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Effect of aqueous extract of strawberry polyphenols on the growth of mesophillic mixed dahi culture (NCDC-167) and probiotic culture *Lactobacillus rhamnosus* GG

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Abstract
Polyphenols can selectively modulate the growth pattern of susceptible microorganisms. Present study was conducted to assess the viability of mesophillic mixed dahi culture (NCDC 167) and probiotic culture *Lactobacillus rhamnosus* GG in their growth medium viz. M17 and MRS with the added strawberry polyphenols aqueous extract. pH of growth medium was observed to be decreased with the addition of polyphenols extract at different concentration (0.25 to 1.0 mg/ml). Growth pattern of the cultures was estimated by measuring the optical density of pH adjusted samples and without pH adjusted samples. Growth of NCDC 167 and *Lactobacillus rhamnosus* GG was observed to be unaffected in the presence of polyphenols. Therefore incorporation of polyphenols in fermented dairy products in combination with probiotic bacteria can be a good option to deliver functional ingredients.

Keywords: Starter culture, dahi, strawberry polyphenols, oxidative stress

Introduction
Fruits and vegetables are the rich source of polyphenolic compounds. These are the plant secondary metabolites that play a vital role in human diet (Shetty, 2005) [1]. Phenolic phytochemicals have certain therapeutic properties like antioxidant, antidiabetic and antihypertensive (Kwon *et al*., 2006) [2]. The incorporation of phenolic compounds in the food and dairy products is increasing with the increase in attractiveness of people for functional foods (Drewnowski and Gomez-carneros, 2000; Balasundram *et al*., 2006) [3, 4]. Strawberry fruit contains high concentration of various kinds of phytochemicals like ellagic acid, ellagitannins, glycosides, gallotannins, falvonols and anthocyanin (Manach *et al*., 2005) [5].
Currently major proportion of functional foods market is contributed by milk and milk based fermented products. Fermented foods also have various health benefits such as lowering in serum cholesterol, mitigation of lactose intolerance, prevention from diarrhea, inhibition of colon cancer and strengthening of the immune system. Fortification of polyphenols in fermented dairy products can boost the health effects. Dahi is a traditional Indian dairy product consumed widely at every household. Studies have been conducted on incorporation of polyphenols in the fermented dairy products. However, lack of information is available on impact of strawberry polyphenols extract on growth of lactic acid bacteria. Hence present study was conducted to examine the effect of strawberry polyphenol addition on the survivability of lactic acid bacteria.

Material and Methods
Materials required
M-17 broth medium and MRS broth were procured from HIMEDIA Laboratories Pvt. Ltd., Mumbai, India. Strawberry pulp was procured from M/S delta Nutritive Pvt. Ltd., Mumbai. Starter culture NCDC 167 (mesophillic mixed dahi culture) and *Lactobacillus rhamnosus GG* were procured from National Collection of Dairy Cultures, National dairy research Institute, Karnal, Haryana.

Preparation of strawberry polyphenols extract
Strawberry polyphenols extract was prepared as per the method given by Cossu *et al*., 2009 [6]. 1000g of the strawberry pulp was added to 1000ml distilled water in beaker of 3000ml

capacity. Then the blending of mixture was done using mechanical stirrer for 30 min. after blending boiling of the mixture was done for 30 min. and centrifuged at 4025g/15 min (Kubota Tokyo, Japan). Collected supernatant was lyophilized (Lyophillizer, Hansil Science industrial). Then extract was stored at -20 ºC for later use.

Estimation of total phenolic content of strawberry polyphenols extract

Total phenol content of strawberry polyphenols extract was estimated as per the methodology developed by Zhang *et al* (2006) [7]. 20 µl of sample/standard solution aws added in 96-well plate. To this 100 µl of Folin-Ciocalteu's reagent (diluted 1:10) was added and mixed. After incubation of 3 minutes 80 µl sodium carbonate solution "(7.5% w/v) was added and incubated for 30 min. under dark conditions. Blank was also taken using distilled water instead of sample. Absorbance was taken against blank at 750nm. Similarly standard curve of gallic acid (10-100 µg/ml) was also prepared.

