Research Article

Available online through
www.ijrap.net

IJRAP
ISSN 2229-3566

ANTI-OXIDATIVE POTENTIAL OF PROBIOTIC BACTERIA FROM INDIAN FERMENTED FOOD

Sukumar Gowri and Ghosh Asit Ranjan*

Centre for Infectious Diseases & Control, Microbial Molecular Biology Laboratory, School of Bio Sciences and Technology, Vellore Institute of Technology University, Vellore- 632014, Tamil Nadu, India

Received on: 20/04/2011 Revised on: 29/05/2011 Accepted on: 16/06/2011

ABSTRACT

Lactic Acid Bacteria (LAB) with probiotic potentials isolated from fermented food of Indian origin were investigated for antioxidant activities. The antioxidant potential of the Cell Free Extracts (CFEs) of the nine probiotic isolates (GS3, GS4, GS7, GS9, GS14, GS16, GS17, GS20 and GS21) were investigated through different assays namely the Reducing power, DPPH free radical scavenging and β-Carotene bleaching assay. The Cell Free Extracts of isolates demonstrated reducing activity in the range of 60 to 92.66 µg/µl ascorbic acid equivalence; with the maximum activity being observed with the CFE of *Pediococcus* GS4 isolate (92.66µg/µl). DPPH free radical scavenging activity of the CFE of isolates was observed in the range of 48.67-91.26%. The β-Carotene bleaching assay was performed on CFE of isolates showing maximum activity in the above two assays. 150 µl of CFE of GS4 exhibited the maximum zone of colour retention of 22.00±1.000 mm and Butylated hydroxy toluene (1mg/100 µl) as positive control exhibited an average zone of colour retention of 27.00±0.577mm. 150 µl CFE of GS17 exhibited lower antioxidant activity in comparison to CFE of GS4 with the average zone of colour retention being 20.30±0.577mm. This extends the functional use of Probiotics as nutraceutical with antioxidant potential and may find therapeutic application for degenerative diseases related to ageing.

KEYWORDS: Fermented food, Lactic Acid Bacteria, Probiotic, Antioxidant activity

***Address for Correspondence**

Dr Asit Ranjan Ghosh, Professor and Asst. Director, Centre for Infectious Diseases & Control, Medical Biotechnology Division, School of Bio Sciences and Technology, Vellore Institute of Technology University, Vellore- 632014, Tamil Nadu, India Email: asitranjanghosh@vit.ac.in, asitranjan@yahoo.com

INTRODUCTION

The growing interest in understanding the role of food in human health has moved from its primary role as a source of energy to the subtle action of biologically active food components on human health. Henceforth functional foods or nutraceuticals are of great demand in present time. The growing scientific evidence suggests that the food supplements containing beneficial bacteria can provide an array of benefits to the host. Probiotic bacteria are widely used in human and animal nutrition and beneficially influence the balance of the intestinal flora of the host. Probiotics is defined as "live microorganism which when administered in adequate amounts confer health benefit on the host"[1]. Probiotic bacteria provide an array of health benefits which include competition, antagonistic effects, enhancement of digestion, strengthening of the immune system and stimulation of Vitamin production[2].

Oxidative damage has been found to play a major role in the development of Atherosclerosis, Cancer, Emphysema, Cirrhosis and Arthritis[3]. Generation of potentially harmful free radicals and Reactive Oxygen Species (such as hydroxyl radicals, hydrogen peroxide) are a result of various endogenous metabolic processes. In addition, other factors such as environmental pollution and UV irradiation could be responsible for the generation of free radicals[4, 5]. The free radicals are a potential cause of oxidative damage leading to cell death and tissue damage[6]. Lin and Yen, 1999 have discussed the role of food containing antioxidants and other antioxidant supplements in the reduction of oxidative damage on humans[7]. Fermentation enhances antioxidant activity[8]. Fermented foods such as *miso*, *natto* and *tempeh* have been reported to have antioxidant potentials [9, 10]. In the current study, Lactic acid bacteria isolated

from Indian fermented food with Probiotic potentials[11, 12] have been evaluated for antioxidant potential.

MATERIALS AND METHODS

Preparation of Cell Free Extract (CFE)

Sterile MRS broth (de Mann Rogosa Sharpe, pH 6.5±0.2 at 25°C) was inoculated with 1% (V/V) of the overnight grown culture of the nine isolates and incubated at 37°C for 18 hours. The CFE (Cell Free Extract) was obtained by centrifugation of the overnight grown culture at 10,000 rpm for 5 min at 4°C. The CFE of the nine isolates were subjected to different antioxidant assays namely Reducing power, DPPH free radical scavenging and β-Carotene bleaching assay.

