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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Selection of Starter Culture for Yogurt Preparation and its Antibacterial Activity

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Abstract

Studies on the symbiotic relationship of six combinations of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for the preparation of yogurt in relation to its anti-microbial activity were carried out. The ratio 2:1 of *L. bulgaricus*: *S. thermophilus* in the inoculum was found best for the preparation of prime quality yogurt with maximum antibacterial activity. The ratio of the two cultures in the final product was 1:1. The yogurt samples were evaluated for curdling time, acidity, diacetyl acetoin contents and antibacterial activity. The antibacterial activity of the best product was comparable in 200 mcg/ml with 100 mcg/ml of Kanamycin and Ampiclox.

Introduction

Yogurt is a fermented product of milk. Yogurt is known and used in nearly every civilization in one type or another. Traditionally yogurt is made from milk that has been heat treated to increase its solid contents and then fermented by specific microorganism's i.e., *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Successful preparation of yogurt depends upon the proper symbiotic relationship between the two organisms. All the combinations for pairing strains cannot produce a high quality product. The satisfactory combination is one which will be amenable for growth under manufacturing conditions (Mann, 1974) and produce a product with good body texture, taste, flavor, etc. Good quality yogurt can be obtained where the mixed cultures contain equal proportion of the two strains during coagulation.

Yogurt has curative properties that were reported by Hoyle and Nichols (1948) and Lightbody and Meanwell (1955). Lightbody and Meanwell (1955) reported the domination among the strain of lactic streptococci results from the production of antibiotics.

The present studies concerned with the suitability of the isolated cultures of *Streptococcus* sp. and *Lactobacillus* sp. for the preparation of yogurt and its curative properties (antibacterial activity).

Materials and Methods

Cultures: The strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were isolated from Dehi sold in local market on M 17 (Terzaghi and Sandine, 1975), MRS (De Man *et al.*, 1960), APT (APHA, 1966) and ROGOSA agar (Sharpe, 1960). The strains of *Lactobacillus* were identified as by Rogosa and Sharpe (1960) where as *Streptococcus* were identified according to Bergey and Holt (1993). The strains of *Lactobacillus* were named as L1, L2 and the *Streptococcus* as S1, S2. These cultures were maintained on nutrient agar and subcultured after every 15 days.

Milk: The buffalo milk having 2.0-2.5% fat contents was used throughout the studies.

Preparation of Yogurt: Two hundred mL milk in 500 mL flasks was steamed for 20 minutes. After cooling to 42°C, the milk was inoculated with 18 hours old cultures. The flasks were incubated at 42±1°C for 4 hours and the coagulation of milk was noted. The flasks were cooled immediately.

Symbiosis Studies: The combination and proportions of *Lactobacillus* and *Streptococcus* varied in yogurt preparation. The following six combinations were prepared:

Combination No.	Strains	Ratio
1	S ₁ L ₁	1:2
2	S ₁ L ₂	2:1
3	S ₂ L ₁	1:1
4	S ₂ L ₁	2:1
5	S ₁ L ₂	1:1
6	S ₂ L ₂	1:2

In each case the curdling was noted and the product was analyzed for acidity, diacetyl acetoin, texture, ratio of two organisms by microscopic examination.

Antibacterial activity: Antibacterial activity was determined in the extract of the product. The extract was prepared by centrifuging the product at 2500 rpm for 10 min. The supernatant liquid was used for antibacterial activity. The organisms used were *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus albus* and *Micrococcus leuteus*.

The test organisms were inoculated on nutrient agar slants and incubated at 37°C for 24 hrs. The 24 hrs old cultures were grown in nutrient broth tubes and incubated at 37°C for 24 hours. One mL of that broth was used as inoculum per Petri plate.

The well diffusion method

was employed for testing antibacterial activity. The diameter of the inhibition zone was measured after 24 hrs incubation at 37°C. The antibiotics Ampiclox and Kanamycin were used for comparison.

Organoleptic evaluation: The organoleptic evaluation of the product was carried out by using Futschik Score Card developed for yogurt.

