OnLine Journal of Biological Sciences 2 (6): 374-377, 2002
ISSN 1608-4127

Optimum Growth Patterns of Wild Strains of *S. thermophilus* and *L. bulgaricus* for Suitable Selection for Yoghurt

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Abstract: Fifteen strains each of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* were screened and out of these, 9 strains of *L. delbrueckii* ssp. *bulgaricus* and 7 strains of *S. thermophilus* produced a desired amount of T.A.% at given period of time and temperature. It was further observed that strains of *L. delbrueckii* ssp. *bulgaricus* produced more developed acidity as compared to strains of *S. thermophilus* in all tested temperatures. Moreover, there was a variation among these strains for their developed acidity. There was an inverse relationship between generation time and acidity. *S. thermophilus* strain showed greater generation time that varied from 90.32 to 110.00 minutes and produced 1.32 to 1.10% T.A. Generation time for *L. delbrueckii ssp. bulgaricus* varied from 70.21 to 80.12 minutes and produced 1.35 to 1.23% T.A. at their respective optimum temperature.

Key words: *L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus*, titrable acidity, generation time

Introduction

In the earlier study (Masud *et al.*, 1991) it has been reported that *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* constituted the predominant micro-flora of market made dahi samples. The symbiotic relationship between *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* has long been utilized in the manufacture of yoghurt and various Italian and Swiss cheese. (Radke-Mitchell and Sandine, 1986). Since lactic acid is the main fermented product of these organisms (Puhan *et al.*, 1973). The rate of acid production depends upon the strain used for inoculation at a particular temperature (Martley, 1983).

In the present investigation an attempt has been made to examine the optimum growth conditions of indigenous strains of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* for further selection of suitable pairs for yoghurt starter.

Materials and Methods

Screening of isolates for sufficient acid production: Fifteen strains each of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* isolated from indigenous dahi samples from local market (Masud *et al.*, 1991) were screened for sufficient acid production by using the procedure of Kosikowki (1977). All the isolates which showed more than 0.7% titratable acidity in 4 hr at 35°C were then examined for their developed acidity (final percentage T.A. minus initial percentage T.A.) at 37, 40 and 45°C.

Optimum growth temperature: Of the isolates examined, two strains each of high and weak of lactic acid producer of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were retained for further studies at these temperature by using the following parameters.

Bacterial counts: Bacterial population was determined by using spectronic 21. Generation time (G) at each temperature of incubation was determined by using following equation:

$$\text{Generation time (G)} = 0.693/k$$

Growth rate (k) was determined by following equation:

$$\text{Growth rate (k)} = 2.303(\log b - \log a)/t$$

where loga and logb are the log-arithmetic absorbancy readings taken during exponential growth and (t) is the time in minutes. pH was determined by using pH meter. Titratable acidity was determined by method of Robinson and Tamine (1976).

Results and Discussion

Screening for the selection of isolates: Fifteen strains each of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* randomly selected among the identified lactic acid bacteria for their ability of acid production in skim milk at 35°C for a given period of 4 hours for further selection (Table 1). It was observed that among the tested strains of *L. delbrueckii* ssp. *bulgaricus* eight strains produced a desired amount of acid production for a given period of time followed by seven strains of *S. thermophilus*. It

Table 1: Culture activity test (T. A. %) for randomly selected lactic acid bacteria in skim milk after 4 hr at 35°C

	S. thermophilus	*L. bulgaricus*
S. No.	T. A. %	T. A. %
1	0.62	0.71*
2	0.72*	0.68
3	0.70*	0.84*
4	0.59	0.73*
5	2.65	0.75*
6	0.72	0.62
7	0.71	0.75*
8	0.49	0.59
9	0.53	0.77*
10	0.74*	0.66
11	0.58	0.68
12	0.62	0.69
13	0.73*	0.77*
14	0.71*	0.69
15	0.67	0.74*

* Produced desired T.A. > 7%

Table 2: Comparison of developed acidity by *S. thermophilus* strains grown in skim milk at different temperatures after 9 hours