Reducing power assay

All the CFE of the isolates were screened for reducing potential by the method described by Oyaizu (1986), with some modifications[13]. 100µl of the CFE was made up to 500 µl using distilled water. To each sample, 1.5 ml of 0.2 M Sodium phosphate buffer (pH 6.6) and 1.5 ml potassium ferricyanide (1%) were added in succession. The mixture was then incubated at 50°C for 20 min followed by the addition of 2.5 ml of Trichloroacetic acid (10%). The contents were centrifuged at 6000rpm for 5 min at 4°C to facilitate uniform mixing. The upper layer of the solution was diluted with 1.5ml distilled water. Finally 300 µl of ferric chloride (0.1%) was added and the absorbance was recorded at 700nm using un-inoculated MRS broth as control. The experiment was performed in triplicates and the average optical density value was calculated. The concentrations of antioxidant in the CFE were expressed in terms of Ascorbic acid equivalence.

DPPH free radical scavenging assay

Free radical scavenging activity of the CFE of isolates was measured using the procedure described by Heo et al., 2005 with slight modifications[14]. To 500 µl of the CFE (sample), 3.0ml of freshly prepared solution of 2, 2-DiPhenyl-2-Picryl Hydrazyl Hydrate (DPPH) at a concentration of 5mg/100ml (ethanol) was added. Control was prepared using 500µl of ethanol added to 3ml DPPH solution, mixed in dark and incubated for 30 min. Absorbance was recorded at 517nm after 30 min of incubation in the dark with un-inoculated MRS broth serving as blank. The readings were recorded in triplicates and the average absorbance value was calculated. The percentage of radical scavenging activity was calculated according to the equation [A_{517} control- A_{517} sample/ A_{517} control] x 100. The concentration of antioxidant in the CFE was read from the standard graph as Ascorbic acid equivalent.

Beta Carotene bleaching assay

The β-Carotene bleaching assay was carried out on the CFE of isolates showing good antioxidant activity in the above antioxidant assays[15]. Ten ml of Linoleic acid solution dissolved in ethanol (2mg/ml) and 10 ml of β-Carotene solution (2mg/ml) in acetone were added to 10ml of molten agar (1.2%). The contents were mixed thoroughly and poured into Petri dishes. The contents were allowed to solidify in dark. Wells of 8mm diameter were made using sterile agar borer. 100 µl and 150 µl of the CFE of GS4 and GS17 were transferred to the wells along with Butylated hydroxy toluene (1mg/100 µl) as positive control and negative control involved the use of un-inoculated MRS broth. Petri dishes were incubated at 45°C for 4 h. The zones of colour retention around wells were measured in mm and the average zone of colour retention was computed from triplicate readings.

RESULTS AND DISCUSSION

Reducing power assay

Reducing power assay measures the reducing ability of the CFE against the oxidative effects of Reactive Oxygen Species (ROS). The data for the reducing power ability of the CFE of isolates has been presented in table 1. The reducing power of the CFE of isolates ranged from 60 to 92.66 µg/µl ascorbic acid equivalents. Among the CFE of isolates, the maximum activity was observed with 100 µl of the CFE of GS4 (92.66 µg/µl) followed by GS17 with 85.33 µg/µl antioxidant concentration as Ascorbic acid equivalence. This is in accordance with the results indicated by Yi Chieh et al., 2006[16]. The report indicated an increase in reducing activity of *Lactobacillus acidophilus* and *Bifidobacterium longam* involved in soybean milk fermentation. The antioxidant activity observed is consistent with the previous reports[17, 18]. Several factors are responsible for the reducing activity of LAB. Peptides of the starter organism could be contributing to the reducing ability in addition to intracellular oxidants[19]. Shimada and coworkers have proposed the hydrogen-donating ability of the LAB isolates as a mechanism for antioxidant activity[20]. Besides these, reductones formed during fermentation could react with the free radicals to stabilize and terminate radical chain reactions[19].

DPPH free radical scavenging assay

DPPH, a relatively stable organic radical has been widely used in the determination of antioxidant activity[21]. Ability to scavenge hydrogen radicals is one of the important mechanisms for antioxidant activity. The scavenging ability of the CFE of the isolates was compared with the standard antioxidant- Ascorbic acid. Table 2 shows the results of the DPPH scavenging potential of the isolates. The antioxidant ability of the

isolates was found to be similar to that of the reducing power assay. The maximum antioxidant activity was observed with CFE of GS4 showing 91.26% followed by GS17 with 89.51% activity. Other isolates showed activity in the range of 48.67-91.26%. The results of the study are consistent with the observations of Bae jin Lee et al., 2010[22]. *Lactobacillus brevis* BJ20 strain isolated from traditional Korean fermented food showed significant scavenging potency in the range of 87.7-92.8%. *Leuconostoc mesenteriodes* and *Lactobacillus plantarum* involved in the fermentation of Chinese cabbage exhibited a maximum of 96.99% scavenging of DPPH radical[23]. DPPH radical scavenging ability is believed to be due to the hydrogen-donating ability of the isolates and the scavenging is visually noticeable as a colour change from purple to yellow[24].