Results and Discussion

The symbiotic relationship of four possible pairs of the yogurt making organisms is given in Table 1. It is observed that lactic acid production was improved in presence of both the cultures in most of the cases. The lactic acid production was high in pairs where S1 is used. The curdling was not occurred in single cultures. Best curdling was seen in 2:1 ratio of *Streptococcus thermophilis* and *Lactobacillus bulgaricus*, of all the combinations. The acidity in the paired culture was more than 0.5% (as lactic acid) which is considered as minimum for use in yogurt preparation.

Table 1: Combined action of the strains of *S. thermophilus* and *L. bulgaricus* on the yogurt formation.

Culture	Ratio	Acidity Lactic acid, %	Final product after 4 hrs
S ₁ L ₁	1:1	0.45	Partially Curdled
	1:2	0.77	Curdled
	2:1	0.50	Curdled
	2:0	0.31	Not Curdled
	0:2	0.42	Not Curdled
S ₁ L ₂	1:1	0.42	Partially Curdled
	1:2	0.71	Curdled
	2:1	0.53	Curdled
	2:0	0.28	Not Curdled
	0:2	0.43	Not Curdled
S ₂ L ₁	1:1	0.31	Curdled
	1:2	0.42	Curdled
	2:1	0.50	Curdled
	2:0	0.41	Not Curdled
	0:2	0.26	Not Curdled
S ₂ L ₂	1:1	0.68	Partially Curdled
	1:2	0.56	Curdled
	2:1	0.58	Curdled
	2:0	0.28	Partially Curdled
	0:2	0,32	Not Curdled

Data regarding the selection of best combination strains with respect to body, texture, flavor and taste is given in Table 2. The data revealed that the curdling time more than 3 hrs in all cases. Only in one case i.e., the combination 3, where the ratio of *Streptococcus* to *Lactobacillus* was 1:1. Here the curdling time was 2 hrs and 50 min. The *Lactobacillus* showed dominance in combinations 1, 3 and 5 where as in rest of the combinations, *Streptococcus* either dominated or remains equal. The overnight storage of

the final product in refrigerator increase the acidity and diacetyl acetoin contents which is due to the dominance of *Streptococcus*. Barnicoat (1935) reported that greater the acidity greater will be the diacetyl amount, the same was observed in our studies. The diacetyl amount impart the strong buttery flavor to the product (Davies, 1938)

Table 2: Effects of different combinations of strains of *S. thermophilus* and *L. bulgaricus* on the final product.

Comb.	Initial Ratio	Final Ratio	Curdling Time (hr-min)	Acidity lactic acid (%)	Diacetyl acetoin (ppm)
1	1:2	1:3	3-15	1.06	68
2	2:1	1:1	3-25	1.15	72
3	1:1	1:3	3-05	0.96	42
4	2:1	1:1	3-30	1.00	65
5	1:1	1:2	3-15	1.03	68
6	1:2	2:1	3-45	1.32	78

Antibacterial activity: Table 3 showed that the extracts of the product of combinations 2 and 4 showed antibacterial activity against all the four strains i.e., *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus albus* and *Micrococcus leuteus*. The extract of the product of combination 1 was found active only against *Staphylococcus aureus* and *Staphylococcus albus* whereas the combination 6 was active against *Staphylococcus albus* and *Micrococcus leuteus*. The yogurt of the combination 3 and 5 were only active in 200 mcg concentration against *Micrococcus leuteus* and *Staphylococcus albus*, respectively. In comparison with Ampiclox and Kanamycin the yogurt extracts were less active against all the bacterial strains. However the antibacterial activity of the yogurt extracts of the combination 2 and 4, in 200 mcg conc. were comparable with standard antibiotics (Table 3 and 4). It was also found that all the extracts had greater activity against *Staphylococcus albus* than other strains under investigation. The results also reveal that the yogurt showed better antibacterial activity for which the incolum used having greater number of *Streptococcus* than *Lactobacillus*. Various workers have reported the production of antibiotics by different *Streptococcus* sp. (Hirsch, 1952; Hirsch and Grinsted, 1951).

Organoleptic Test: Data presented in Table 5 showed that the yogurt prepared from the combinations 2 and 4 are of excellent quality, combination 1 is very good whereas the combination 3, 5 and 6 yielded a product of good quality. The results of the organoleptic evaluation also revealed that the excellent quality yogurt had equal ratio of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in the final product. Also reported that equal ratio of *Lactobacillus Streptococcus* at the time of coagulation, yield good yogurt.

