Mixed Culturing of *Rhodospirillaceae* in Effective Microorganisms

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Abstract

Studies were initiated to elucidate the mechanisms involved in culturing photosynthetic bacteria found in solutions of Effective Microorganisms (EM), The study isolated a few types of purple nonsuphur bacteria from a culture of EM. An important species identified in the culture on the basis of the standard methods used for photosynthetic bacteria was *R. palustris*. The mechanisms of interaction between this species, *Lactobacillus plantarum* and *Saccharomyces cerevisiae*, which are two important species found in EM were determined under in vitro conditions. *L. plantarum* and *S. cerevisiae* promoted the growth of *R. palustris* even in the presence of molasses, although its growth is generally inhibited by molasses, which is the basis of EM. The survival of *R. palustris* was poor under conditions found in EM. In contrast, this species could survive the low pH in a nutrient rich medium or in one containing extracts of *S. cerevisiae* or *L. plantarum*. The results presented the potential of using lactic acid bacteria and yeast to promote the growth of photosynthetic bacteria in mixed culture as required for producing solutions of EM.

Introduction

The solutions of Effective Microorganisms (EM) contain a mixture of beneficial and food culturing microbes, cultured in one medium, which is generally molasses (Higa, 1996). However, all species found in EM do not grow well in the presence of molasses.

The primary ingredient in EM is photosynthetic bacteria. The efficacy of EM is determined on the basis of the populations of photosynthetic bacteria found in EM solutions. However, the survival and growth of this species is affected adversely by molasses. Hence other species are required to modify the molasses based medium or release growth promoting substances to maintain populations of the photosynthetic bacteria in EM.

EM also contains *Lactobacilli* and yeasts. The effect of these on the development of photosynthetic bacteria has not been clearly identified. Hence experiments were developed to determine the influence of yeast and *Lactobacilli* on the growth of photosynthetic bacteria when cultured alone or in combination. The photosynthetic bacterium used was *Rhodopseudomonas palustris*, while the *Lactobacillus* used was *L. plantarum* and the yeast was *Saccharomyces cerevisiae*.

Materials and Methods

The studies carried out at the Department of Horticulture, University of the Ryukyus, Okinawa, Japan in 1994 and 1995 consisted of three experiments. The species of microorganisms used in these studies were as follows:

Lactobacillus plantarum (IFO 3070), and *Saccharomyces cerevisiae* (IFO 02203) were the standards, and the purple nonsulphur photosynthetic bacteria *Rhodospseudomonas palustris* (RHE 1101) was separated from solutions of Effective Microorganisms.

Experiment 1. The effect of *L. Plantarum* on the growth of *R. palustris*

The pre-culturing of the microbes used in this study, namely *L. plantarum* and *R. palustris* was carried out under the following conditions:

L. plantarum was pre-cultured in 10 ml of GYP media at 30^{o}C for 3 days in the absence of light.

R. palustris was precultured in 10 ml of a standard media (described by Van Niel, 1971, quoted by Kyan, 1996) at 30^{o}C at an illumination of 5000 lux. The containers used were sterilized tubes, which could be utilized for spectrophotometry.

The experiment had four treatments, which were replicated 20 times, in a completely randomized design. The treatments were a mixture of *R. palustris* and *L. plantarum*, a mixture of *R. palustris* and the metabolites of *L. plantarum*, *R. palustris* with non-cultured YM medium and *R. palustris* on

the standard medium. The last treatment served as the control.
In the first mixture, 0.1 ml of the *L. plantarum* culture was added to that of *R. palustris*. In the second, the culture of *L. plantarum* was filtered through a millipore (0.22 um) to remove organisms and 0.1 ml of the filtrate added to the culture of *R. palustris*.
All four treatments were maintained at 30^{o}C and at an illumination of 5000 lux. At 24 hour intervals all cultures were checked by spectrophotometry at an absorbance of 650 nm for the growth of *R. palustris*. The highest absorbance was treated as the maximum growth in terms of numbers of microbes. The time taken for the absorbance values to transform into the logarithmic phase was considered to be the lag phase.

Experiment 2 The effect of *Sacchromyces cervisiae* on the growth of *R. palustris*
This experiment was similar to that described earlier, with the replacement of *L. plantarum* with *S. cervisiae*. The pre-culturing of *S. cerevisiae* was carried out by culturing this microorganism in a YM medium for 3 days at 30^{o}C in the absence of light. Thereafter, inoculation was carried out as described in the previous experiment. The treatments were *R. palustris* alone on a standard medium, *R. palustris* to which either uncultured YM medium or YM medium with cultures and filtered or non-filtered *S cervisiae* was added. The four treatments were maintained under conditions similar to that of Experiment 1. The data recorded was also similar to that described earlier.

Experiment 3. Effect of mixed cultures of *L. plantarum* and *S. cervisiae* on the growth of *R. palustris*.
The pre-culturing of a mixture of *L. plantarum* and *S. cervisiae* was carried out using molasses having a brix value in the range of 80-90. The environmental conditions were 30^{o}C in the absence of light for 3 days. Thereafter, 0.1 ml of this mixed culture was added to that of *R. palustris* with or without filtration, to develop the four treatments, namely *R. palustris* cultures on a standard medium, *R. palustris* cultured on a standard medium to which molasses was added or *R. palustris* cultured on a standard medium to which either filtered or non filtered mixtures of *Lactobacillus* and *Saccharomyces* were added. Thereafter, the mixed cultures were maintained at 30^{o}C at an illumination of 5000 lux and the absorbance measured daily as described in Experiment 1.
All data of the experiment were subjected to appropriate statistical analyses to evaluate the significance of observed differences.

