The Use of Sweet potato (*Ipomoea batatas*) to Develop a medium for cultivation of *Lactobacillus reuteri*

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Abstract

The sweet potato is rich with many nutrients that could support the growth of lactobacilli bacteria. The objective of this study was to develop a medium for Lactobacilli using sweet potato as basic component. Fresh sweet potato was baked at 400˚C for 1 h, cooled, peeled, blended with 50% (w/v) distilled water, centrifuged and supernatant was collected to form sweet potato medium (SPM). A group of components in table 2 was added to the supernatant to form positive SPM (PSPM) and negative SPM (NSPM) then autoclaved at 121˚C for 15 min. Three strains of *Lactobacillu reuteri* were used in this study and the growth in SPMs was compared to that in MRS. Batches of MRS, PSPM, NSPM, and SPM at 10 ml were inoculated with individual *L. reuteri* strain at 2.7 log CFU/ml. Samples were incubated for 12 h at 37˚C and the bacterial growth was monitored by measuring the optical density at 3 h interval. At the end of incubation, final bacterial populations and final pH values were determined. Our results showed that *L. reuteri* populations reached 10.18 log CFU/ml in PSPM, 10.06 log CFU/ml in MRS, and 9.08 log CFU/ml in NSPM. The pH values were 4.17 in PSPM, 4.35 in MRS, and 4.53 in negative SPM. Thus results indicated that similar growth of *L. reuteri* was observed in both positive SPM and MRS media. In conclusion, sweet potato can support the growth of *L. reuteri* and could be used as a low cost medium for industrial applications of LAB.

Introduction

Lactic acid bacteria (LAB), including *Lactobacillus*, are among the most important groups of bacteria due to the ability to produce organic acids, antimicrobial compounds, and functional compounds. This group of bacteria is nutritionally fastidious and required several nutrients and growth factors with many variations in nutritional requirements can occur between species and even strains of the same species. The most common standard laboratory medium is deMan-Rogosa-Sharpe (MRS) which was invented 1960. The use of existing media including MRS is limited to academic purposes owing their high cost, alternative media with lower cost and higher density cell is highly demanded. Sweet potato (*Ipomoea batatas* (L.) Lam.) the starchy root, is rich with many nutrients such as vitamins (A and C), menials (iron and potassium), and fiber in addition to several useful components that could support the growth of LAB. In addition, sweet potato is abundant agricultural product in the state of North Carolina with growing in production. The State produces 40% of total production in the United States with an average of 36,000 acres per year. Therefore, the objective of this study is to develop a medium for *Lactobacillus reuteri* using sweet potato as basic component.

Materials and Methods

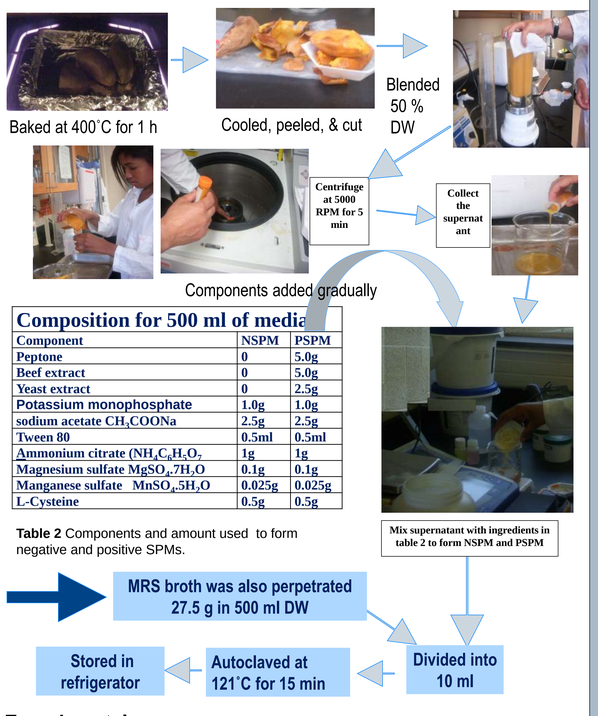
Bacterial culture activation and preparation

1.Strains of *Lactobacillus reuteri* (Table 1), stored at -80˚C freezer.
2.Activated by transferring 100 µl culture to 10 ml MRS broth, anaerobic incubation at 37˚C for 48 h.
3.Individual strain was streaked on MRS agar, incubated under anaerobic conditions at 37˚C for 24 h.
4.Isolated colony transferred to 10 ml MRS , anaerobic incubation at 37˚C for 24 h.

Table 1 Strains of *Lactobacillus reuteri* .

