Biological Forum – An International Journal **15(1): 588-591(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Survival and Bio-preservation Studies of Lactic Acid Bacteria in Different Bedding Material

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(Received: 09 December 2022; Revised: 11 January 2023; Accepted: 16 January 2023; Published: 23 January 2023)
(Published by Research Trend)

ABSTRACT: Lactic acid (LA) bacterial viability on a bedding material is dependent on temperature, humidity, the composition of the material, light and other physical factors. The present study deals with the viability of a potent lactic acid bacterial consortium in different bedding materials and bio-preservation ability of consortium on onion. The sawdust inoculated LA bacterial consortium had the highest population (128.66 × 10^3 CFU /g) on 45th day and pH of coir pith treated with consortium was found to be the lowest (2.58) on 45th day. The onion bulbs were artificially inoculated with spoilage organisms and LA bacterial consortium using the pinprick method. The onion bulbs treated with consortium had the least weight loss (8.28 %), no spoilage, the least decrease in pH (5.39) and TSS (4.0) at the end of 28th day of storage. Hence, sawdust as the bedding material inoculated with LA bacterial consortium can be used as an effective bio-protectant.

Keywords: Lactic acid bacteria, Consortium, Bedding material, Pinprick method, pH, TSS.

INTRODUCTION

Lactic acid bacteria are Gram positive, catalase variable, microaerophilic to anaerobic bacteria. LA bacteria produce lactic acid as the main organic acid. Their shape can either be a rod or spherical (bacilli or cocci) and can tolerate low pH ranges. They possess a vital role in food fermentations, as probiotic products and in vaccine administration. They also play a crucial role in human health as their antimicrobial properties make them effective to inhibit food borne pathogens such as bacteria, yeasts and molds. The antimicrobial activities of LA bacteria are mainly based on the production of metabolites such as lactic acid, organic acids, hydrogenperoxide and bacteriocins. They reduce the environment's pH, thereby inhibiting pathogens that cannot tolerate acidic environments from growing (Coral *et al*., 2022).

Biopreservation is the use of natural substances derived from microorganisms, plants or animals with the aim of increasing shelf life of the product. Bio-preservative should have antimicrobial activity against abroad range of spoilage organisms and should not have a negative influence on consumers health. LA bacteria are 'Generally Recognized As Safe' according to the U.S. Food and Drug Administration and Qualified Presumption of Safety" by the European Food Safety Authority (Zheng *et al*., 2020).

The bacterial survival or multiplication depends on the type of bedding material. The factors affecting the viability of inoculated LA bacteria are ambient temperature, pH, humidity and bedding management. The bedding material itself may already have inherent physical, biochemical or nutritional properties that could sustain or promote bacterial growth. It provides microniche for the colonization of LA bacteria and protects from environmental factors (Eckes *et al*., 2001; Bey and Reneau 2002). The shavings, clean sand and chopped straw are traditionally used as bedding materials. Hence, an attempt was to identify suitable bedding material for supporting the viability and maintaining the antimicrobial properties of lactic acid bacteria, when used as bio-preservative agent.

MATERIAL AND METHODS

Viability of LA bacteria and pH of bedding material

The LA bacterial isolates were screened for antimicrobial activity against potent spoilage organisms (*Bacillus spizizenii* UASBMIC_004 and *Aspergillus welwitschiae* UASBMIC_F2). The LA bacterial isolates were identified as *Levilactobacillus brevis* UASBMIC_001 and *Levilactobacillus brevis* UASBMIC_002 (Unpublished data). The bedding materials (coirpith, bagasse and sawdust) were chopped and sterilized and allowed to cool down. The carriers were mixed with 48 h old culture at 10 % and allowed to shade dry. The viability of bacteria was studied in different bedding materials for 45 days of storage at room temperature (28^{o}C).

Pinprick method: The bulbs were pricked with sterile cork borer and four holes with a width of 3 mm diameter were drilled (Prajapathi and Patil 2016). These holes were inoculated with 30 µL of culture and incubated at 28^{o}C for 28 days with sawdust as the bedding material. The experimental design was CRD

with destructive sampling units at 14 and 28 days intervals having nine treatments with 16 bulbs, each bulb representing a replicate (Table 1).