Results and Discussion
Experiment 1.
The absorbance characteristics of the different cultures of microbes are presented in Table 1. The highest absorbance determined at 650 nm was seen in the culture containing *R. palustris* and the filtered culture of *Lactobacillus*. This indicated that the use of filtered *Lactobacillus* culture, which contained the metabolites of the bacterium and not the bodies of the organisms, promoted the growth of the photosynthetic bacteria.

Table 1. The Effect of *L. plantarum* on the Growth of *R. palustris*.

Treatment	Populations of Microbes* (nm)+SE	Growth Rates of Microbes*(nm/day)+SE	Lag Phase (Hrs)*
R. palustris (control)	0.80 + 0.006c	0.60 + 0.006b	48
R. palustris + L. plantarum	0.99 + 0.007b	0.77 + 0.037a	72
R. palustris + L. plantarum	1.08 + 0.017a	0.61 + 0.045b	72
R. palustris + GYP medium	0.83 + 0.012c	0.50 + 0.033d	72

*Values based on absorbace at 650 nm.
Means followed by the same letter within a column are not significantly different (p=0.05) as per Duncans Multiple Range Test.

The lowest absorbance values were observed in the culture containing the photosynthetic bacteria in a GYP or standard medium. The cultures which contained *R. palustris* with Lactobacillus had absorbance values that were greater than when *R. palustris* was grown alone. This suggested that the growth of the photosynthetic bacterium is promoted by the inclusion of *Lactobacillus*.
The rate of growth of the bacterium, as determined by absorbance per day was highest in the culture containing *R palustris* with the unfiltered culture of *Lactobacillus*. This was followed by the culture containing the strained culture of *Lactobacillus*. Again, the lack of *Lactobacillus* reduced the rates of growth of the photosynthetic bacteria significantly, thereby confirming the useful role of *Lactobacillus* in promoting the development of *R. palustris*, which is a vital component of EM solutions.
The lag phase, which denotes the dormant period was lowest when *R. palustris* was grown on a standard medium. All other cultures had similar lag phases (Table 1). This suggested that the growth of *R. palustris* is initiated when grown alone in a standard medium. However, the total growth of the photosynthetic bacteria is promoted by inclusion of *Lactobacillus*, in either a strained or unstrained form. This could be attributed to the development of a suitable acidic medium for the development of photosynthetic bacteria or to the release of growth promoting substances which are developed by this species (Sakakibara, l994). These factors need further study.

Experiment 2.
The absorbance of the different cultures are presented in Table 2. The data was very similar to that of Experiment 1, where the inclusion of *Saccharomyces* promoted the growth of the photosynthetic bacterium. This was clearly visible in total absorbance, rates of growth and even in the reduction of the lag phase. Thus, the requirement of another organism to promote the growth of the photosynthetic bacteria, which is the most important organism in EM, was clearly seen.

Table 2. The Growth of *R. palustris* as Affected by *S. cerevisiae*.

Treatment	Populations of Microbes* (nm)+SE	Growth Rates of Microbes*(nm/day)+SE	Lag Phase (Hrs)*
R. palustris (control)	0.80 + 0.006c	0.60 + 0.006b	48
R. palustris + S. cerevisiae	0.88 + 0.008a	0.73 + 0.009a	48
R. palustris + S. cerevisiae (filtered)	0.89 + 0.012a	0.72 + 0.013a	48
R. palustris + YM medium	0.83 + 0.009b	0.40 + 0.018c	72

*Values based on absorbance at 650 nm.
Means followed by the same letter within a column are not significantly different (p=0.05) as per Duncans Multiple range test.

Experiment 3.
The addition of mixed cultures of both lactic acid bacteria and yeast promoted the growth of the photosynthetic bacterium (Table 3). The total numbers of the photosynthetic bacterium, as denoted by the absorbance was highest with the inclusion of the filtered or non filtered cultures of the other two microbes. The total growth rates were also higher and lag phases reduced significantly. This clearly presented the creation of a suitable environment by *Lactobacillus* and *Saccharomyces* for the development of *R. palustris*, which is the most important organism in EM (Higa, 1996). The causal mechanisms of this phenomenon however needs to be confirmed, although the release of growth promoting substances by *Lactobacillus* and *Saccharomyces* cannot be precluded. In contrast, the presence of molasses alone, which is a basic ingredient of EM is added, the development of the photosynthetic bacterium was the lowest. Hence, the development of good quality EM, which contains both photosynthetic bacteria and molasses requires yeasts and *Lactobacilli*, to maintain populations of the photosynthetic bacterium.

Table 3. The Influence of *L. plantarum* and *S. cerevisiae* on the Growth of *R. palustris*.

Treatment	Populations of Microbes* (nm)+SE	Growth Rates of Microbes*(nm/day)+SE	Lag Phase (Hrs)*
R. palustris (control)	0.80 + 0.006b	0.60 + 0.006c	48
R. palustris + Mixed culture#	0.94 + 0.018a	0.67 + 0.022b	48
R. palustris + Mixed culture# (filtered)	0.99 + 0.021a	0.76 + 0.031a	48
R. palustris + Molasses medium	0.78 + 0.039b	0.48 + 0.016d	120

*Values based on absorbance at 650 nm.

Mixed culture indicates a mixture of *L. plantarum* and *S. cerevisiae*.

Means followed by the same letter within a column are not significantly different (p=0.05) as per Duncans Multiple range test.

References

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