

Original Research Article

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Development of a Liquid Formulation of Lignolytic Fungi for Increased Shelf Life

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ABSTRACT

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High lignin content is one of the major reasons for the recalcitrance of rice stubble, and microbial degradation is an eco-friendly method to manage this large fraction of rice residue, which accumulates in the field. In the present study, three fungal isolates with promising lignolytic potential were selected to develop and optimize a suitable liquid formulation comprising a lignolytic consortium of fungi. For making the liquid formulation, 3% jaggery medium containing 0.05% yeast extract was selected as the base. Various polyvinylpyrrolidone (PVP) concentrations from 0-2.5% were added into the medium and tested for viability of the consortium, and the isolates individually for one year. The study revealed that PVP at 1.5%, 2% and 2.5% concentration maintained the viability of the cells for one year. Individual isolates as well as consortium followed a similar trend. However, in the treatment which did not contain PVP, viable cells were found to reduce sharply after three months. So, the study revealed that a liquid formulation containing 3% jaggery, 0.05% yeast extract and 1.5% PVP is most suitable for better shelf life and viability of the consortium.

Introduction

Rice is categorized as the most important human food crop in the world, accounting for 20% of global ingested calories. Paddy cultivation produces 660 million tons of rice, along with 800 million tons of residues (Borah *et al.*, 2016). Rice stubble constitutes part of the root system and just above the ground portion and is highly rich in cellulose and lignin. It is incorporated into the soil during field preparation and allowed to decompose (Vibha and Sinha, 2007), but it has very high lignin and cellulose content,

particularly the roots. Lignin constitutes a relatively stable component of soil organic matter because of its recalcitrant chemical structure and its resistance to microbial degradation. Lignin is reported to slow down the mineralization of nutrients from crop residues on the time scale of a cropping season. Lignin concentration and the lignin/N ratio are widely used as indicators for the degradability of litter. The disposal of rice stubbles after harvest of rice crop remains a major concern in all rice-growing areas. The states follow rice-wheat cropping patterns like in Punjab and Haryana, rice stubble

management is a burning issue (Kumar and Joshi, 2015). The most common practice adopted by farmers to manage rice stubble and straw is burning, especially in areas where the next crop is grown in a short time. This method offers less time for its incorporation into the soil for decomposition. Burning of straw leads to emission of trace gases like CO₂, CH₄, CO, N₂O and SO₂. Moreover, a large amount of particulates are also released through rice stubble burning. Approximately, India emits 144719 mg of total particulate matter from open-field burning of rice straw annually (Kumar *et al.*, 2015). For addressing this issue, there is a need for developing hyperlignolytic consortium specifically for recalcitrant rice stubble. Competitiveness and viability of mycelial products during preservation also requires food base for survival. (San Martin *et al.*, 1997; Lewis and Papavizas, 1985; Vikineswary *et al.*, 1997). Generally, liquid formulations sustain the shelf-life up to 18-24 months (Sharma *et al.*, 2010). In this study, optimization of a formulation for the consortium of lignolytic fungal isolates was undertaken. These fungal strains have been isolated and identified from degrading rice stubble previously (Sruthy, 2020).

Materials and Methods

Lignolytic fungal isolates, LN-1 LN-14 and LN-19, already identified as *Aspergillus terreus*, *Aspergillus fumigatus* and *Alternaria* spp. (Sruthy, 2020) were used for developing a formulation. All the three isolates were co-inoculated, two/three at a time, on the same PDA plates and checked for any zone of inhibition or incompatibility while growing. Seven days old fungal isolates were then inoculated on media containing 3% jaggery and 0.05% yeast extract. The cultures were blended individually after 10 days of growth and different concentrations of filter sterilized polyvinylpyrrolidone (PVP) was added. 30 ml

of each of the sets were transferred into screw cap tubes and three tubes were withdrawn from the lot at monthly intervals and checked for the viable count, by serial dilution and plating. For mixed cultures, equal quantity of three cultures were mixed together and the same procedure was followed.

Results and Discussion

Development of formulation requires a suitable substrate whose nutrients can sustain the growth of the organism, over long periods of storage and transport to the place of use from manufacturing units. In the present investigation, routinely used substrates such as jaggery and yeast extract were selected, as they are accessible, effective carbon sources and economically feasible for mass scale production (Vijayendra *et al.*, 2004). Besides nutrition, the formulation needs to be amended with a polymer. A wide variety of polymers, such as PVP are used for inoculant production, as they can restrict heat transfer and high water activities and improve shelf life (Mugnier and Jung, 1985). PVP is a well known polymer, having a hydrophobic alkyl backbone along with hydrophilic pendant groups, thereby readily absorbs up to 40% of water by its weight. Its use as a binder is because in solution, it has excellent wetting properties, readily forms films, or useful as a coating (Hiremath *et al.*, 2019).