Propagation of cultures

NCDC 167 and Lactobacillus rhamnosus GG were propagated in M-17 and MRS broth medium at 30ºC/24 hr. then the propagated active cultures were stored in glycerol stock at -20ºC. Each culture was activated prior to use.

Preparation of testing culture solution with added polyphenols

Survivability of both the cultures NCDC-167 and *Lactobacillus rhamnosus GG* was estimated as per the procedure given by Tabasco *et al*., 2011 [21]. Strawberry polyphenols extract was added to the broth medium of M-17 and MRS at different concentration ranged from 0.25 to 1 mg/ml at pH 3.57 (natural extract pH) and at adjusted pH 7.0. Then both cultures were added @1.5% and incubated. Absorbance was measured at 600 nm after every 2 hours up to 24 hrs. till turbidity was appeared.

Result and Discussion

Strawberry polyphenols extract analysis

Water soluble strawberry polyphenols extract was examined for total phenolic content, total monomeric anthocyanin and total flavonoid content. Phenolic content of extract was observed to be 10 mg/ml of extract. Total monomeric anthocyanin content was observed to be 164±1.15 µg cyanidin-3-glucoside equivalents/mL of extract and pH of the aqueous extract was 3.57 (0.05% solution w/v).

Phenolic content in fruits and vegetables have the capability to serve as antioxidant. Estimation of phenolic content by Folin-Ciocalteu assay is based on redox reaction. It is estimation of not only phenol compounds but also other compounds like carotenoids, vitamins, sugar and amino acids. Phenolic content in strawberry is mainly contributed by high phenolic acid content along with anthocyanin content (Skrede *et al*., 2004) [10]. It has been reported that strawberry is rich source of phenolic phytochemicals with antioxidant and anti-proliferative properties (Wang *et al*., 1996, Guo *et al*., 2003) [9, 11]. Similarly, Shah (2008) observed the phenol content in aqueous and lipophillic fraction of strawberry to be 16.18 µM GAE/g and 4.14 µM GAE/g, respectively.

Major anthocyanin present in strawberries are pelargonidin (Plg) and cyaniding (Cy) aglycons and specifically are Plg-diglucoside, Cy-glucoside, Plg-glucoside, and Plg-rutinoside (Seeram, 2006a) [13]. Heo and Lee (2005) [14] reported the total anthocyanin content in strawberry is 19.430 ± 1.11 mg of cyanidin- 3- glucoside/100g of fruit. Similarly Wang *et al* (2000a) [15] observed as 38.9 mg/100g of fresh matter and Clifford and Scalbert (2000) [16] reported the anthocyanin on the basis of fresh matter ranged 15-35 mg/100g. Flavonoids are abundant in plants and most common group of phenolic compounds in human diet. These exhibit many properties like antioxidant, ACE inhibitory, antiviral and antimutagenic. These have tendency to chelate Fe^{3+}, Fe^{2+} and Cu^{2+}. glucosides and glucuronides of quercetin and kaempferol aglycons are the major flavonoids in strawberry (Da *et al*., 2008) [17].

Effect of polyphenols on the growth of Lactic acid Bacteria (NCDC 167 and *Lactobacillus rhamnosus* GG)

Strawberry polyphenols addition at different concentrations 0.25 mg/ml, 0.5 mg/ml and 1.0 mg/ml to the broth medium (MRS) of *Lactobacillus rhamnosus GG* caused a decrease in pH from 6.6 to 6.1, 5.7 and 5.2. Similarly polyphenols extract addition to M-17 broth of mesophillic mixed dahi culture (NCDC 167) caused a decrease in pH range from 7.0 to 6.5, 5.9 and 5.4. The viability of the cultures was also observed without addition of polyphenols extract at the corresponding pH values for both the mesophillic mixed dahi culture and *Lactobacillus rhamnosus GG*. Results displayed in fig (a) to fig (h) for *Lactobacillus rhamnosus GG* and mesophillic mixed dahi culture (NCDC 167) revealed that on incubation of these cultures with strawberry polyphenols extract at different phenolic concentrations 0.25 mg/ml, 0.5 mg/ml and 1.0 mg/ml, the growth of both the culture was not much affected negatively.

