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Short Communication

Optimal Medium Composition Suitable for Enhancement of Biofertilizer's Shelf Life

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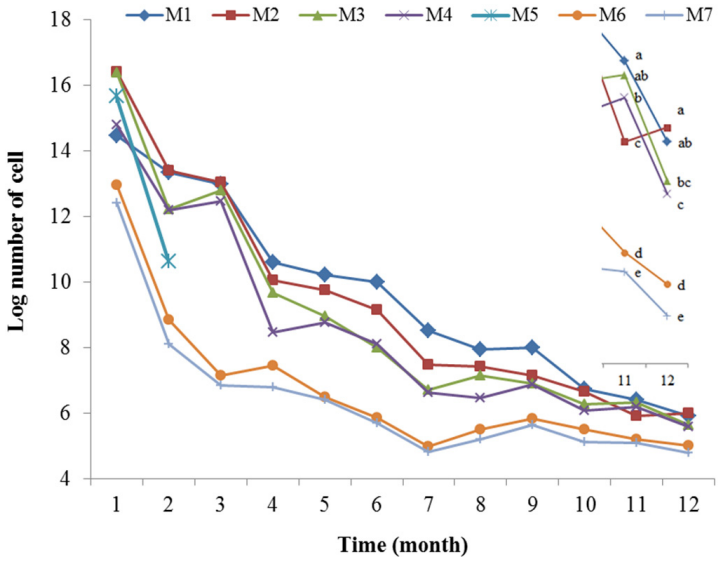
Biofertilizers are increasingly available in the market as one of the alternatives to chemical fertilizers. The supply of a high number of viable microorganisms is important for farmers. *Lysobacter capsici* YS1215 producing chitinases and gelatinases, isolated from soil in Korea, was evaluated for the establishment of an optimal medium condition of its shelf life under an *in vitro* condition. In this study, the population density of a biofertilizer (*L. capsici* YS1215) in media containing crab shell and gelatin powder (M1, M2, M3 and M4) was observed to be higher than that of populations grown in TSB (Tryptic soy broth) media (M5, M6 and M7) during experimental period. In addition, the population density at 11 months was over 10^6 CFU mL^{-1} in M1, M3 and M4, but under 10^6 CFU mL^{-1} in M2, M5, M6 and M7. The best optimal medium for the shelf life was M1 (2.6×10^6 CFU mL^{-1}) containing both chitinous and gelatinous materials at 11 months. Therefore, this study provided results of the appropriate medium composition for the enhancement of the shelf life of *L. capsici* YS1215.

Key words: Shelf life, Biofertilizer, Crab shell powder, Gelatin



The $\log_{10}$ (CFU mL^{-1}) profiles of different compositions of liquid biofertilizer (*Lysobacter capsici* YS1215) over a storage period of 12 months. M1: Crab shell powder medium, M2: Half of M1, M3: One fifth of M1, M4: One tenth of M1, M5: Half of TSB medium, M6: One fifth of TSB medium, M7: One tenth of TSB medium.

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Introduction

In the agricultural industry of South Korea, recent trends have been observed towards converting conventional agricultural practices to organic agriculture and eco-agricultural practices, because of the increasing consumer demand for environmentally friendly agricultural products (Jeong et al., 2005). Chemical fertilizers and fungicides are the most commonly utilized means of increasing crop productivity and pest management efficacy. However, there are problems with the use of these methods, such as the development of pathogen resistance to fungicides and environmental damage. Without the use of synthesized chemicals, a successful transition from conventional to organic/environmentally-friendly farming often requires making improvements to soil quality in order to improve plant growth and the efficacy of disease control methods. In such situations, biofertilizers offer an alternative means of replenishing soil fertility and productivity when the aim is to improve crop nutrition and control crop pests without the use of pesticides (Allison, 1973; Parr and Hornick, 1992; Parr et al., 2002).

Biofertilizer is commonly considered to be liquid or solid fertilizer that contains living microorganisms whose activity is expected to influence the soil ecosystem and produce nutritional substances for crop plants (Parr et al., 2002). Various biofertilizers are available, with differences among them being mainly a matter of the raw materials used, their manner of utilization, and the source of the microorganisms included (Higa and Parr, 1994; Ngampimol and Kunathigan, 2008). Thus, the concentration of the biofertilizer as determined by the rate of bacterial growth will vary according to the components of the culture media used. Therefore, low-cost materials of good quality are needed in order to produce biofertilizers with an excellent shelf life even under adverse storage conditions.

