Co-Existence of Photosynthetic Bacteria, *Streptomyces* and Lactic Acid Bacteria in Solutions of Effective Microorganisms

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Abstract

The coexistence of photosynthetic bacteria, lactic acid bacteria and *Streptomyces*, as required for the production of solutions of Effective Microorganisms (EM) is difficult, due to the different environments required by these species. However, the production of EM required that they coexist together, Hence studies were conducted to evaluate possible methods of developing an environment conducive for the co-existence of these three species as required for EM.

The presence of *Lactobacillus plantarum*, increased acidity of the media, which was not conducive for the other two species. In contrast, culturing of photosynthetic bacteria with *Streptomyces* in a fish extract medium proved a possible alternative for the coexistence of these two species. Thereafter, the inclusion of lactic acid bacteria to this medium with molasses and shaking the culture under dark conditions at 30^{o}C proved to be the best condition for the coexistence of these three species. All other conditions such as culturing under light or in the absence of light, without shaking proved futile. Therefore, the study illustrated the possibility of culturing these three important species in preparing the solutions of EM.

Introduction

Solutions of Effective Microorganisms (EM) contain many species of beneficial microbes (Higa, 1996). These include Photosynthetic bacteria, Lactic acid bacteria, Yeast, Ray Fungi and *Actinomyces*. These microorganisms, multiply and develop beneficial effects when applied to agricultural soils. For example, some species of *Lactobacilli* develop antagonistic effects towards *Fusarium oxysporium*, spp. *Lycosperscii*, which develop with in Solanaceous crops. Another type of Ray fungi, *Streptomyces* has antagonistic effects towards *Fusarium* (E.I. Abayed et al 1993). Hence, such diseases can be controlled by the use of mixtures of these organisms such as EM, under practical situations.

In practical situations, the mixing of beneficial microorganisms cause problems, as the environment required for one species can be toxic for another. This is found in EM, where *Lactobacilli* develops acidity in the medium, which is not conducive for the growth and survival of *Streptomyces*. Hence a study was initiated to identify possible methods of maintaining both *Lactobacilli* and *Streptomyces* in one culture solution as required in EM, using species of photosynthetic microbes which are found in this solution of beneficial microorganisms.

Materials and Methods

The program of research conducted consisted of three experiments, using three species of organisms. *Lactobacilli* was represented by *Lactobacillus plantarum* (IFA 3070), *Streptomyces* by *Streptomyces griseus* (IFO 3358) and the third species used was *Rhodobactor sphaeroides* (IFA 12203), which was a photosynthetic bacterium. These species were procured from the Institute of Fermentation, Okasa. (IFO, Japan.)

All experiments were carried out at the Department of Agriculture, University of the Ryukyus, Okinawa, Japan, over the period January -December 1994.

Experiment l. Coexistence of *S. griseus* and *L. plantarum*

The two species were pre-cultured in the following manner.

S. griseus was grown in a glycerine-asparagin broth contained in test tubes of 200 ml capacity for 7 days at 30^{o}C, in total darkness. *L. plantarum* was grown in a GYP broth contained in above mentioned tubes for 24 hours at 30^{o}C in the absence of light.

In the mixed culture, a molasses + fish extract broth was prepared by mixing 3 g of molasses (brix value 80-90), 3 g of fish extract in 100 g of distilled water. The pH of this broth was adjusted to 7

with 0.1 NHCl.

To 100 ml of this MF broth contained in a 250 ml conical flask, 1 ml of each of the cultures of *S griseus* and *L plantarum* were added and the mixture left standing at 30^{o}C in total darkness. This was repeated three times and each flask was treated as a replicate.

The development of colonies of *L. plantarum* and *S. griseus* were counted at 12 hour intervals by sub-culturing samples of the mixture on GYP agar and glycerine-asparagin plates respectively. This procedure was carried out for 72 hours. At each sampling, the pH of the mixture was also determined using a standard pH meter (Horiba Co., Japan).

Experiment 2. Development of *S. griseus* and *R. sphaeroides* in Mixed Cultures

The pre-culturing of *S. griseus* was carried out in the manner described in Experiment 1. *R. sphaeriodes* was grown on a Peptone - Yeast extract broth contained in 200 ml test tubes for 7 days at 30^{o}C under light (5000 lux).