Rehman *et al.*: Yogurt, symbiotic relationship, antimicrobial activity.

Table 3: Antibacterial activity of yogurt extracts of different culture combinations (Zone Diameter in mm)

Organisms	Concentration	Culture combinations					
		1	2	3	4	5	6
<i>Bacillus subtilis</i>	100	No activity	6.00	No activity	7.50	No activity	No activity
	200	No activity	13.50	No activity	18.20	No activity	No activity
<i>S. aureus</i>	100	5.50	8.0	No activity	6.00	No activity	No activity
	200	10.60	15.00	No activity	17.50	5.00	No activity
<i>S. albus</i>	100	5.50	12.50	No activity	8.50	No activity	No activity
	200	12.00	20.50	No activity	20.00	No activity	No activity
<i>M. leuteus</i>	100	No activity	13.50	No activity	6.50	No activity	5.00
	200	No activity	18.00	5.50	15.50	No activity	5.80

Table 4: Antibacterial activity of Ampiclox and kanamycin in 100 mcg concentration

Organisms	Zone Diameter in mm	
	Ampiclox	Kanamycin
<i>Bacillus subtilis</i>	16.50	19.00
<i>Staphylococcus aureus</i>	18.00	23.75
<i>Staphylococcus albus</i>	21.25	26.75
<i>Micrococcus leuteus</i>	22.50	27.50

Table 5: Score card for yogurt prepared from different combinations

Comb. No.	1	2	3	4	5
1	2	2	3	3	10
2	2	3	4	5	14
3	1	1	2	3	7
4	2	2	3	5	12
5	1	1	1	3	6
6	1.5	1	2	4	8.5

Excellent = 12-15 Very Good = 9-11 Good = 6-8;
1 = Appearance Max. No. 2; 2 = Consistency Max., No. 3;
3 = Taste Max. No. 4; 4 = Flavour Max. No. 6; 5 = Total
Max. No. 15.

The quality of yogurt depends upon the successful symbiosis of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in the product. An excellent quality yogurt should have *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in equal ratio. The antibacterial activity of yogurt depends upon the ratio of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in the inoculum. Greater the number of *Streptococcus thermophilus* in the inoculum higher will be the antibacterial activity.

References

- APHA., 1966. Recommended Methods for the Microbiological Examination of Foods. 2nd Edn., American Public Health Association, Washington, DC., USA., Pages: 205.
- Barnicoat, C.R., 1935. The determination of diacetyl and acetyl methyl carbinol. *Analyst*, 60: 653-662.
- Bergey, D.H. and J.G. Holt, 1993. *Bergey's Manual of Determinative Bacteriology*. Williams and Wilkins, Baltimore, ISBN: 9780683006032, Pages: 787.
- Davies, W.L., 1938. The aroma and flavour of butter and starters. *Agric. Progress*, 15: 30-34.
- De Man, J.C., D. Rogosa and M.E. Sharpe, 1960. A medium for the cultivation of lactobacilli. *J. Applied Bacteriol.*, 23: 130-135.
- Hirsch, A. and E. Grinsted, 1951. The differentiation of the lactic streptococci and their antibiotics. *J. Dairy Res.*, 18: 198-204.
- Hirsch, A., 1952. The evolution of the lactic streptococci. *J. Dairy Res.*, 19: 290-293.
- Hoyle, M. and A.A. Nichols, 1948. Inhibitory strains of lactic streptococci and their significance in the selection of cultures for starter. *J. Dairy Res.*, 15: 398-408.
- Lightbody, L.G. and L.J. Meanwell, 1955. The growth of lactic acid streptococci in mixed starter cultures. *J. Applied Bacteriol.*, 18: 53-65.
- Mann, E.J., 1974. The yogurt story-A review. *Cult. Dairy Prod. J.*, 9: 13-13.
- Rogosa, M. and M.E. Sharpe, 1960. An approach to the classification of the lactobacilli. *J. Applied Bacteriol.*, 22: 329-340.
- Sharpe, M.E., 1960. Selective media for the isolation and enumeration of lactobacilli. *Lab. Pract.*, 9: 223-227.
- Terzaghi, B.E. and W.E. Sandine, 1975. Improved medium for lactic streptococci and their bacteriophages. *Applied Microbiol.*, 29: 807-813.