Chitinous materials from marine waste have been used as a source of material for the growth of microorganisms that produce chitinolytic and proteolytic enzymes (Liu et al., 2003; Wang et al., 2006; Lee et al., 2014). Gelatin is derived from collagen obtained from various animal by-products, and is a good source of growth-promoting substances for bacteria (Stewart et al., 1938). The use of these inexpensive substrates may help to both reduce the cost of producing the biomass portion of biofertilizers and enhance the shelf life of those biofertilizers. However, to date, we have not found any research investigating the shelf life of biofertilizer that uses both *Lysobacter capsici*, a chitin- and gelatin-degrading microorganism, and chitinous and gelatinous ingredients. *Lysobacter capsici* YS1215 was previously isolated in Korea. It produced the chitinolytic and gelatinolytic enzymes, and showed antifungal and nematicidal activities (Lee et al., 2013a; Lee et al., 2013b; Lee et al., 2014). Hence, the objective of this study is to investigate the best optimal medium composition for the shelf life of the biofertilizer (*L. capsici* YS1215) using media supplemented with crab shell and gelatin powder, or with commercial TSB media.

Materials and Methods

The experiment was conducted using *L. capsici* YS1215, which is an antagonistic to root knot nematodes and is isolated from the crab shell-rich soil of Suncheon City (Jeolanamdo, South Korea). Its shelf life was studied under seven different medium conditions at room temperature. The different media were either composed of crab shell powder (Purne, Korea), gelatin powder (Geltec, Korea), complex fertilizer (21-17-17; N:P:K; Dongbu Hitek, Korea), sucrose (BackSul, Korea), $FeCl_3 \cdot 6H_2O$, yeast extract, and amino fertilizer (Purne, Korea), or TSB (Tryptic soy broth; LAB). The different medium compositions are listed in Table 1. To determine the shelf life, *L. capsici* YS1215 was grown on TSB media in 250-mL Erlenmeyer flasks at 30°C for 5 d on a rotary shaker (140 rpm). After 5 d, aliquots of the 8 mL cultured biofertilizer were individually moved to small vials (10 mL) and stored at the room temperature. The viability of the biofertilizer was then measured using the Standard Plate Count (SPC) method on TSB agar media. This was carried out monthly for up to 12 months. In each treatment, colony- forming unit (CFU) was counted, using three replicates. The results were interpreted in terms of CFU mL^{-1}.

Table 1. Different medium compositions for an assay of the shelf life of liquid biofertilizer (*Lysobacter capsici* YS1215).

Substrate and Nutrient	M1	M2	M3	M4	M5	M6	M7
Crab shell powder (kg ton^{-1})	0.8	0.4	0.16	0.08			
Gelatin powder (kg ton^{-1})	0.2	0.1	0.04	0.02			
Complex fertilizer (kg ton^{-1})	3.0	1.5	0.6	0.3			
Sucrose (kg ton^{-1})	3.0	1.5	0.6	0.3	-	-	-
Amino fertilizer (L ton^{-1})	4.0	2.0	0.8	0.4			
$FeCl_3 \cdot 6H_2O$ (g ton^{-1})	13.5	13.5	13.5	13.5			
Yeast extract (g ton^{-1})	15.0	7.5	3.0	1.5			
TSB (kg ton^{-1})	-	-	-	-	1.5	0.6	0.3

Results and Discussion

The effect of different medium formulations on the bacterial density and shelf life of the biofertilizer (*L. capsici* YS1215) is presented in Fig. 1 and 2. After one month, the bacterial density was highest (2.5×10^{16} CFU mL^{-1}) in the M2 treatment, while the lowest bacterial density was observed in the treatment using TSB medium (2.6×10^{12} CFU mL^{-1}). This result could be due to differences among the chemical compositions of the different media. Gutierrez-Correa et al. (1999) showed that