	S. thermophilus			*L. bulgaricus*		
	Temperature (°C)			Temperature (°C)		
S. No.	37	40	45	37	40	45
1	0.46	0.47	0.48	1.01	1.06	1.08
2	0.44	0.46	0.51	0.94	0.97	1.09
3	0.92	0.92	1.30	0.88	0.93	1.07
4	0.98	0.88	0.86	1.00	1.03	1.06
5	0.86	0.87	1.00	0.99	0.94	0.85
6	1.00	0.85	0.81	0.92	0.96	1.02
7	0.85	0.87	1.01	1.02	1.04	1.07
8				0.83	0.90	1.00

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Table 3: Optimum temperature for growth, acid production and generation time for *S. thermophilus* and *L. bulgaricus* strains grown in skim milk (Temperature for growth 45°C)

Strains	Generation time (Min)	Acidity (%)
S. thermophilus (H^1)	96.86	1.28
S. thermophilus (H^2	90.23	1.32
S. thermophilus (w^1)	110.00	1.10
S. thermophilus (w^2)	106.29	1.13
L. bulgaricus (H^1)	78.23	1.32
L. bulgaricus (H^2)	70.21	1.35
L. bulgaricus (W^1)	80.12	1.23
L. bulgaricus (w^2)	80.00	1.25

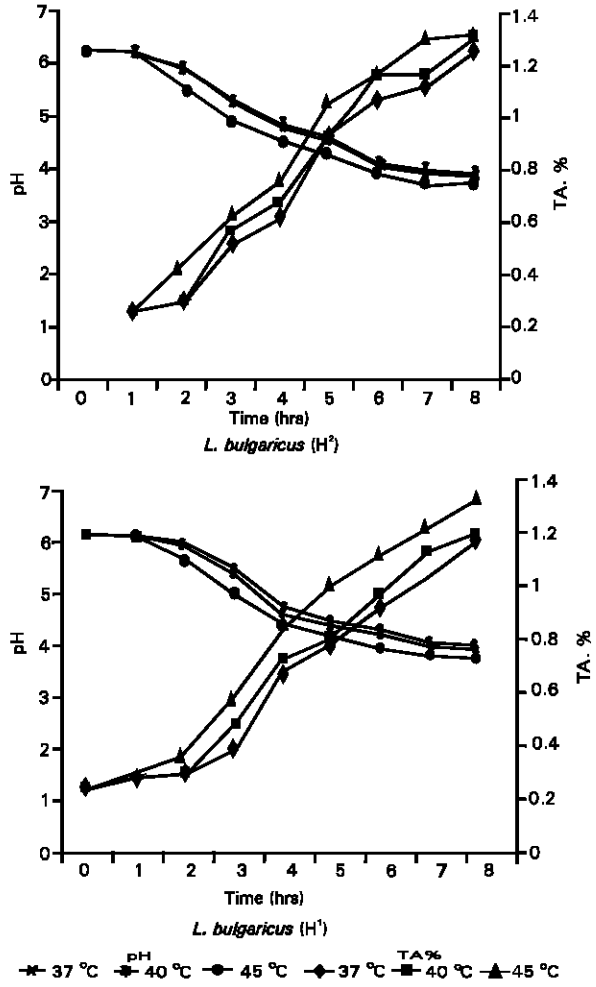

Fig. 1: Rate of acid production and their corresponding pH values with time among the tested strains of *L. bulgaricus* (H^1 and H^2) at different temperatures in skim milk

could be seen that *L. delbrueckii* ssp. *bulgaricus* strains produced a high rate of acid production as compared to *S. thermophilus*. This may be due to their genetic variation. Similar views are expressed by Jeffrey (1985). It was further observed that 8 strains of *S. thermophilus* and 7 strains of *L. delbrueckii* ssp. *bulgaricus* were considered to be slow starter strains which may be due to that as the lactic acid is not produced by a starter culture from lactose at the desired rate of speed therefore, the culture is called "slow". Almost any condition leading to an