Ruggeri *et al*. (2008) [18] have reported no effect of polyphenols extract on the growth of S. thermophillus at concentration 40 mg/125g of yoghurt. Similarly Kahlil *et al*., 2010 also estimated the effect of gallic acid and catechin on the viability of proven probiotic cultures and observed that strain was unaffected upto 0.8% gallic acid and 0.3% catechin. Chodak *et al*., 2008 [20]) also reported that the catechin and chlorogenic acid had a stimulatory effect on *L. casei* growth while the quercetin had opposite effect up to an incubation time ≥ 6 hrs. Tabasco *et al*. 2011) [21] also observed the maximal growth of *Lactobacillus plantarum, Lactobacillus casei* and *Lactobacillus bulgaricus* in the presence of grape seed extract.

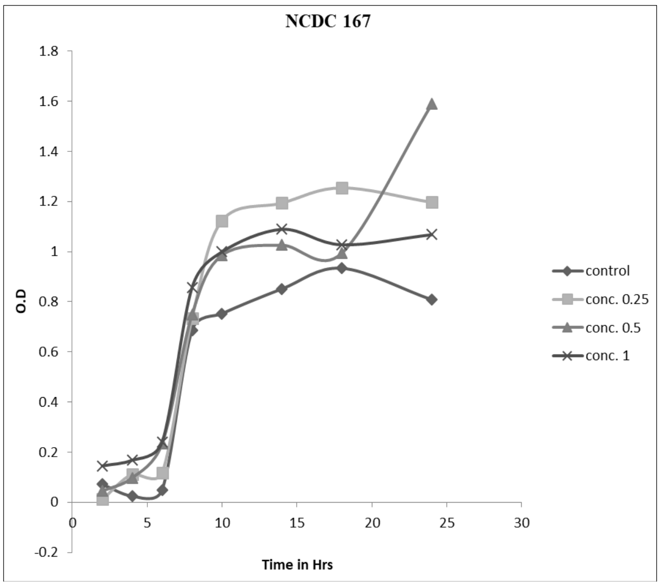

Fig (a): Growth of NCDC 167 at pH 7.0 with different polyphenols concentration

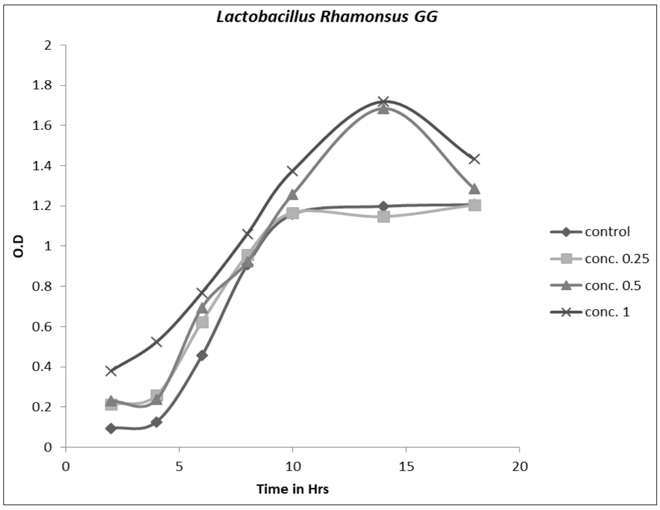

Fig (b): Growth of *Lactobacillus rhamnosus* at pH 6.5 with different concentration of polyphenols

