IJBPAS, July, 2022, 11(7): 3287-3296

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

HOME-FERMENTED YOGHURT AS A PROBIOTIC SUPPLEMENT TO CURE ANXIETY AND INSOMNIA

SRUTHI C[1*] AND SHRUTHI S[2]

1: Assistant Professor, Department of Biotechnology, Jyoti Nivas College Autonomous, Karnataka-560102, India

2: Department of Biotechnology, Jyoti Nivas College Autonomous, Karnataka – 560102, India

***Corresponding Author: Dr. Sruthi Cyriac: E Mail:** sruthicyriac@jyotinivas.org

Received 16th June 2020; Revised 24th July 2020; Accepted 22nd Nov. 2021; Available online 1st July 2022

https://doi.org/10.31032/IJBPAS/2021/11.7.6235

ABSTRACT

Today, most people suffer from insomnia and anxiety. Home-fermented yoghurt is a natural source of probiotic bacteria which can help in reducing anxiety and insomnia by regulating the gut brain axis. The present study aimed at isolating the colonies of rod-shaped gram-positive bacteria obtained from yoghurt, optimizing growth conditions and analyzing probiotic properties. The optimum growth conditions were observed to be at pH6-7 and temperature 25-35° C. They also exhibited excellent properties such as gamma-haemolysis, resistance to antibiotics and antimicrobial susceptibility. A study conducted to analyze its effect on anxiety and insomnia showed that out of 41.8% people who do not consume yoghurt 45.7% people had issues falling asleep, 61.9% had issues of waking up multiple times and 76.2% had anxiety and unspecified stress amidst sleep. Whereas, out of 58.2% people who consume yoghurt regularly the percentage was lesser. At the end of a controlled study conducted on 10 individuals for 8 weeks, significant results were produced. The present study shows that consumption of home-fermented unprocessed yoghurt on a regular basis can control anxiety and insomnia and can also improve overall health.

Keywords: Anxiety; Insomnia; Probiotic; Yoghurt; Survey

INTRODUCTION

Anxiety is a condition of emotional distress and excessive worry which has become a persistent issue over the last decade. Studies show that 33.7% of the population experience anxiety disorder during their lifetime **[1]**. Insomnia is a state of sleeplessness. Both onset insomnia and maintenance insomnia have increased at alarming rates over the last 15 years **[2]**. Studies show that insomnia is highly prevalent in anxiety disorders and vice versa **[3]**. There exists a bidirectional communication between the central and the enteric nervous system, which links the emotional and cognitive centers of the brain with peripheral intestinal functions, called the Gut-Brain Axis (GBA) **[4]**. The diverse range of microorganisms, naturally present in the gut, establish a connection with the brain through the GBA called the Microbiome-Gut-Brain (MGB) axis, impairment of which affects significant centers of the brain resulting in a disrupted state of mind **[5]**. Probiotics are living microorganisms that have a health benefit on the host when administered. These microbes help in activating the gut micro biome to restore a healthy MGB axis and brings about a balance in the emotional state of mind. It can reduce anxiety, depression, insomnia, stress and also improves the overall health of the individual **[6]**.

Yoghurt is an excellent source of various bacteria that help in the restoration of the MGB axis and thus provide a healthier state of mind **[7]**. Unfortunately, commercial yoghurt contains harmful additives and preservatives. The results of a study indicated higher levels of benzoic acid and sorbic acid than those set by regulatory agencies, used in yoghurt production **[8]**. Fermentation of milk into yoghurt is an easy process as well involving no mandatory expertise and is an easily affordable, unprocessed, safe source of a wide range of probiotic bacteria **[9]**.

The current study aimed at introducing use of unprocessed yoghurt as a probiotic supplement as it iseasily available, affordable and would produce least side effects with significant results in curing anxiety and insomnia. Objectives of the study were to test the various probiotic characteristics of microbes present in home-fermented yoghurt – by optimization of conditions, haemolytic property, antibiotic susceptibility and antimicrobial activity – and to study its effect on anxiety and insomnia by means of a survey.

MATERIALS AND METHODS

A. Isolation and Analysis of probiotic properties of bacteria in yoghurt

Isolation of bacteria from home-made yoghurt on MRS agar

de Man, Rogosa and Sharpe (MRS) agar, measuring 50ml was prepared and autoclaved **[10]**. The solution was poured into 2 petri plates and allowed to solidify. A stock solution of home-fermented yoghurt was serially diluted to 10^{-5} and 10^{-6}. 0.1ml of these samples were inoculated on the agar plates using an L-rod and incubated.