Per cent physiological loss of weight and per cent spoilage. A laboratory level weighing balance (Shimadzu India Pvt. Ltd) was used to determine the total weight of the packet with onion bulbs on the 14th and 28th day of storage at 28°C. The results were expressed in terms of % weight loss and % spoilage.

$$\text{Spoilage per cent} = \frac{\text{Number of onion bulbs spoiled}}{\text{Total number of onion bulbs}} \times 100$$

pH and total soluble solids (°Brix). The pH and TSS were measured on the 14th and 28th day of storage at 28 °Cusing pH meter and refractometer, respectively (Prajapathi and Patil 2016).

Table 1: *In situ* pinprick treatments of onion bulbs in bedding materials using lactic acid bacteria and spoilage microorganisms.

Treatments	Treatment details	Treatment code
T_1	Control- water	C
T_2	*Lactobacillus acidophilus* NCIM 2903	LA
T_3	*Levilactobacillus brevis* UASBMIC_001 + *Levilactobacillus brevis* UASBMIC_002	Consortium
T_4	*Bacillus spizizenii* UASBMIC_004	BS
T_5	*Aspergillus welwitschiae*UASBMIC_F2	AW
T_6	*Lactobacillus acidophilus* NCIM 2903 + *Bacillus spizizenii* UASBMIC_004	LA+BS
T_7	*Levilactobacillus brevis* UASBMIC_001 + *Levilactobacillus brevis* UASBMIC_002 + *Bacillus spizizenii* UASBMIC_004	Consortium+ BS
T_8	*Lactobacillus acidophilus* NCIM 2903 + *Aspergillus welwitschiae* UASBMIC_F2	LA+AW
T_9	*Levilactobacillus brevis* UASBMIC_001 + *Levilactobacillus brevis* UASBMIC_002 + *Aspergillus welwitschiae*UASBMIC_F2	Consortium+ AW

Statistical analysis. The Opstat 2.0 was used for the data analysis, significant different groups were calculated with the One-way Analysis of variance (ANOVA). The Duncan Multiple Range Test (DMRT) was used for multiple comparisons and the level of significance was set at $p \leq 0.05$ (Panse and Sukhatme 1967).

RESULT AND DISCUSSION

This study was mainly focused to identify suitable bedding material capable of supporting the viability as well as the antimicrobial properties of LA bacteria. The identification of convenient carrier bedding material helps us to support LA bacteria as bio-protective agents. The bedding material will provide micropores for the colonization of LA bacteria and protect from environmental stress. The results of the current study are presented and discussed here.

pH of bedding material inoculated with LA bacteria. The pH of coir pith treated with consortia was found to be the lowest (2.58) on 45th day followed by coir pith treated with *Lactobacillus acidophilus* NCIM 2903 (2.70). The pH of bagasse treated with consortium observed the least decrease in pH of 3.66 followed by bagasse treated with *Lactobacillus acidophilus* NCIM 2903 (2.76) (Table 2). The organic acid produced by LA bacteria or by utilizing the carbohydrate source present in bedding material should be the cause for decreased pH. Similar reports of a decrease in pH were observed with kitchen waste (Ma *et al*., 2010); compost material (Quyen *et al*., 2015); lignocellulosic sillage of *Peninisetumsinese* (Junfeng *et al*., 2018).