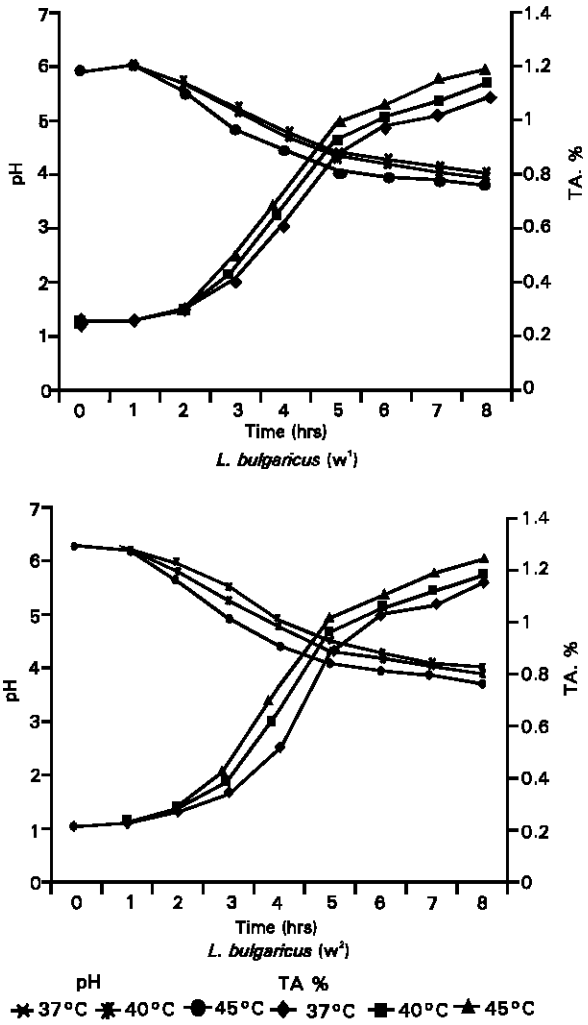

Fig. 2: Rate of acid production and their corresponding pH values with time among the tested strains of *L. bulgaricus* (w^1 and w^2) at different temperatures in skim milk

impairment in the growth of starter culture bacteria results in a "slow" starter (Kosikowski, 1982).

Comparison among strains of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* for their developed acidity: Later on these bacterial strains were compared individually at different temperatures i.e., 37, 40 and 45°C by using their developed acidity (final T.A. minus initial T.A.) for further selection of starter culture for yoghurt making. The results of (Table 2) revealed that temperature had a considerable effect on the growth of *S. thermophilus* strains however, there was no difference between 37 and 40°C respectively. It was further observed that there was also a pronounced differences among the strains for their developed acidity on their respective temperature. It was also noted that their rate of acid production increases with the increase of temperature. More acid production was recorded at higher temperature.

On the basis of these observations these strains were further divided into two groups for their developed acidity. The strain No 3, 5, 7, produced higher amount of developed acidity and are ranked as high (H) while 4, 1, 2 and 6 produced a relatively less developed acidity are ranked as weak (w). Similar results were also recorded for the examined strains of *L. delbrueckii* ssp. *bulgaricus*.