Gram Staining of the isolated colonies

The isolated bacteria were Gram stained and visualized under the microscope **[11]**.

Optimization of pH and temperature for the isolated bacteria

Optimization of conditions was done by modifying the work of Ashrafuzzaman *et al*. **[12]** Twelve test tubes with each 5ml nutrient broth were taken. Seven of these test tubes were adjusted to a pH of 3-9 respectively. The test tubes were autoclaved and the inoculum was added to all the test tubes. The 7 test tubes were incubated at 30^0C. The remaining 5 were incubated at temperatures 5^0, 25^0, 30^0, 40^0 and 50^0C respectively. The absorbance was measured after an incubation of 24 hours at 600nm wavelength using a spectrophotometer.

Culturing isolated bacteria on blood agar to test haemolytic properties

Haemolytic property of the microbes was assessed by modifying the protocol followed by Halder *et al*. **[13]**. 25ml of Nutrient agar was prepared and autoclaved. 2ml of mammalian blood was added to the nutrient agar and poured to a petri plate. The inoculum was streaked and the haemolysis capacity was analyzed by viewing the plates under UV light.

Test for antibiotic susceptibility of the isolated bacteria

Nutrient broth, measuring 25ml, was prepared and autoclaved. The broth was then cooled and a pinch of Streptomycin (2mg/100ml conc.) was added to the broth. The broth was inoculated with the isolated sample. Absorbance was measured after an incubation of 24 hours at 600nm wavelength using a spectrophotometer **[12]**.

Test for antimicrobial activity of the isolated bacteria

MRS agar, measuring 25ml, was prepared and autoclaved. A pour plate was made with *Staphylococcus aureus* and a well was made in the center. The well was filled with the isolated sample. The plate was incubated for 24hours and was observed for zone of inhibition **[12].**

B. Survey and Analysis - to study the effect of yoghurt on human health

A survey study was conducted to assess the effect of yoghurt on human health by modifying the work of Kitano *et al*. **[14]**.

General Survey

A general survey was conducted within the age group of 18-60 to analyze the sleep patterns and level of anxiousness in regular

and irregular consumers of yoghurt. The survey was conducted on 5 aspects: (i) Ability to fall asleep (ii) Ability to stay asleep - no. of times individual wakes up during sleep (iii) General feeling of anxiety and stress (iv) Inability to sleep due to experience of anxiety amidst sleep, and (v) Experience of frequent headaches. The data thus obtain was analyzed.

Controlled study using convenience sampling technique

A group of 20 individuals (age 18-60) experiencing insomnia and frequent anxiety were selected for the study. It was made sure that the subjects did not have disorders such as lactose intolerance, dairy allergy or any other discomfort pertaining to the consumption of yoghurt. Ten subjects were treated as the control. The remaining 10 individuals were asked to consume 100g yoghurt after consumption of food at night before sleep for 3 weeks and the quantity was increased to 150g for 5 weeks. A questionnaire **(Table 1)** was made and the subjects were asked to score the intensity/ frequency on scale of 1-10 (1 being lowest and 10 being highest). This was done at the beginning and at the end of 8 weeks. Observations were made as follows:

a. The mean score of all individuals along with percentage change for every category was calculated before and after the study.
b. The cumulative scores of each individual before and after the 8 weeks study was calculated in order to evaluate the improvement of the overall sleep patterns and levels of anxiety of each individual. The data thus obtained was plotted on a graph.

RESULTS AND DISCUSSION

A. Analysis of probiotic properties of bacteria in yoghurt

Isolation of bacteria from home-fermented yoghurt

The bacteria isolated on the MRS agar were observed as white pin-headed colonies **(Figure 1)**.

Gram staining of the isolated bacteria

The isolated bacteria were found to be Gram- positive bacilli **(Figure 2)**.

Optimisation of pH and temperature

The absorbance at different pH and temperature conditions **(Table 2)** indicated the optimum conditions for growth of the isolated bacteria to be pH 6 and temperature 25°C (optimum range of 25°-35°C).

Haemolytic properties of isolated bacteria

The blood agar plate displaying gamma hemolysis **(Figure 3)**, appearing brownish indicates they are non-pathogenic probiotic bacteria.

Antibiotic susceptibility of the isolated bacteria

Thebroth containing antibiotic showed+ 0.080absorbance. This shows that the

bacteria are susceptible to antibiotics and show significant growth resisting them.

Antimicrobial activity of the isolated bacteria

The incubated plate showed an 8mm wide zone of inhibition **(Figure 4)**.