Table 2: pH of the bedding materials inoculated with LA bacteria

Sr. No.	Treatments	pH at interval (days)			
		0	15	30	45
1.	*Lactobacillus acidophilus* NCIM 2903 + Coirpith	6.65^{a}	3.4^{ab}	2.96^{b}	2.7^{b}
2.	*Lactobacillus acidophilus* NCIM 2903+ Bagasse	5.69^{b}	3.18^{b}	2.76^{b}	2.76^{b}
3.	*Lactobacillus acidophilus* NCIM 2903+ Sawdust	6.42^{a}	3.46^{ab}	2.83^{b}	2.73^{b}
4.	Consortium + Coirpith	6.65^{a}	3.33^{ab}	2.81^{b}	2.58^{b}
5.	Consortium + Baggase	5.69^{b}	3.66^{a}	3.66^{a}	3.66^{a}
6.	Consortium + Sawdust	6.42^{a}	3.36^{ab}	3.13^{b}	2.87^{b}

Note: Numerical values are expressed as mean of three replicates. Treatments with different superscripts in the same column represent a significant difference as determined by DMRT ($p \leq 0.05$).

Viability of LA bacteria in bedding material. The highest population (128.66×10^3 CFU) was observed in sawdust treated with consortium on the 45th day, even though the initial population of consortia was low (26.66×10^9 CFU). The lowest population (12.66×10^3 CFU) was observed in coir pith treated with *Lactobacillus acidophilus* NCIM 2903 on 45th day (Table 3). The carrier material was non-toxic, inert, provided micropores for adhesion, protection from environmental stress; these could have led to the survivability of LA bacterial isolates.

Table 3: Viability of LA bacteria in bedding materials.

Sr. No.	Treatments	Population in CFU /g			
		Initial (10^9)	15th day (10^8)	30th day (10^6)	45th day (10^3)
1.	*Lactobacillus acidophilus* NCIM 2903 + Coirpith	116.00 ^{a} (10.81)	3.00 ^{b} (2.00)	0.00 ^{d} (1.00)	12.66 ^{c} (3.69)
2.	*Lactobacillus acidophilus* NCIM 2903+ Bagasse	116.00 ^{a} (10.81)	0.00 ^{d} (1.00)	0.00 ^{d} (1.00)	0.00 ^{d} (1.00)
3.	*Lactobacillus acidophilus* NCIM 2903+ Sawdust	116.00 ^{a} (10.81)	5.30 ^{a} (2.51)	1.66 ^{b} (1.63)	16.66 ^{b} (4.20)
4.	Consortium + Coirpith	26.66 ^{b} (5.26)	0.66 ^{c} (1.29)	0.33 ^{c} (1.15)	19.66 ^{b} (4.54)
5.	Consortium + Baggase	26.66 ^{b} (5.26)	0.00 ^{d} (1.00)	0.00 ^{d} (1.00)	0.00 ^{d} (1.00)
6.	Consortium + Sawdust	26.66 ^{b} (5.26)	3.00 ^{b} (2.00)	2.66 ^{a} (1.91)	128.66 ^{a} (11.38)

Note: Numerical values are $\sqrt{x+1}$ transformed values and expressed as mean of three replicates. Treatments with different superscripts in the same column represent a significant difference as determined by DMRT ($p \leq 0.05$).

Per cent physiological loss of weight (PLW), per cent spoilage and chemical properties of onion bulb under *in situ* in bedding material. The onion bulbs treated with *Aspergillus welwitschiae* UASBMIC_F2 noted maximum loss in weight (11.48 %) followed by *Bacillus spizizenii* UASBMIC_004 (10.98 %) and minimum loss in weight was observed in control (8.35 %) followed by consortium (8.38 %) after 28 days. The onion bulbs treated with *Bacillus spizizenii* UASBMIC_004 and *Aspergillus welwitschiae* UASBMIC_F2 both showed the highest spoilage of 50 and 80 %, respectively and no spoilage was observed in control and consortiumon the 28th day of storage. The pH of the onion (5.73) was decreased in all treatments during the storage period of 28 days. The onion bulbs treated with *Bacillus spizizenii*UASBMIC_004 recorded the highest decrease in pH (4.33) followed by *Aspergillus welwitschiae* UASBMIC_F2 (4.49) and the lowest decrease in pH (5.39) was observed in consortium on the 28th day of storage. The TSS of onion bulbs (5) decreased in all the treatments during the storage period of 28 days. The onion bulbs that were treated with *Aspergillus welwitschiae* UASBMIC_F2 observed the highest decrease in TSS of 2.0 followed by *Bacillus spizizenii* UASBMIC_004 (2.5) on the 28th day of storage. The least decrease in TSS was observed in control, LA and consortium treatments with a TSS of 4.0 on the 28th day of storage (Table 4). The pH and TSS might have decreased due to respiration of vegetables, microbial activity and transpiration. Parallel results of decreasing pH and TSS was observed in minimally processed onion bulbs (Carolina *et al*., 2022). The per cent spoilage and physiological loss of weight (PLW) of onion may have increased due to relative humidity in environment and microbial spoilage. Similar reports of increasing PLW and rotting were observed in non-radiated onion bulbs during the storage period of 144 days (Pallavi *et al*., 2020).