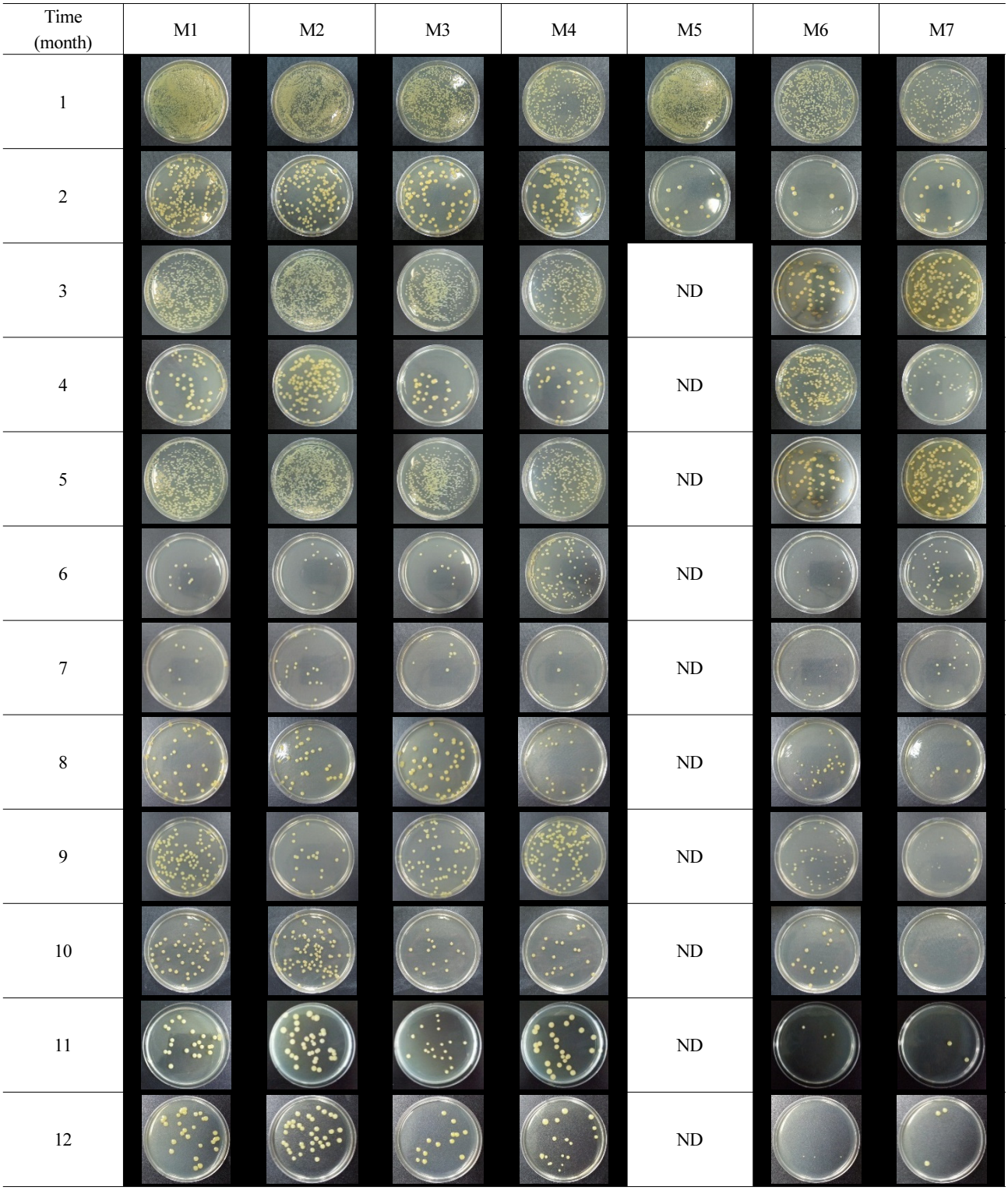

Fig. 1. Microbial profile of liquid biofertilizer (*Lysobacter capsici* YS1215) on TSA plates inoculated with media of different compositions. M1: Crab shell powder medium, M2: Half of M1, M3: One fifth of M1, M4: One tenth of M1, M5: Half of TSB medium, M6: One fifth of TSB medium, M7: One tenth of TSB medium. ND: No detection.