B. Survey and Statistical Analysis - to study the effect of yoghurt on human health

General survey

Out of a population of 150 people within the age group of 18-60, 58.2% (87/150) were regular consumers of yoghurt while 41.8% (63/150) were people who do not consume yoghurt regularly.

Overall, it is seen that the percentage of population under stress, anxiety and insomnia is significantly high in the case of irregular consumers of yoghurt. Particularly, the percentage of population experiencing insomnia due to anxiety amidst sleep is drastically high in the case of irregular consumers (76.2%) in comparison to the regular consumers (49.5%).

Controlled study analysis using convenience sampling technique

a. The percentage change in the mean score for each category calculated was negative, Negative percentage change indicates a net decrease in the percentage of population with respect to each category. It clearly proved that regular consumption of home-fermented yoghurt over a period of 8 weeks can produce considerable improvement in the anxiety and insomnia levels of the individual **(Table 4)**.

   The total scores calculated before and after 8 weeks for each individual clearly shows a decrease over 8 weeks **(Figure 5)**.

Table 1: Questionnaire used for the controlled study

QUESTIONNAIRE Indicate your agreement or disagreement with the following sentences using numbers 1-10 (1- Very Low----10- Very High)		
1.	I have an issue falling asleep	
2.	I have an issue staying asleep (waking up multiple times)	
3.	I experience stress	
4.	I experience sleeplessness due to anxiety and unspecified stress at night	
5.	I experience frequent headaches (excluding cases of migraine)	

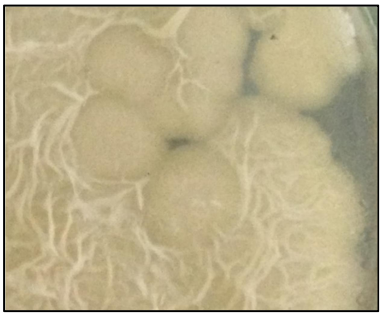

Figure 1: White pin-headed colonies observed on MRS Agar

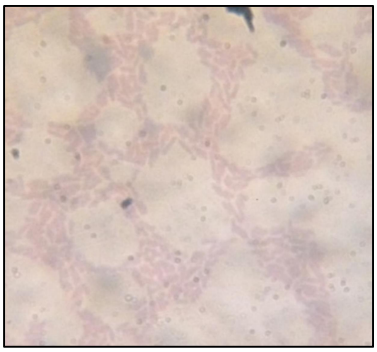

Figure 2: Colonies observed under 100X magnification after Gram Staining

Table 2: Absorbance values at different pH and temperature conditions

pH	O.D at 600nm	Temperature (^{0}C)	O.D at 600nm
3	0.021	5	0.021
4	0.032	25	0.032
5	0.049	30	0.049
6	0.208	35	0.208
7	0.105	40	0.105
8	0.076	50	0.076
9	0.050		

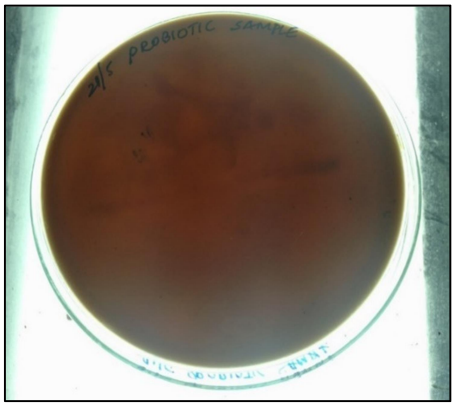

Figure 3: γ-haemolysis observed on blood agar with inoculation of isolated bacteria

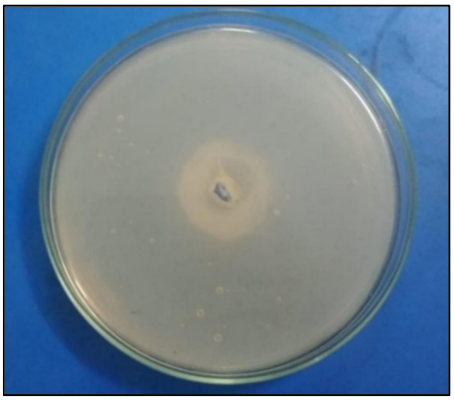

Figure 4: 8mm zone of inhibition on agar plate with *Staphylococcus aureus* inoculated with isolated bacteria

Table 3: Percentage of population that experience sleep related issues in consumers

	Regular consumers		Irregular consumers	
Difficulty falling asleep	32/87	37.3%	29/63	45.7%
Difficulty Staying asleep (waking up multiple times)	40/87	45.5%	39/63	61.9%
General stress	47/87	54.3%	41/63	65.6%
Inability to sleep due to anxiety amidst sleep	43/87	49.5%	48/63	76.2%
Experience of frequent headaches	15/87	16.7%	15/63	24%