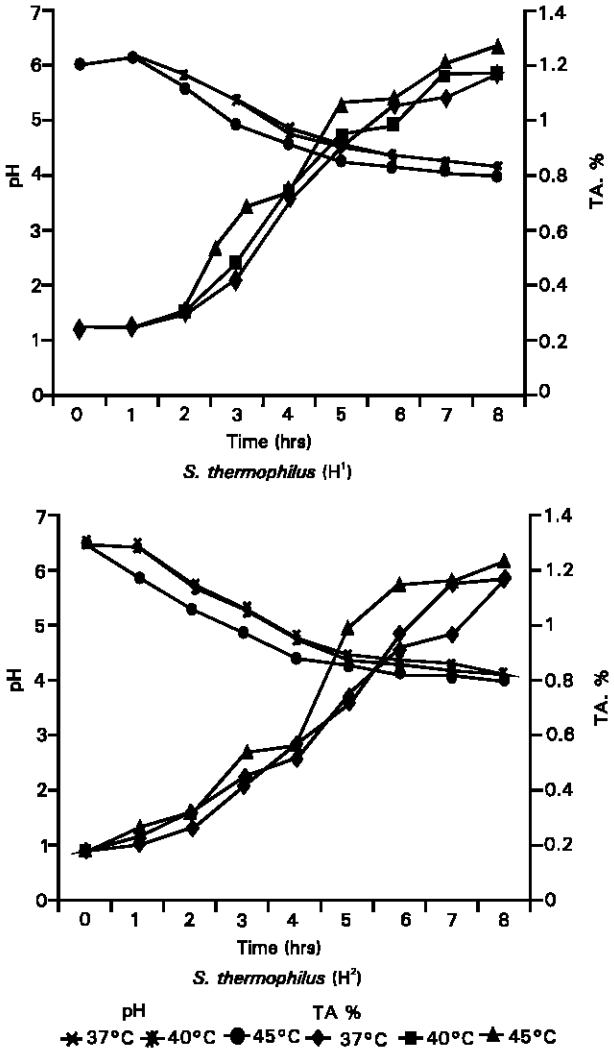

Fig.3: Rate of acid production and their corresponding pH values with time among the tested strains of *S. thermophilus* (H^1 and H^2) at different temperature in skim milk

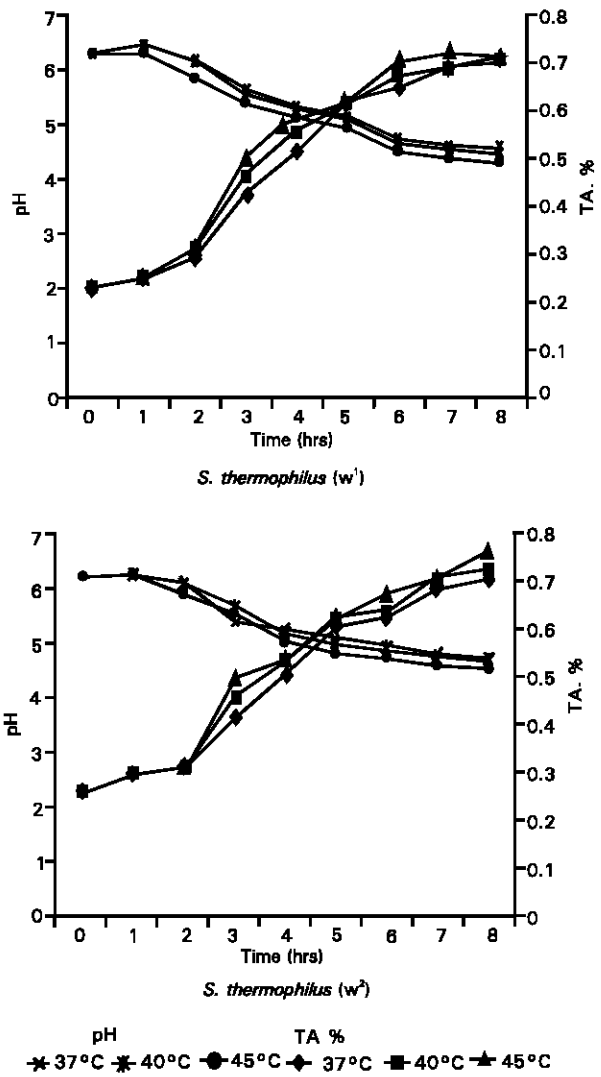

Fig.4: Rate of acid production and their corresponding pH values with time among the tested strains of *S. thermophilus* ($w^1$and w^2) at different temperature in skim milk

It was observed (Table 2) that temperature also has a marked effect on the growth of *L. delbrueckii* ssp. *bulgaricus* strains are in descending order i.e. 45°C < 40°C < 37°C respectively. Furthermore, there was also a marked difference among the strains for their developed acidity. The strains No.1, 7, 2, 3 and 4 produced higher amount of developed acidity and are ranked as (H) while 5, 8 and 6 produced low developed acidity are ranked as (w).