Beta Carotene bleaching assay

In the β-Carotene/Linoleic acid model system, β-Carotene undergoes rapid de-colourization when the Linoleic acid is subjected to oxidation or in the absence of an antioxidant. Table 3 shows the results of Beta Carotene bleaching assay. 150µl of CFE of GS4 gave the maximum antioxidant activity by exhibiting maximum zone of colour retention (22±1.0mm) in comparison to positive control with the zone of colour retention of 27± 0.577mm. CFE of GS17 was also observed to possess antioxidant potential through the retention of colour. 150µl of CFE of GS17 showed higher antioxidant activity compared to 100µL of GS17. Yoghurt bacteria such as *S. salvarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 have been reported to have specific effect on oxidative stress[25]. The peptide hydrolysates from fermentation could be responsible for minimizing oxidative stress when analyzed for their antioxidant activity by the β-Carotene bleaching assay[26]. The comprehensive evaluation of the antioxidant potential using different tests has shown the importance of endogenous compounds of LAB.

CONCLUSION

From the studies carried out, it can be inferred that Probiotic bacteria isolated from Indian fermented food have demonstrated antioxidant potential studied through different assays. However, the isolates showed variation in their antioxidant potentials. This extends the application of probiotics as a nutraceutical with antioxidant potential and may find therapeutic potential in the management of degenerative diseases.

ACKNOWLEDGEMENT

The authors acknowledge the Management of Vellore Institute of Technology University for providing the Laboratory facilities for carrying out the research work.

REFERENCES

1. FAO/WHO. Health and Nutritional properties of Probiotics in food including powder milk with Live Lactic Acid Bacteria, Report from joint Food and Agriculture Organization (FAO) of the United Nations/World Health Organization (WHO) expert consultation, Argentina, 2001; pp. 1-34.
2. de Baets L, Iwaarden PV, Meeus N, Schimmel H, Philipp W, Emons H. First certified reference materials for molecular finger printing of two approved probiotic *Bacillus* strains. Int J Food Microbiol 2009; 129 Suppl 1:16-20.
3. Jacob RA. Nutrition, health and antioxidants. Inform 5 1994; 1271-1275.
4. Ames BN, Shigenaga MK, Hagen TH. Oxidants, antioxidants, and the degenerative diseases of ageing. Proceedings of the National Academy of Sciences of the United States of America 1993; 90:7915-7922.
5. Lenaz G. Role of mitochondria in oxidative stress and ageing. Biochimica et
Biophysica Acta 1998; 1366:53-67.
6. Kehrer JP. Free radicals as mediators of tissue-injury and disease. Critical Reviews in Toxicology 1993; 23: 21-48.
7. Lin MY, Yen CL. The beneficial effects of Lactic acid bacteria on human health. Journal of Chinese Nutrition Science 1999; 20:367-380.
8. Kusznierewicz B, S´ miechowska A, Bartoszek A, Namies´nik J. The effect of heating and fermenting on antioxidant properties of white cabbage. Food chemistry 2008; 108: 853-861.
9. Esaki H, Onozaki H, Osawa T. Antioxidative activity of fermented soybean products. In: Huang MT (Editor). Food Phytochemicals for Cancer Prevention I, Fruits and Vegetables. Washington DC: American Chemical Society; 1994. p. 353–360.
10. Sheih IC, Wu HY, Lai YJ, Lin CF. Preparation of high free radical scavenging *tempeh* by a newly isolated *Phizopus* sp.R-96 from Indonesia. Food Sci Agric Chem 2000; 2: 35-40.
11. Gowri Sukumar and Asit Ranjan Ghosh. Study of the probiotic potential of Lactic acid bacteria isolated from a variety of Indian fermented food. Journal of Pharmacy Research 2010; 3 Suppl 9: 2254-2257.
12. Gowri Sukumar and Asit Ranjan Ghosh. *Pediococcus* spp. – a potential probiotic isolated from *Khadi* (an Indian fermented food) and identified by 16S rDNA sequence analysis. African J Food Sci 2010; 4 Suppl 9: 597-602.
13. Oyaizu M. Antioxidant activities of browning reaction prepared from glucosamine. Jpn J Nutr 1986; 44: 307-315.
14. Heo SJ, Park EJ, Lew KW, Jeon YJ. Antioxidant activities of enzyme extracts from brown seaweeds. Bioresource Technol 2005; 96:1613-1623.
15. Graven EH, Deans SG, Svoboda KP, Mavi S, Gundidza MG. Antimicrobial and antioxidative properties of volatile (essential) oil of Artemsia afra Jacq. Flavour and fragrance Journal 1992; 7 Suppl 3:121-123.
16. Yi-Chieh Wang, Roch-Chui Yu, Cheng-Chun Chou. Antioxidative activities of soymilk fermented with Lactic acid bacteria *Bifidobacteria*. Food Microbiol 2006; 23: 128-135.
17. Berghofer E, Grzeskowiak B, Mundigler N, Sentall WB, Walcak JJ. Antioxidative properties of faba bean-, soybean- and oat *tempeh*. Int J Food Sci Nutr 1998; 49: 45–54.
18. Chung YC, Chang CT, Chao WW, Lin CF, Chou ST. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. J Agric Food Chem 2002; 50:2454–2458.