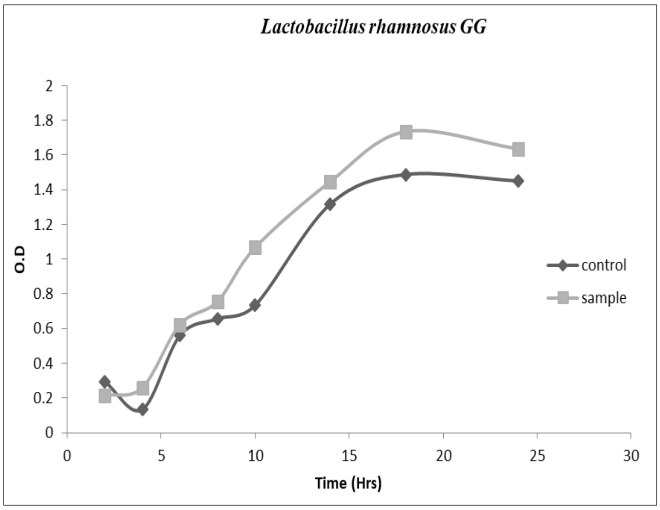

Fig (c): Growth of *Lactobacillus rhamnosus GG* at pH 6.1 with polyphenol concentration at 0.25 mg/ml

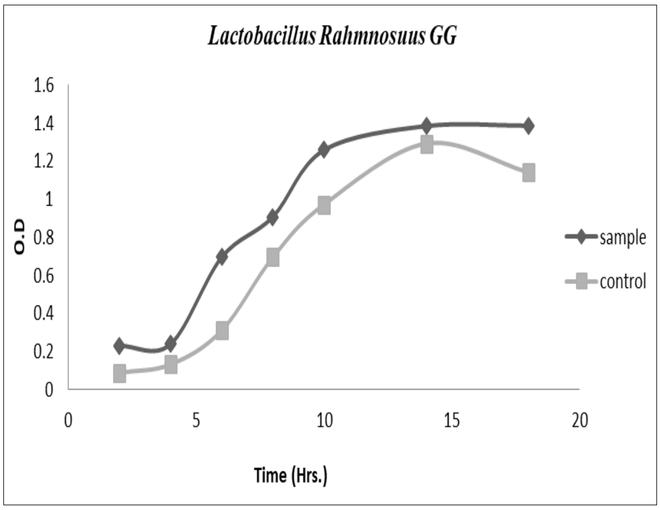

Fig (d): Growth of *Lactobacillus rhamnosus GG* at pH 5.7 with polyphenol concentration 0.5 mg/ml

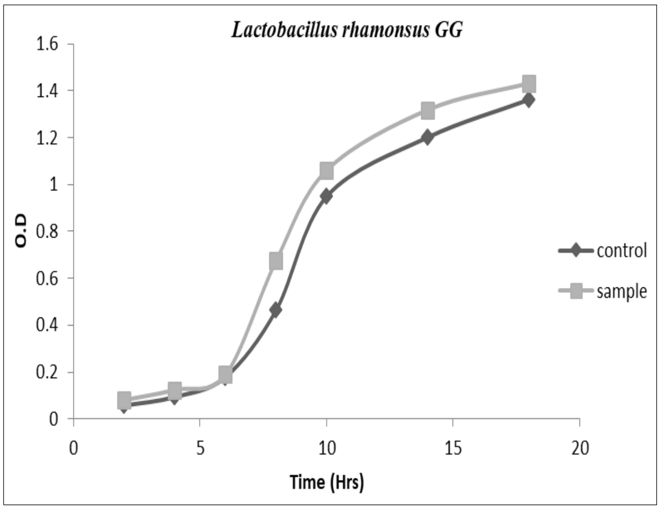

Fig (e): Growth *of Lactobacillus rhamnosusa* at pH 5.2 with polyphenol concentration 1.0 mg/ml