In the mixed culture, 1 ml each of the media containing the two microbial species were added to 100 ml of the MF broth made up as described in Experiment 1, and contained in 250 ml conical flasks. The mixed cultures were kept at 30^{o}C under full light (5000 Iux).

At 12 hour intervals, sub-samples of the mixture were sub-cultured on either glycerine - asparagin agar or on Hoshinno's modified agar for determining the colonies of *S. griseus* and *R. sphaeroides* respectively. This procedure was carried out for a period of 7 days, the process was repeated three times and each was considered a replicate.

Experiment 3. Mixed Culturing of *L. plantarum, S. griseus* and *R. sphaeroides*

R. sphaeroides was pre-cultured on a P Y medium as described in Experiment 2. Thereafter, 1 ml of this culture was added to 100 ml of the MF broth described in Experiment 1, and kept under light (5000 lux) at 30^{o}C for two days.

The second step was to pre-culture *S. griseus* as described in Experiment 1. Thereafter, 1 ml of the culture containing *S. griseus* was added to the MF broth containing *R. sphaeroides*, This mixture was continuously shaken at 120 rpm and 30^{o}C for another two days.

The final step was to pre-culture *L. plantarum* as described in Experiment 1. Thereafter, 1 ml of this culture was added to the MF broth containing the other two species along with 40 ml of 3 percent molasses (brix 80-90) and shaken at 120 rpm at 30^{o}C for another two days.

At the end of this procedure, three samples of the MF broth, each containing 30 ml were poured into individual test tubes of 40 ml volume and tightly sealed. One of the test tubes was kept standing under continuous light (5000 lux). The second was kept standing in total darkness. The third was shaken continuously at 120 rpm in the absence of light. This process of culturing lasted for 48 hours (Details presented in Figure 1).

Sampling was carried out at two day intervals beginning from the initiation of culturing the species (*R. sphaeroides*) on the MF medium (Step 1). Sub samples were obtained at different stages and cultured on an agar medium to determine the colonies of the three species. In addition, the pH of the mixed culture was also determined on each occasion as described in Experiment 1. The total number of samplings was six over a total of 10 days. This facilitated the determination of the three species after mixed culturing on two occasions with samples drawn and the test tubes maintained at different conditions. This process was repeated three times to facilitate replication.

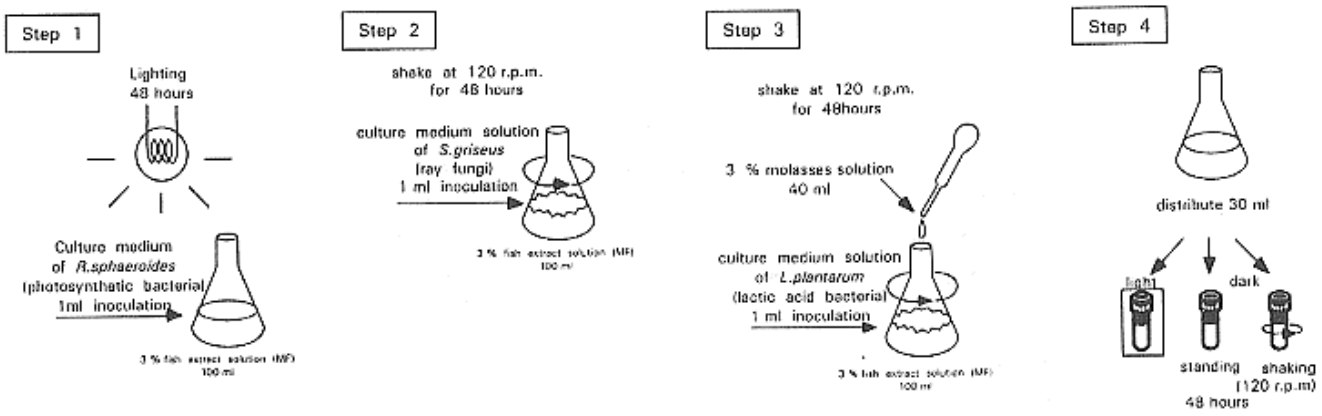

Figure 1. The Procedure of Experiment 3.

Results and Discussion

Experiment 1.

Lactobacillus is a very important microbe in EM. However, the presence of *Lactobacillus* reduced the pH of the medium rapidly (Figure 2) to a value below 2 within 36 hours, although the numbers of colonies of this species increased exponentially.