Table 4: Per cent physiological loss of weight (PLW), per cent spoilage and chemical properties of onion bulb under stored *in situ* in bedding material.

Treatments	14th day				28th day			
	Spoilage (%)	Physiological loss of weight (%)	pH	TSS (° Brix)	Spoilage (%)	Physiological loss of weight (%)	pH	TSS (° Brix)
T_1	0 (1.00^d)	5.20^e	5.30^a	5.00^a	0 (1.00^f)	8.35^e	5.28^a	4.00^a
T_2	0 (1.00^d)	6.12^d	5.36^a	4.50^b	20 (4.57^d)	9.15^d	5.35^a	4.00^a
T_3	0 (1.00^d)	5.35^e	5.45^a	5.00^a	0 (1.00^f)	8.38^e	5.39^a	4.00^a
T_4	20 (4.58^b)	7.85^b	5.18^a	3.00^e	50 (7.13^b)	10.98^b	4.33^b	2.50^d
T_5	30 (5.56^a)	8.15^a	5.21^a	2.50^f	80 (8.99^a)	11.48^a	4.49^b	2.00^e
T_6	10 (3.31^c)	6.97^c	5.30^a	3.50^d	20 (4.58^d)	9.12^d	5.12^a	3.00^c
T_7	0 (1.00^d)	6.95^c	5.32^a	4.00^c	10 (3.31^e)	8.78^{de}	5.19^a	3.50^b
T_8	10 (3.31^c)	7.12^c	5.23^a	3.50^d	30 (5.56^c)	9.89^c	5.08^a	3.00^c
T_9	0 (1.00^d)	7.06^c	5.29^a	4.00^c	10 (3.31^e)	9.65^c	5.12^a	3.50^b

Note: Numerical values are angular transformed and treatment with different superscripts in the same column represent a significant difference as determined by DMRT ($p \leq 0.05$). T1- Control- water, T2- LA, T3- Consoritum, T4 –BS, T5- AW, T6-LA +BS, T7- Consortium+BS, T8-LA+AW, T9- Consoritum+BS. Consoritum- *Levilactobacillus brevis* UASBMIC_001 + *Levilactobacillus brevis* UASBMIC_002, LA- *Lactobacillus acidophilus* NCIM 2903, BS- *Bacillus spizizenii*UASBMIC_004, AW- *Aspergillus welwitschiae*UASBMIC_F2.

CONCLUSION AND FUTURE SCOPE

In the present study, the lactic acid bacterial consortium survivability was best in sawdust bedding material and was able to maintain a sufficient population up to 45th day. The pH of the sawdust material decreased moderately in comparison with other bedding material. LA bacterial consortium increased the shelf life of onion for 28 days after the artificial inoculation of spoilage organisms. Further research can be taken up on the probiotic properties of LA bacterial consortium, molecular identification of genes involved in antimicrobial compound production.

Acknowledgement. I thank co-authors for their ideas, valuable suggestions and contribution during this work. I thank Department of Agricultural Microbiology, UAS, GKVK, Bangalore for providing support during the study.
Conflict of Interest. None.

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How to cite this article: Bharath Kumar N., Suvarna V.C., Girish H.C. and Rashmi S. (2023). Survival and Bio-preservation Studies of Lactic Acid Bacteria in different Bedding Material. *Biological Forum – An International Journal, 15*(1): 588-591.