The viable count of isolate LN-1 at different concentrations of PVP for 1 year is depicted in Table 1& figure 1. The results indicated that the viable count of isolate LN-1 dropped rapidly in formulations which had no added PVP (from 10.66 to 5.57 log₁₀ CFU), 0.5% PVP (from 10.66 to 6.88log₁₀ CFU) and 1% PVP (from 10.66 to 8.20 log₁₀ CFU) from third month onwards. In the formulation not containing PVP, viable spores were not detected from 7th month onwards. In formulations added with 0.5% and 1% PVP,

viable spore was not detected from 9th and 11th month respectively. The formulation with PVP added at 1.5%, 2% and 2.5% were able to maintain the viable cells up to 12 months (\log_{10} CFU/ml was found to be more than or equal to 4 in this treatment at 12th month). So, the formulation added with 1.5% PVP was identified to be the most suitable for better shelf life of the consortium. In a related study, a liquid formulation for *Azospirillum brasilense* developed by Vendan and

Thangaraju (2006) in the presence of PVP, reported 10^8 cells/ml up to 10 months.

Similarly, Table 2 and figure 2 show the viable count of lignolytic isolate LN-14 on different concentrations of PVP. A similar result was obtained in isolate LN-14, in which formulations with 1.5%, 2% and 2.5% PVP have shown better shelf life as compared to other treatments.

Table.1 Viable count of isolate LN-1 at different concentrations of PVP for 1 year

Concentration of PVP	Viable count (\log_{10} of CFU/ml) of isolate LN-1 treatments at one month interval												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0.5%	10.66	9.82	8.77	6.88	6.08	5.72	4.45	3.86	2.08	ND	ND	ND	ND
1%	10.66	9.86	9.20	8.20	8.04	7.49	6.57	6.71	4.49	3.62	1.53	ND	ND
1.5%	10.66	9.93	9.62	8.97	8.92	8.76	8.18	7.76	6.98	6.15	5.61	4.61	4.04
2%	10.66	9.93	9.66	8.99	8.97	8.77	8.28	7.84	6.96	6.32	5.64	4.15	4.15
2.5%	10.66	9.95	9.65	9.04	9.04	8.71	8.26	7.76	6.26	5.45	4.04	4.04	4.04
No PVP	10.66	8.30	6.23	5.57	4.85	3.26	2.58	ND	ND	ND	ND	ND	ND

Table.2 Viable count of isolate LN-14 at different concentrations of PVP for 1 year

Concentration of PVP	Viable count (\log_{10} of CFU/ml) of isolate LN-14 in formulations at one month interval												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0.5%	10.71	9.87	8.86	6.97	6.38	5.83	4.91	3.94	2.68	ND	ND	ND	ND
1%	10.71	10.00	9.43	8.43	8.23	7.67	6.87	6.89	4.76	3.83	1.85	ND	ND
1.5%	10.71	9.98	9.76	9.15	9.08	8.79	8.62	7.95	7.46	6.69	5.95	4.77	4.28
2%	10.71	9.99	9.69	9.20	9.15	8.81	8.64	7.96	7.51	6.72	5.86	4.72	4.08
2.5%	10.71	9.99	9.68	9.28	9.11	8.83	8.67	7.89	6.57	5.57	4.18	3.72	3.72
No PVP	10.71	9.36	6.38	5.64	4.95	3.30	2.69	1.51	ND	ND	ND	ND	ND

Table.3 Viable count of isolate LN-19 at different concentrations of PVP for 1 year

Concentration of PVP	Viable count (\log_{10} of CFU/ml) of isolate LN-19 in formulations at one month interval												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0.5%	10.63	10.18	8.51	6.77	6.23	5.56	4.11	3.66	2.20	ND	ND	ND	ND
1%	10.63	10.26	9.26	8.26	8.11	7.52	6.28	6.68	4.45	3.20	1.43	ND	ND
1.5%	10.63	10.23	9.57	9.08	9.04	8.61	8.32	8.03	7.57	6.23	5.43	4.67	4.23
2%	10.63	10.28	9.61	9.04	8.99	8.59	8.46	8.04	7.59	6.36	5.49	4.79	4.32
2.5%	10.63	10.26	9.65	8.97	8.93	8.59	8.46	7.28	6.46	5.28	3.85	3.04	3.04
No PVP	10.63	9.49	6.32	5.04	4.08	3.15	2.53	ND	ND	ND	ND	ND	ND