Table 4: Calculations of the mean scores and percentage change form questionnaire

Mean Scores			
Category	Before (x)	After (y)	Percentage change $\frac{y-x}{x} \times 100$
Issue falling asleep	7.7	3.7	-51.94%
Issue staying asleep (waking up multiple times)	6.9	4.3	-37.68%
General stress	7.5	4.9	-34.67%
Feeling anxiety amidst sleep	7.9	3.8	-51.89%
Experience of frequent headache (excluding migraine)	4.1	3.2	-21.95%

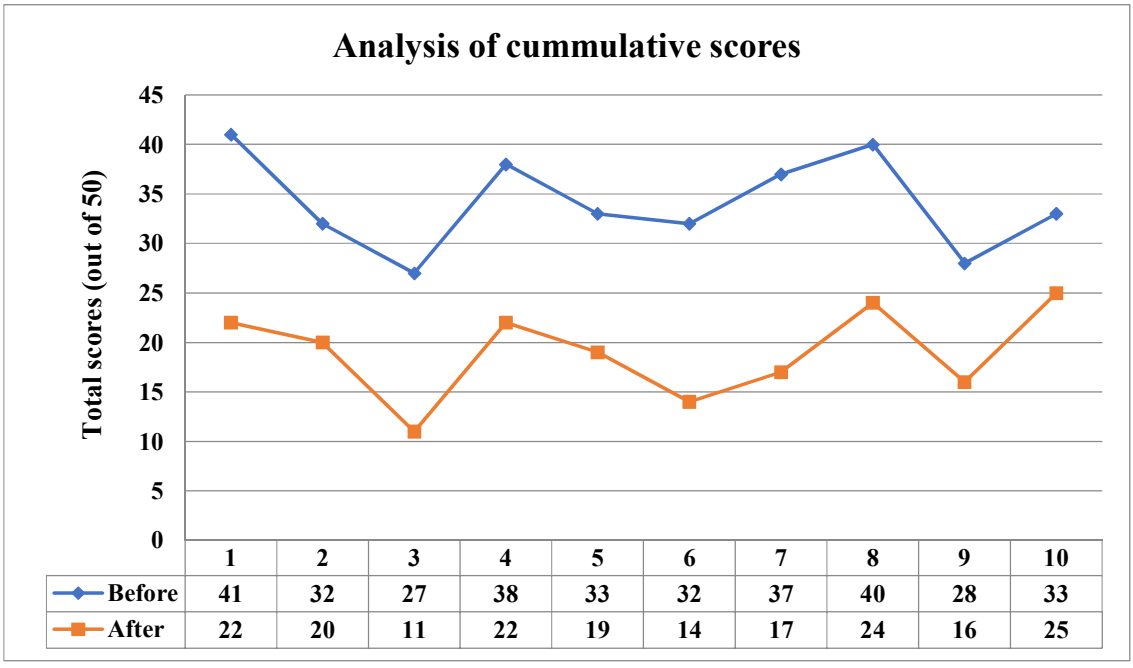

Figure 5: Chart showing the overall improvement in scores before and after the study over 8 weeks

CONCLUSION

The use of probiotic supplements is a rising trend due to its beneficiary properties and fewer side effects produced. However, synthetically manufactured supplements invariably contain components that produce side effects in the long run. Various harmful additives have been isolated from commercial yoghurt like non- nutritive sweeteners **[15]**, and additives are found to be added as protective agents for preservation **[16]**. Thus home-fermented yoghurt is definitely a safer, un-adulterated supplement, showing significant probiotic properties.

As against the work of Sharifan *et al*. **[17]** and Jaatinen *et al*. **[18]**, the present study indicated that normal yoghurt without any fortification or presence of bioactive compounds also had significant improvement in insomnia and quality of life. The survey study showed that the percentage of population facing sleep and anxiety issues could be significantly reduced by consumption of yoghurt regularly. Therefore, it can be concluded that regular consumption of home-made unprocessed yoghurt can be used as an effective probiotic supplement to cure anxiety and insomnia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

I would like to thank my project guide Dr. Sruthi Cyriac and Dr. Geeja S Kurian, Head of Department of Biotechnology, and all my teachers for their continued guidance. Above all I would like to thank Dr. Sr. Elizabeth C S for giving me the opportunity to do this project. I would also like to thank my parents, family and friends for their encouragement and support throughout the project work.