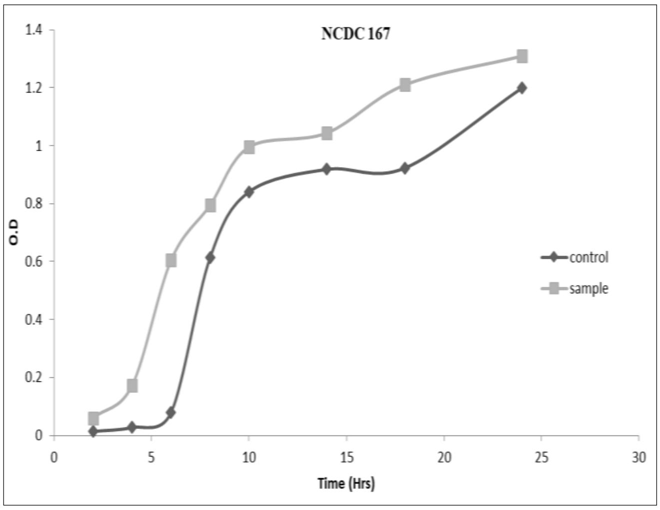

Fig (f): Growth of NDC 167 at pH 6.0 with polyphenol concentration 0.25 mg/ml

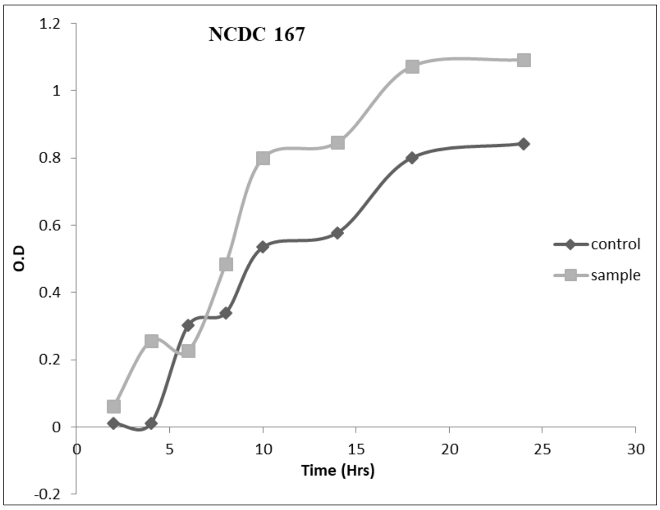

Fig (g): Growth of NCDC 167 at pH 5.9 with polyphenol concentration 0.5 mg/ml

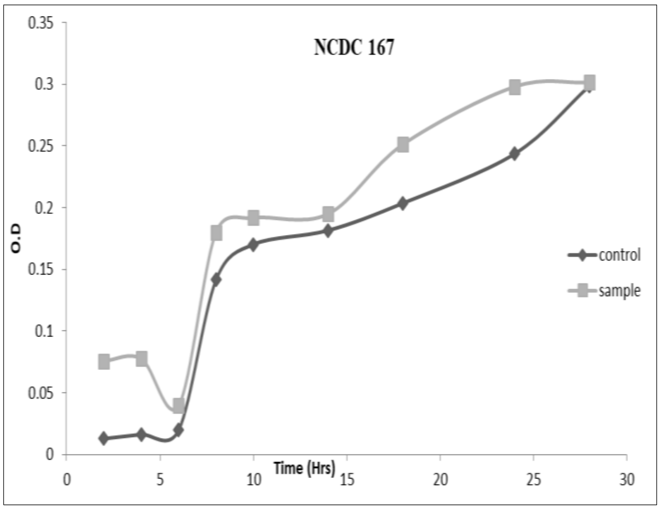

Fig (h): Growth of NCDC 167 at pH 5.4 with polyphenol concentration 1.0 mg/ml

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