Strain no.	Source/Reference
CF2F-7F	Child fecal isolate
DSM20016	Mother's milk
SD2112	Mother's milk

Sweet potato media and MRS preparation:

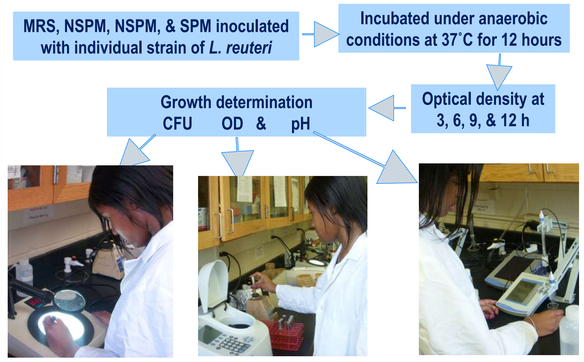


Composition for 500 ml of media

Component	NSPM	PSPM
Peptone	0	5.0g
Beef extract	0	5.0g
Yeast extract	0	2.5g
Potassium monophosphate	1.0g	1.0g
sodium acetate CH_3COONa	2.5g	2.5g
Tween 80	0.5ml	0.5ml
Ammonium citrate ($NH_4C_6H_5O_7$	1g	1g
Magnesium sulfate $MgSO_4.7H_2O$	0.1g	0.1g
Manganese sulfate $MnSO_4.5H_2O$	0.025g	0.025g
L-Cysteine	0.5g	0.5g

Table 2 Components and amount used to form negative and positive SPMs.

Experimental assay:



Results

Initial log CFU/ml			Initial pH			
CF2F-7F	DSM20016	SD2112	MRS	P-SPM	N-SPM	SPM
2.73	2.94	2.81	6.55	6.53	6.65	5.67

Table 3 Initial bacterial populations for *L. reuteri* strains and initial pH values for MRS, PSPM, NSPM, and SPM.

L. reuteri Strain	pH value after 12 hours MRS	P-SPM	N-SPM	SPM
CF2F-7F	4.23	3.99	4.21	4.41
DSM20016	4.34	4.04	4.33	4.35
SD2112	4.49	4.49	4.71	4.82

Table 4 Final pH values in MRS, PSPM, NSPM, and SPM after 12 h of incubation due to the growth of *L. reuteri* strains.

L. reuteri Strain	CFU log/ml after 12 hours MRS	P-SPM	N-SPM	SPM
CF2F-7F	10.13	10.39	9.09	8.01
DSM20016	10.14	10.18	9.28	8.11
SD2112	9.92	9.97	8.85	7.79

Table 5 Final bacterial populations in MRS, PSPM, NSPM, and SPM after 12 h of incubation for individual strains of *L. reuteri* strains.

L. reuteri	3 hours				6 hours			
Strain	MRS	PSPM	NSPM	SPM	MRS	PSPM	NSPM	SPM
CF2F-7F	.002	.045	.07	.02	0.039	040	0.74	0.18
DSM20016	.006	.05	.07	.03	0.008	0.35	0.77	0.23
SD2112	.02	.045	.07	.03	0.111	0.47	0.63	0.21

Table 6 Optical density reading for MRS, PSPM, NSPM, and SPM after 3h and 6h of incubation.

L. reuteri Strain	9 hours MRS	P-SPM	NSPM	SPM	12 hours MRS	PSPM	NSPM	SPM
CF2F-7F	0.753	0.926	0.704	0.599	1.28	1.43	1.01	0.943
DSM20016	0.713	0.845	0.693	0.545	1.112	1.101	0.911	0.812
SD2112	0.811	1.007	0.757	0.699	1.21	1.23	0.931	0.873

Table 7 Optical density reading in MRS, PSPM, NSPM, and SPM after 9h and 12h of incubation.

Discussion

➢PSPM showed the lowest pH value followed by MRS, NSPM, then SPM (Tables 4).

➢Growth of *Lactobacillus* in PSPM was slightly higher than those in MRS whereas NSPM and SPM showed lower growth than MRS (Tables 5).

➢ Change in turbidity due to the growth of *Lactobacillus* shows higher change in PSPM, followed by MRS, NSPM, then SPM (Tables 6,7).

➢After 12h of incubation, *Lactobacillus* reach the exponential growth phase in PSPM, NSPM, and MRS media.

➢Sweet potatoes are rich with carbohydrate, Vitamins, minerals, and some protein to support lactobacilli growth.

➢Since sweet potatoes are rich in several nutrients, PSPM shows no significant differences in lactobacilli growth compare to MRS

➢Nitrogen is required for lactobacilli growth, NSPM and SPM are not enhanced with any nitrogen sources, growth of lactobacilli in NSPM and SPM were lower than those in MRS but with less than 1 log CFU/ml.

Conclusion

➢Sweet potatoes (a rich source of many nutrients) can support the growth of lactobacilli (a group of fastidious bacteria) and thus SPM need to be enhanced with different nitrogen sources to support same or bitter lactobacilli growth than those in MRS.

➢Sweet potatoes could be used to develop a low cost medium for laboratorial and industrial applications of lactic acid bacteria

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