19. Yang JH, Mau JL, Ko PT, Huang LC. Antioxidant properties of fermented soybean broth. Food Chem 2000; 71:249–254.
20. Shimada K, Fujikawa K, Yahara K, Nakamura T. Anti-oxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992; 40: 945–948.
21. Katalinic V, Milos M, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem 2006; 94 Suppl 4:550–557.
22. Bae-Jin Lee, Jin-Soo Kim, Young Mi Kang, Ji Hyun Lim, Young-Mog Kim, Myung-Suk Lee, Min Ho Jeong, Chang-Bum Ahn, Jae-Young Je. Antioxidant activity and γ- amino butyric acid (GABA) content in sea tangle fermented by *Lactobacillus brevis* BJ20 isolated from traditional fermented foods. Food Chem 2010; 122: 271-276.
23. Yu- Ping sun, Cheng-Chun Chou, Roch- Chui Yu. Antioxidant activity of Lactic acid bacteria fermented Chinese cabbage. Food Chem 2009; 115: 912-917.
24. Baumann J, Wurn G, Bruchlausen FV. Prostaglandin synthetase inhibiting O_2^- radical scavenging properties of some flavonoids and related phenolic compounds. Naunyn, Schmiedebergs. Archives Pharmacology 1979; 308:27–32.
25. Chu-Chyn OU, Jiunn-Liang KO, Meei-Yn-Lin. Antioxidative effects of Intracellular extracts of yoghurt bacteria on Lipid peroxidation and intestine 407 cells. Journal of Food and Drug analysis 2006; 14 Suppl 3: 304-310.
26. Sakanaka S, Tachibana Y, Ishihara N, Juneja LR. Antioxidant properties of casein calcium peptides and their effects on lipid oxidation in beef homogenates. J Agri Food Chem 2005; 53: 464-468.

TABLE 1: REDUCING POWER ACTIVITY OF THE CFE OF THE ISOLATES AS ASCORBIC ACID EQUIVALENTS (µg/µl)

Isolate No.	Average Optical Density at 700nm	Concentration (µg/µl)
GS3	1.106	76.66
GS4	1.375	92.66
GS7	0.997	65.33
GS9	1.030	69.33
GS14	0.993	62.66
GS16	1.219	81.33
GS17	1.259	85.33
GS20	1.172	79.33
GS21	0.860	60.00

TABLE 2: FREE RADICAL SCAVENGING ACTIVITY OF THE CFE OF ISOLATES BY DPPH FREE RADICAL SCAVENGING ASSAY

Isolate No.	Average Optical Density at 517nm	Concentration of antioxidant µg/µl	% antioxidant activity
GS3	0.427	80	72.36
GS4	0.135	110	91.26
GS7	0.491	75	68.22
GS9	0.455	78	70.55
GS14	0.502	74	67.51
GS16	0.240	99	84.46
GS17	0.162	107	89.51
GS20	0.322	91	79.16
GS21	0.793	50	48.67
Control	1.545	0	0

TABLE 3: AVERAGE ZONES OF COLOUR RETENTION IN BETA CAROTENE BLEACHING ASSAY (mm)

Test substance	Average Diameter of zone of colour retention (mm)
GS4 CFE (100 µl)	20.00±0.000
GS4 CFE (150 µl)	22.00±1.000
GS17 CFE (100 µl)	18.67±0.577
GS17 CFE (150 µl)	20.30±0.577
Positive control (Butylated hydroxy toluene) 1mg/100 µl	27.00±0.577
Negative control(blank broth)	0

Source of support: Nil, Conflict of interest: None Declared