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Modelling of Growth Profile of Three Probiotic Single Strain Starter Cultures (L.acidophilus (La-5), Bifidobacterium (BB-12), S.thermophilus (STB-01)) through Turbidity Measurement Technique

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Abstract
Probiotics are one or more mixture of viable microorganisms which have beneficial effects on animals and human beings through propagation gastrointestinal microflora. Some instances for health benefits of these products are: alleviating gastrointestinal disorders, diarrehea, food allergies, infection of Helicobacter pylori, lactose intolerance, candidiasis, serum cholesterol, and improving immune system balance, mineral uptake and protecting the consumer from different cancers such as colon, bladder and gastrointestinal cancers.To achieve these neutraceutical purposes, a large population of probiotics(107- 108 cfu/g) should remain alive during storage of these products up to expiring date.In this research production of probiotic ABT yogurt is taken into consideration. Single strains of two probiotic starter cultures, Bifidobacterium(BB-12) and L. acidophilus(La-5), and one single strain of S. thermophilus (STB-01) for reducing the fermentation time are used. In probiotic products the method of counting probiotic bacteria have a significant effect. Traditional microbiological methods require wide range of time and a lots of facilities. Modelling of growth profile of bacteria with the data obtained from turbidity measurement would be a helpful method for fast counting of microbial communities.
Keywords: analyze ; Broth media ; Colony Count Unit; Direct-Vat-Set(DVS); Durbin-Watson statistics

1.introduction

Traditional microbiological methods for the study of microbial communities, i.e. counts on differential and selective media are time-consuming (Kristo, *et al*, 2003). The present investigation studied growth profile of three single strains *L.acidophilus* (LA-5), *Bifidobacterium*(BB-12), *S.thermophilus*(STB-01) through turbidity measurement technique. Modelling of the starter growth profiles from these measurements. The models provided rapid method for estimating bacterial population in probiotic ABT yoghurt(Sadar, 2002).

2.Materials and Methods

2.1.Starter cultures

The commercial single strain starter cultures(Chr. Hansen Ltd. Denmark*) Lactobacillus acidophilus*(La-5) ,*Bifidobacterium*(BB-12) and *S.thermophilus* (STB-01) were used in this study. The starter cultures were in freeze-dried direct to-vat set form and were stored at -18ºC.

2.2. Statistical analysis

The data were analysed with balanced analysis of variance with SPSS version 13 for windows(SPSS Inc. NY, USA).

2.3.Turbidity measurement technique

In this technique three broth media, MRS+ 0.05% cystein hydrochloride monohydrate broth, MRS-broth and M17 broth were used.

L.acidophilus, Bifidobacterium and S.thermophilus were directly inoculated (DVS) in MRS+ 0.05% cystein hydrochloride monohydrate broth, MRS-broth and M17 broth respectively.The amount of inoculation was 0.2 g/L. After inoculation all these three media were placed in a 37°C incubator equipped with a 120-rpm shaker. Because of anaerobic treatment for L.acidophilus and Bifidobacterium anaerobic nitrogen jars were used.

After time intervals: 15 min, 30 min, 1h, 2h,3h,4h, 5h,6 h,7h and 8 h growth profiles of these bacteria were investigated.

At selected times taking samples were taken from each media and optical density(O. D.) was measured at 580 nm with spectrophotometer device were read.

Also microbial analyses were done using a spectrophotometer(Hach D/R 2000). For every starter growth profile a model which is dependent on optical density (OD) and amount of bacterial growth (colony count unit) was obtained. The first order responses models were fitted to each of the responses based on the following equation:

$$yi = \beta_0 + \beta_1 X_i + e_i$$

where β_0 represents intercept term, β_1 the linear effects and e_i the random error, while the X_i are the independent coded varaiables(colony count). Considering Durbin-Watson statistics (Tables 1,2 and 3) which in all cases is above p=0.05% , A linear relation between the two variables (OD and colony count) was proven.

Table 1. Durbin-Watson statistics for *Bifidobacterium*

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.708[a]	.501	.429	.1229801	.501	7.017	1	7	.033	.774

a. Predictors: (Constant), colony count

b. Dependent Variable: OD

Table 2. Durbin-Watson statistics for *L.acidophilus*

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.758[a]	.574	.513	.1796542	.574	9.436	1	7	.018	1.022

a. Predictors: (Constant), Colony count

b. Dependent Variable: OD

Table 3. Durbin-Watson statistics for *S. thermophilus*

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.262[a]	.069	-.064	.6609611	.069	.517	1	7	.495	1.321

a. Predictors: (Constant), STB-01

b. Dependent Variable: Colony count

In model coefficients all of the components can be obtained (Table 4,5 and 6). From these componenets all three models cab be concluded .

Table 4. Model coefficients of *Bifidobacterium*

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	3.099	1.008		3.073	.018	.715	5.483
	colony count	-.384	.145	-.708	-2.649	.033	-.726	-.041

a. Dependent Variable: OD

Table 5. Model coefficients of *L.acidophilus*

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-3.141	1.189		-2.642	.033	-5.952	-.330
	Colony count	.547	.178	.758	3.072	.018	.126	.969

a. Dependent Variable: OD

Table 6. Model coefficients of *S. thermophilus*

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	1.031	.748		1.378	.211	-.739	2.800
	Colony count	-.084	.113	-.272	-.749	.478	-.351	.182

a. Dependent Variable: OD

Bifidobacterium model: OD = 3.099 – 0.384(CC)
L.acidophilus model: OD = -3.141 + 0.547(CC)
S. thermophilus model: OD = 1.031 – 0.084(CC)

3. Conclusions

From the resulted models amount of different yoghurt samples can be obtained. By inoculation of 0.2 g/L of every ABT yoghurt samples to suitable growth media and measurement of its optical densities at 580 nm wave length , bacterial population can be estimated with above models.

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