REFERENCES

[1] Bandelow B. & Michaelis S. (2015). Epidemiology of anxiety disorders in the 21st century. *Dialogues in clinical neuroscience,* 17(3), 327–335.

[2] Calem M., Bisla J., Begum A., Dewey M., Bebbington P. E., Brugha T., TewartR. (2012). Increased prevalence of insomnia and changes in hypnotics use in England over 15 years: analysis of the 1993, 2000, and 2007 National Psychiatric Morbidity Surveys. *Sleep,* 35(3), 377–384.

[3] Staner L. (2003). Sleep and anxiety disorders. *Dialogues in clinical neuroscience,* 5(3), 249–258.

[4] Carabotti M., Scirocco A., Maselli M. A. & Sever C. (2015). The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Annals of gastroenterology*, 28(2), 203–209.

[5] Jiang Hai-yin & Zhang Xue& Yu Zheng-He & Zhang Zhe & Deng Min &

Zhao Jian-Hua & Ruan Bing. (2018). Altered gut microbiota profile in patients with generalized anxiety disorder. *Journal of psychiatric research*. 104. 130-136.

[6] Clapp M., Aurora N., Herrera L., Bhatia M., Wilen E., & Wakefield S. (2017). Gut microbiota's effect on mental health: The gut-brain axis. *Clinics and practice*, 7(4), 987.

[7] Korpela K., Salonen A., Vepsäläinen O., Suomalainen M., Kolmeder C., Varjosalo M., de Vos W. M. (2018). Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome,* 6(1), 182.

[8] Mohamad M., Dina I., Jesele K., Bassam H., Amal M., Zeina K. & Imad T. (2008).Levels of benzoic and sorbic acid preservatives in commercially produced yoghurt in Lebanon. *Journal of Food, Agriculture & Environment,* Vol.6 (1): 62-66.

[9] Balamurugan R., Chandragunasekaran A. S., Chellappan G., Rajaram K., Ramamoorthi G., & Ramakrishna B. S. (2014). Probiotic potential of lactic acid bacteria present in homemade yoghurt in southern India. *The Indian journal of medical research*, 140(3), 345–355.

[10] Man J. C., de Rogosa M., and Sharpe M. E. (1960). A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* 23**,** 130–135.

[11] Bartholomew, J. W., & Mittwer, T. (1952). The Gram Stain. Bacteriological reviews, 16(1), 1–29. https://doi.org/10.1128/br.16.1.1-29.1952

[12] Ashrafuzzaman Md & Momtaz Farhana & Foysal Javed & Ali Md & Rahman Md. Mahbubur. (2015). Optimization of probiotic activity of Lactobacillus acidophilus characterized from commercial yoghurt. *International Journal of Biosciences*. 94-99.

[13] Halder D., Mandal M., Chatterjee S. S., Pal N. K., & Mandal S. (2017). Indigenous Probiotic Lactobacillus Isolates Presenting Antibiotic like Activity against Human Pathogenic Bacteria. *Biomedicines*, 5(2), 31.

[14] Kitano N., Tsunoda K., Tsuji T., Osuka Y., Jindo T., Tanaka K., & Okura T. (2014). Association between difficulty initiating sleep in older adults and the combination of leisure-time physical activity and consumption of milk and milk products: a cross-sectional study. *BMC geriatrics*. 14, 118.

[15] Boakes, R. A., Kendig, M. D., Martire, S. I., & Rooney, K. B. (2016). Sweetening yoghurt with glucose, but not with saccharin, promotes weight gain and increased fat pad mass in rats. *Appetite*, 105, 114–128.

[16] Ismail, E. A., Aly, A. A., & Atallah, A. A. (2020). Quality and microstructure of freeze-dried yoghurt fortified with additives as protective agents. *Heliyon*, 6(10), e05196.

[17] Sharifan, P., Khoshakhlagh, M., Khorasanchi, Z., Darroudi, S., Rezaie, M., Safarian, M., Vatanparast, H., Afshari, A., Ferns, G., Ghazizadeh, H., & Ghayour Mobarhan, M. (2020). Efficacy of low-fat milk and yogurt fortified with encapsulated vitamin D3 on improvement in symptoms of insomnia and quality of life: Evidence from the SUVINA trial. *Food science & nutrition*, 8(8), 4484–4490.

[18] Jaatinen, N., Korpela, R., Poussa, T., Turpeinen, A., Mustonen, S., Merilahti, J., & Peuhkuri K. (2014). Effects of daily intake of yoghurt enriched with bioactive components on chronic stress responses: a double-blinded randomized controlled trial, *International Journal of Food Sciences and Nutrition*, 65: 4, 507-514.