The increase in acidity of the medium resulted in the total elimination of *S. griseus*, which is a vital component in EM (Figure 2). The lowering of the pH to a value below 2 destroyed all colonies of *S. griseus*. This clearly presented a situation where two important microorganisms in EM, namely *Lactobacillus* and *Streptomyces* could not be cultured together in the same medium.

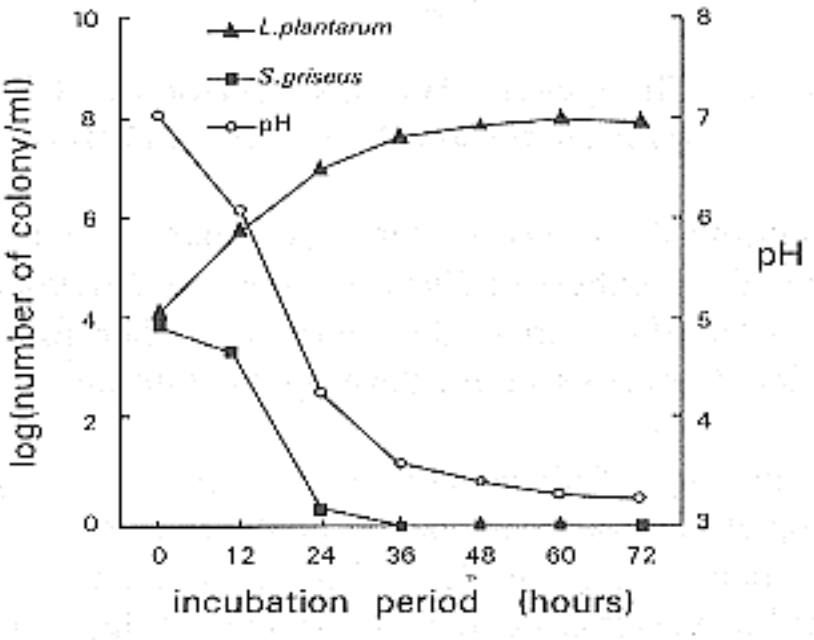

Figure 2. Changes in pH and Coexistance of Colonies of *L. plantarum* and *S. griseus*.

Experiment 2.

The culturing of photosynthetic bacteria and *Streptomyces* together illustrated the opposite phenomenon (Figure 3). The numbers of colonies of *R. sphaeroides* grew with time. The colonies of *S. griseus* also developed with time and the rate of development was more rapid than that of *R. sphaeroides*. In addition, the completion of the rapid phase of growth of *Streptomyces* coincided with the depression observed in the associated photosynthetic bacteria. However, the results showed

that these two species, which are also vital components could be cultured together to develop solutions of EM.

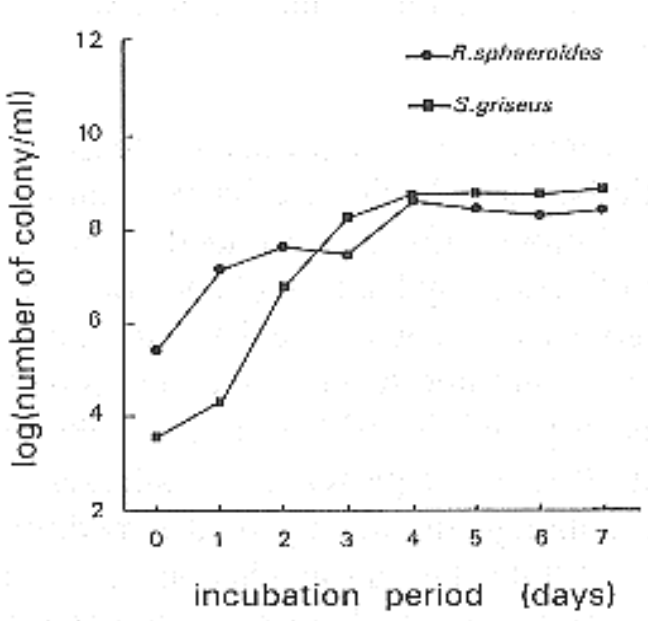

Figure 3. Coexistance of Colonies of *R. sphaeroides* and *S. griseus*.

Experiment 3.