Table.4 Viable count of consortium at different concentrations of PVP for 1 year

Concentration of PVP	Viable count (\log_{10} of CFU/ml) of consortium in formulations at one month interval												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0.5%	10.69	9.98	8.83	6.88	6.28	5.69	4.67	3.84	2.73	ND	ND	ND	ND
1%	10.69	10.11	9.34	8.34	8.08	7.62	6.75	6.57	4.51	3.64	1.61	ND	ND
1.5%	10.69	10.18	9.59	9.04	9.04	8.71	8.57	7.96	7.04	6.32	5.49	4.71	4.32
2%	10.69	10.20	9.65	9.11	9.08	8.72	8.62	7.94	7.26	6.58	5.68	4.68	4.26
2.5%	10.69	10.18	9.59	9.04	8.97	8.69	8.59	7.76	6.40	5.54	3.96	3.51	3.51
No PVP	10.69	9.20	6.56	5.52	4.65	3.19	2.23	1.61	ND	ND	ND	ND	ND

Fig.1 Viable count of isolate LN-1 at different concentrations of PVP for a period of one year

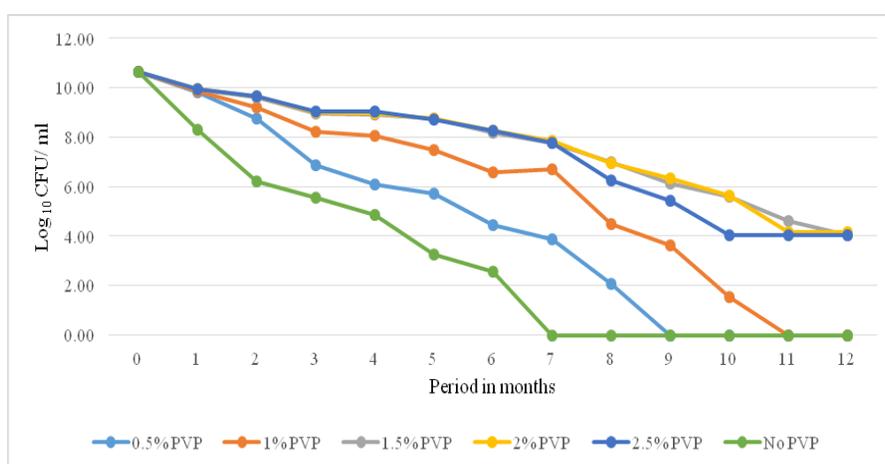


Fig.2 Viable count of isolate LN-14 at different concentrations of PVP for a period of one year

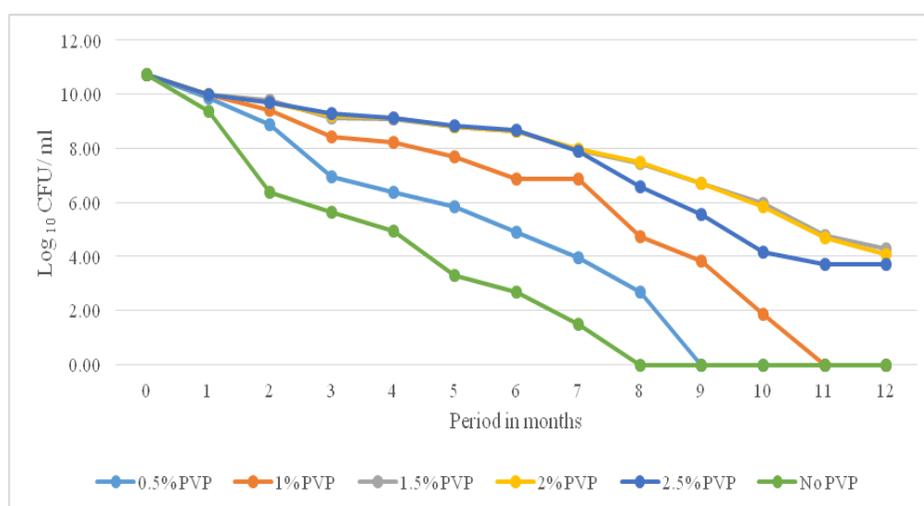


Fig.3 Viable count of isolate LN-19 at different concentrations of PVP for 1 year