Comparative studies of indigenous starter culture for their optimum growth condition: The starter culture used for the preparation of a fermented product have been selected on the basis of their acidification rates and organoleptic quality of final product (Batt, 1986). In manufacturing yoghurt with the defined starter the *S. thermophilus* is used in conjunction with *L. bulgaricus* as starter, cocci forming the greatest proportion of the population at the time of inoculating the milk. The production of acid in the early stages of yoghurt making depends substantially on the activity of cocci while lactobacilli are responsible for continuing the acidification process. These properties depend upon the strains used for inoculation.

The results obtained from this study related with optimum growth temperature indicated that there is a correlation between generation time and acidity and both these factors depend upon the optimum growth temperature (Table 3). The strains that have less generation time produce more acid at its respective optimum temperature. It is further observed that there was a difference in generation time between the species of *Streptococci* and *Lactobascilli*. The generation time of *L. delbrueckii* ssp. *bulgaricus* strains are less than *S. thermophilus* therefore *L. delbrueckii* ssp. *bulgaricus* produced more acid than *S. thermophilus*. It was further observed for *S. thermophilus* that coagulation of milk occurred sooner at temperatures much higher than those temperatures at which the greatest amount of cell growth occurred. This indicates that the optimum temperature for growth did not coincide with the temperature at which the rate of acid production is the greatest. These results support the view of Radke-Mitchell and Sandine (1986) and Masud *et al.* (1992).
It was earlier reported by Sampolinski *et al.* (1978) that optimum growth temperature is one of the unique characteristics of bacteria. This trait would be expected to influence the growth compatibility of strains, especially those with different optimum

growth temperature. It was also observed that all examined strains of *S. themophilus* and *L. delbrueckii* ssp. *bulgaricus* have maximum growth at 45°C as compared to other tested temperatures. Moreover, their was also a difference in their generation time among the examined strains of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*. This may be due to the differences in their genetic make up. Jeffery (1985) reported that some of the properties essential for successful milk fermentation are encoded by genes located on plasmid DNA. Furthermore, it was reported by Yu *et al.* (1983) that if the plasmid profile of the two organism is similar or even identical, there may be difference in their nucleotide composition, nucleotide sequence or even both. *L. delbrueckii* ssp. *bulgaricus* have 43°C optimum temperature and are thermophilic in nature and have more generation time and acidity at 43°C. Similar observations are also reported by Breed *et al.* (1957); Martly (1983) and Radke - Mitchell and Sandine (1986). This study also showed that there was an inverse relationship between titratable acidity and pH values of each strain at different time intervals (Fig. 1,2,3,4). Growth temperature is correlated with the pH and acidity. It was observed that there was variation for their rate of acid production among the examined strains of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*. Maximum acidity recorded after 8 hour of incubation at 45°C for the strains of *S. thermophilus* varies from 0.72 to 1.28% while for *L. delbrueckii* ssp. *bulgaricus* strains it varies from 1.23 to 1.35%. The difference was again presumably due to the generation time and genetic make up. The acidity for *L. delbrueckii* ssp. *bulgaricus* at 45°C varies from 0.22 to 1.97 %. These results are also in line with the findings of Masud *et al.* (1992). The present study further revealed that there was a gradual increase in acidity with the passage of time by the individual strains at their optimum temperatures. It also appeared that there was no marked difference among the examined strains for rate of acid production and there was a considerable diversity between the individual strains of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* with respect to their rates of acid production confirming the observations of Accolas *et al.* (1980) and Masud *et al.* (1992). This diversity is probably more significant than the generalization that the *L. delbrueckii* ssp. *bulgaricus* exhibit maximum rate of acid production as compared to the other strains. These results also support the findings of Mohannan *et al.* (1983).

On the basis of the present investigation it may be concluded that strains of *S. thermophilus* have greater generation time as compared to the strains of *L. delbrueckii* ssp. *bulgaricus*. There was direct relationship between generation time and titratable acidity at all examined temperatures. The results further suggested that in order to produced a good quality product a balanced growth of the two organisms is essential. Therefore, further study is needed to determine a suitable pair of starter culture for preparation of yoghurt.

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