The incubation of the three species together under the adopted conditions produced different results (Figure 4). In all instances, the pH of the media declined to values below 2 within 24 hours of sub-culturing in the small tubes, irrespective of the environment provided. This could be attributed to the development of Lactic acid by the *Lactobacillus* species.

The growth of the three species under light showed the development of *Rhodobactor* and *Streptomyces*. However, the *Lactobacilli* populations, which are important in EM declined (Figure 4a). Hence, this conditions was considered unfavourable for the production of EM.

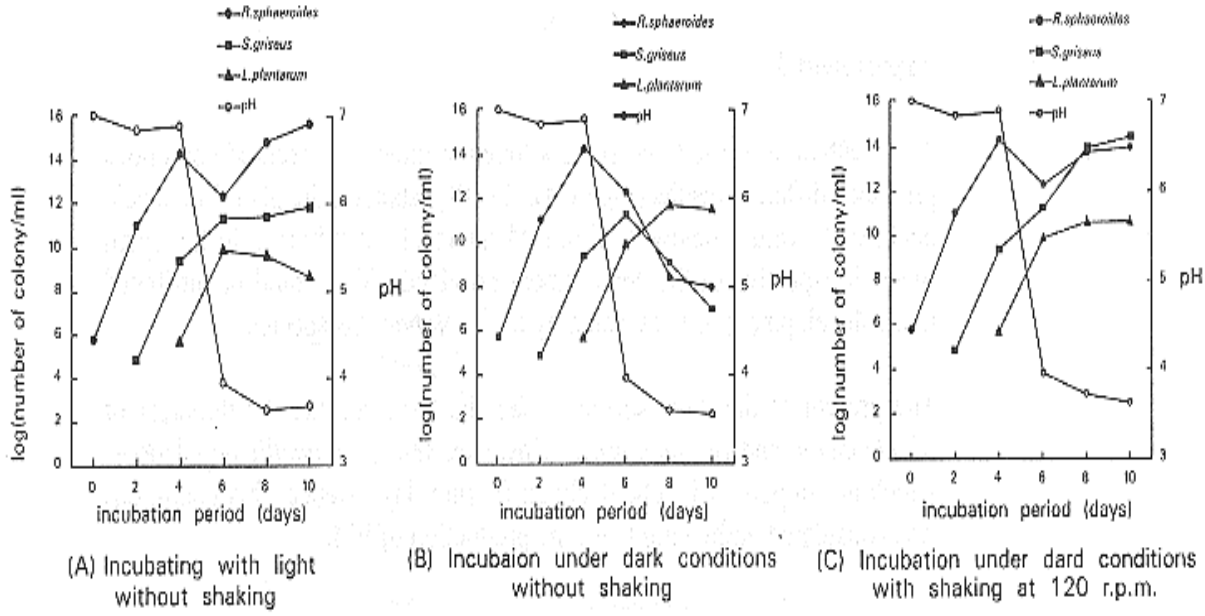

Figure 4. Changes in pH and Coexistance of *L. plantarum*, *R. sphaeroides* and *S. griseus* in Different Environments.

Maintaining the mixed culture under dark conditions with any movement reduced the numbers of *Rhodobactor* and *Streptomyces*, while the colonies of *Lactobacillus* increased (Figure 4b). Therefore, this condition was also considered not suitable for developing solutions of EM.

The culturing of all species in the absence of light, but with continued shaking at 120 rpm produced to be the most conducive environment for the three species (Figure 4c). The populations of *Rhodobactor* colonies showed a marginal depression on the 6th day, which corresponded to the day of mixing. However, this was overcome by the 8th day. In contrast, the other two species grew with time, thus indicating the possibility of co-existence of these three important species, which are vital components of EM solutions.

Conclusions

The studies carried out to evaluate possible methods of culturing photosynthetic bacteria, lactic acid bacteria and *Streptomyces* together for developing solutions of EM provided very interesting results. The development of acidity by lactic acid bacteria suppressed *Streptomyces*. In contrast, *Streptomyces* could coexist with photosynthetic bacteria. In mixed cultures of all three species, the only possibility of the three species coexisting as required in EM was to shake the cultures under dark conditions, at 30^{o}C in the presence of molasses. This provided a suitable environment, although the acidity of the medium increased due to lactic acid bacteria. While the causal phenomenon for this observation needs further study, the results of these experiments highlight the possibility of culturing these three important species, which are vital components in EM.

References

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