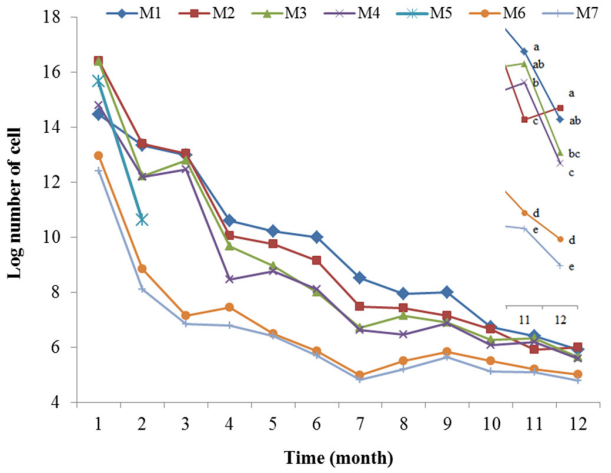

Fig. 2. The $\log_{10}$ (CFU mL^{-1}) profiles of different compositions of liquid biofertilizer (*Lysobacter capsici* YS1215) over a storage period of 12 months. M1: Crab shell powder medium, M2: Half of M1, M3: One fifth of M1, M4: One tenth of M1, M5: Half of TSB medium, M6: One fifth of TSB medium, M7: One tenth of TSB medium.

the nutritional status of the substrate was a determining factor for the successful mixed-culture fermentation of *Trichoderma* and *Aspergillus*. When the chitin content of the growth media for *Saccharomyces cerevisiae* was increased, the cell wall mass was enhanced to about three times that seen in cell cultures cultivated in mineral media (Aguilar-Uscanga and Francois, 2003). Similarly, marine wastes such as shrimp shell powder and crab shell powder were found to be good substrates for both the production of chitinase enzyme and the growth of microorganisms (Wang and Yeh, 2006; Wang et al., 2006; Annamalai et al., 2011). Therefore, our result indicated that the combination of appropriate mixed chemical substances increased the shelf life of *L. capsici* YS1215. In addition, crab shell and gelatin powder contained in the media might affect the improvement of the shelf life as they might be used for long periods of time by *L. capsici* YS1215 as each carbon and protein source, resulting in delaying the depletion of nutrients in the media. According to Mawdsley and Burns (1994), the shelf life of a biofertilizer increased with carbon sources. Crab shell powder generally contains 10 to 20% chitin, which is used as carbon source by chitinase producing microorganisms (Crini et al., 2009).

In different compositions of liquid media, population counts (CFU) slowly began to decline at 2 months, with the rate of decline varying among the different treatments. At 3 months, no live population remained in the M5 treatment. In the M3 and M4 treatments, the bacterial density had increased slightly from that observed at 2 months. The bacterial density declined in all remaining treatments. The population density of the biofertilizer declined continuously but at different rates, from 3 months after the experiment has begun until the end of the 12-month study period. At 11 months, the population density of the biofertilizer was over 10^6 CFU mL^{-1} the crab shell and gelatin powder-amended media (M1, M3, and M4), and under 10^6 CFU mL^{-1} in the M2 and TSB media (M5, M6 and M7) treatments. In South Korea, commercial liquid biofertilizer must have a bacterial density of 1×10^6 CFU mL^{-1} in order to be considered fit for sale. Therefore, when M1, M3, and M4 are used, manufacturers can lengthen the period over which the fertilizer is sold. After 12 months, the maximum population density was recorded in the M2 treatment (9.8×10^5 CFU mL^{-1}), while the lowest was observed in the M7 treatment (6.3×10^4 CFU mL^{-1}). Densities in all treatments were under 10^6 CFU mL^{-1} after 12 months. Thus, the optimal medium with which to maximize the shelf life of the biofertilizer at 11 months was the M1 medium that was composed of the high concentration of nutrients. However, the population density in M1 and M2 at 11 months was statistically not significant. Generally, a decline in population of the biofertilizer during storage period may be attributed to the depletion of nutrients, moisture, and autolysis of cells (Gaind and Gaur, 2003; Mugilan et al., 2011). Therefore, our result might indicate that the shelf life in the crab shell and gelatin powder-amended media (M1, M2, M3 and M4) was more enhanced than in TSB media (M5, M6 and M7) because they might delay the occurrence of environmental and physiological problems encountered to the biofertilizer.

Conclusion

This study provided results of a suitable medium for the enhancement of the shelf life by different medium compositions. The maximal shelf life was observed in the M1 medium at 11 months. The complex of various chemical components including chitinous and gelatinous materials is more effective than commercial TSB media on the shelf life of the biofertilizer organisms (*L. capsici* YS1215).

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