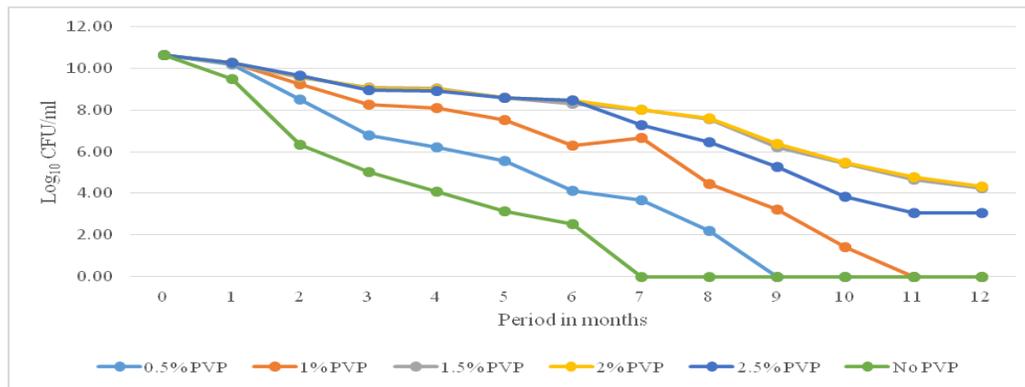
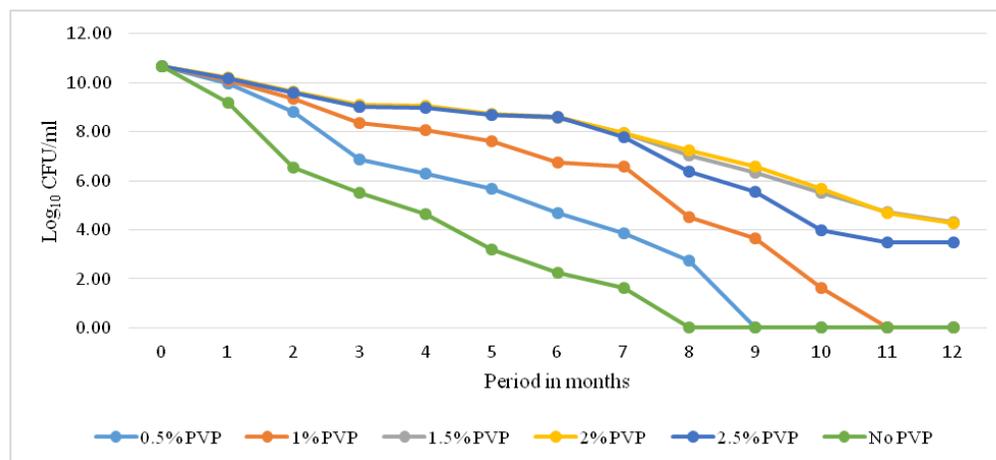


Fig.4 Viable count of lignolytic consortium at different concentrations of PVP for 1 year



In Control, viable cells were not detected from 8th month onwards, whereas, in formulations with 0.5% and 1% PVP from 9th and 11th month onwards, no cell was detected. All the treatments except formulations containing PVP 1.5% and above, the viability count was observed to reduce sharply. As shown in Table 3 & figure 3, isolate LN-19 also followed the same trend. The viable count of isolate LN-19 dropped rapidly in formulations added no PVP (10.71 to 5.64 log₁₀ CFU/ml), 0.5% PVP (10.71 to 6.97 log₁₀ CFU/ml) and 1% PVP (10.71 to 8.83 log₁₀ CFU/ml) from third month onwards. From 7th month onwards, no viable spore was detected in the formulation without any PVP. In

formulations added with 0.5% and 1% PVP, viable spore was not detected from 9th and 11th month respectively. The formulation containing PVP at 1.5%, 2% and 2.5% were recorded to maintain viable cells for 12 months.

As displayed in Table 4 and figure 4, the consortium of three lignolytic fungal isolates was found to be viable in formulations containing 1.5%, 2% and 2.5% PVP till 12th month. Control was not detected with viable cells from 8th month onwards, whereas, in formulations with 0.5% and 1% PVP from 9th and 11th month onwards no cell was detected. In all the treatments, except formulations

containing PVP 1.5% and above, the viable count was observed to reduce sharply. So, in this experiment, PVP at 1.5% was observed to be most optimal for maintaining the viability of lignolytic consortium upto one year. Gopal and Baby (2016) reported that, PVP at 2.5 % concentration was the most suitable amendment for improving the shelf life of phosphate solubilizing bacteria. Polyvinylpyrrolidone (PVP) contributes to metabolism of organism by maintaining water around the cells (Singleton *et al.*, 2002, Deaker *et al.*, 2004). PVP in the presence of gum arabic is known to protect cells against toxic principles (Mugnier and Jung, 1985). From the present study, it can be concluded that a formulation containing 3% jaggery and 0.05% yeast extract amended with 1.5% PVP is the most suitable and effective to maintain the shelf life of all the three lignolytic fungi for 12 months, and maintain viable cells up to a period of 12 months.

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