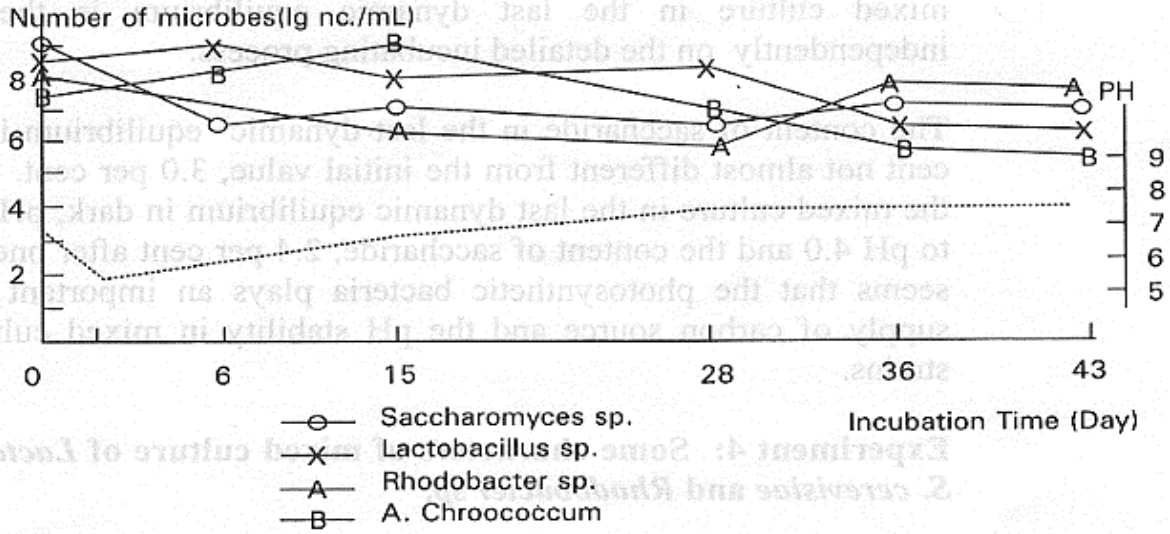

Fig. 9. Change of pH and Number of Strains with Incubation Time in Mixed Culture of *Lactobacillus sp., S. cerevisiae. Rhodobacter sp.* and *A. chroococcum.*

As shown in Fig. 9. aerobic *A. chroococcum* can multiply with anaerobic *Lactobacillus sp., S. cerevisiae* and *Rhodobacter sp.* under the anaerobic condition and their mixed culture is kept in the stable dynamic equilibrium since the 36th day after incubation.

Conclusions

Zymogenic microorganisms – *Lactobacillus sp.* and *S. cerevisiae* are able to coexist with synthetic microorganism *Rhodobacter sp.*, forming the stable dynamic equilibrium of microflora.

The mixed culture of *Lactobacillus sp., S. cerevisiae* and *Rhodobacter sp.* incubated in molasses medium reached the stable dynamic equilibrium, in which the number of each strain is $1x10^8$, $8x10^8$ and $5x10^7$ cells/ml respectively and pH is 6.5, It reaches again the stable dynamic equilibrium as far as lighting, although changing the dynamic equilibrium according to the change of pH in mixed culture artificially.

Under the dynamic equilibrium, pH of mixed culture is 6.5 regardless of the detailed incubating process, but the number of each strain is dependent on the detailed process.

Also, the mixed culture *of Lactobacillus sp., S. cerevisiae, Rhodobacter sp.* and *A chroococcum* in the molasses medium reaches the stable dynamic equilibrium of microflora, in which the number of each strain is $5x10^6$, $2x10^7$, $3x10^7$ and $2x10^6$ cells/ml respectively and the value of pH is 6.5.

References

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