Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 6 Issue 10 October 2023

Research Article

Degradation of 4-Nitroquinoline-1-Oxide by Lactic Acid Bacteria

Priyanka Chaturvedi[1], Rimjhim, Pathak[2], Subhash Chandra Biyan[3], Himani Parihar[1] and Prem Saran Tirumalai[1]*

[1]Dayalbagh Educational Institute, Faculty of Science, Department of Agriculture Sciences, Uttar Pradesh, India

[2]Dayalbagh Educational Institute, Faculty of Arts, Department of Home Science, Uttar Pradesh, India

[3]Central Lab, Patanjali Food and Herbal Park Ltd, Uttarakhand, India

***Corresponding Author:** Prem Saran Tirumalai, Dayalbagh Educational Institute, Faculty of Science, Department of Agriculture Sciences, Uttar Pradesh, India.

DOI: 10.31080/ASMI.2023.06.1303

Received: August 17, 2023

Published: September 19, 2023

Abstract

Colorectal cancer is a major global health concern, with a strong link to processed meat consumption and the formation of carcinogenic compounds. This study explores the potential of selected probiotic strains, including *Lactobacillus plantarum, Lactobacillus casei*, and *Bifidobacterium bifidum*, in degrading a specific carcinogen called 4-Nitroquinoline-1-Oxide (4-NQO). The research investigates the effect of 4-NQO on the growth of the test organisms individually and as a consortium, as well as the degradation of 4-NQO by the test organisms. The study employs culture methods and High-Performance Liquid Chromatography (HPLC) to analyze the growth patterns and degradation of 4-NQO over specific time intervals. The results show minimal impact of 4-NQO on the growth of *Lactobacillus casei*, while *Lactobacillus plantarum* demonstrates a slight decline in growth. *Bifidobacterium bifidum* exhibits consistent growth patterns in the presence of 4-NQO, indicating resilience to the carcinogen. Additionally, the consortium of cultures shows similar trends to the individual strains. These findings also contribute to understanding the potential role of selected probiotic species in the degradation of 4-NQO and its implications for cancer mitigation.

Keywords: 4-Nitroquinoline-1-Oxide (4-NQO); *Lactobacillus casei*; *Lactobacillus plantarum*; *Bifidobacterium bifidum*

Introduction

According to the American Cancer Society's Facts and Figure 2020-2022 report, colorectal cancer ranks third among cancers worldwide and has a high number of cases [1]. In the United States alone, approximately 147,950 cases were recorded between 2020 and 2022. There is growing evidence linking diet to colorectal cancer, particularly the role of processed meat. High protein and nitrate content in processed meat may contribute to the formation of Heterocyclic Aromatic Compounds (HCA) during cooking. HCAs are carcinogenic and therefore increase the risk of colorectal cancer [2-4].

Recent studies have also shown a connection between meat intake, especially when cooked at high temperatures, and colon/pancreatic cancer, with HCA potentially playing a role [5]. HCA was first discovered in cooked foods over 25 years ago by Sugimura *et al* [6], and 4-Nitroquinoline 1-oxide (4-NQO), an HCA was reported to be carcinogenic [7,8].

Considering the widespread use of high cooking temperatures for meat, the presence of 4-NQO in a meat-based diet may be a possibility and the reason for carcinogenesis. While reducing

processed meat consumption can be a preventive measure, the use of microbes for mitigation can also be promising. The reduction of tumorous hazards has been reported as one of the health benefits conferred by probiotic microbes in the diet [9]. There have been reports that *Bifidobacterium longum* when administered in the diet with food mutagen 2-amino-3-methyl 3H-imidazo[4,5f] quinoline (IQ), inhibited liver and colon cancer that could be induce by the mutagens [10]. Another study states that the oral supplementation of *Lactobacillus acidophilus* NCFB1748 and *Bifidobacterium longum* BB536 decreased the bioavailability of 3-amino-1,4-dimethy5H-pyrido –[4,3-b] indole [Trp-P-1],3-amino-1-methyl-5H-pyrido-[4,3-b] indole [Trp-P-2] [11]. There are also reports citing the role of *Lactobacillus rhamnosus* in effectively transforming 4-NQO into inactive compounds [12]. These reports and many others establish the role of probiotic strains in cancer mitigation. This study aims to understand the possible role of selected commonly used probiotic species, namely *Lactobacillus plantarum*, *Lactobacillus casei*, and *Bifidobacterium bifidum* in the degeneration of 4-NQO.

Material and Methods

Cultures and chemicals

4-nitroquinoline-1-oxide (4-NQO; Product number. N8141; Sigma Aldrich), (CAS Number- 56-57-5) was used in the study. A stock solution of 1000 ppm of 4-NQO in Dimethyl sulfoxide (DMSO) was prepared, which was then further diluted as required for the study. *Lactobacillus casei* (ATCC 12116) was procured from the National Collection for Industrial Microbes (NCIM), Pune, India., *Lactobacillus plantarum* (ATCC 8014), and *Bifidobacterium bifidum* (ATCC 29521) were procured from the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) Culture Collection (NRRL), USA. These cultures were further revived and identified using biochemical tests and refrigerated for further use. Tryptone Soy Broth (Himedia- M011) was used for culture studies.

Experimental design

Study on the effect of 4-NQO on Test organisms

4- Nitroquinoline1-oxide (4-NQO) was studied for its effect on the test organisms *Lactobacillus casei* (LC), *Lactobacillus plantarum* (LP), and *Bifidobacterium bifidum* (BB) individually and collectively as consortia. The effect was observed on the growth pattern by enumerating the culture concentrations at specific time intervals (0hr, 24hr, 48hr and 72hr). Each of the test organisms, LP, LC and BB were processed as detailed below. The test organism(s) were inoculated in Tryptone Soy Broth (TSB) medium with and without 4-NQO. 1ml of the test organism(s) were inoculated to 100ml TSB and the same to be referred to hereafter as control. Similarly, another set of 100ml TSB with 100µl of 4-NQO (in DMSO) was inoculated with 1ml of the test organism(s). Both the test(s) and the control(s) were incubated at 37°C. For reference the tests hereafter will be referred to as T-LP, T-LC, T-BB, T-Co corresponding to Test with *L. plantarum*, *L. casei, B. bifidum* and Consortia and the controls will be referred to as C-LP, C-LC, C-BB and C-Con corresponding to Control of *L. plantarum*, *L. casei, B. bifidum* and Consortia. The cell density of the test organism(s) was estimated at specified time intervals from the initial 0hrs (start of the test) and at 24, 48 and 72hrs in both the control (C) and test (T) conditions. This was indicative of the growth pattern of the test organisms unperturbed as control(C) and in the presence of the carcinogen, 4-NQO. The comparison of the growth pattern of control to the test, estimated as cell density, to be indicative of the effect of the 4-NQO on the test organisms. The cell density estimation was done by standard culture method [13]. The design of the experiment is as shown in Table 1.

Test Organism	**Control**				**Test**			
Lactobacillus plantarum(LP)	0hr	24hrs	48hrs	72hrs	0hr	24hrs	48hrs	72hrs
	LP-C				LP-T			
Lactobacillus casei(LC)	0hr	24hrs	48hrs	72hrs	0hr	24hrs	48hrs	72hrs
	LC-C				LC-T			
Bifidobacterium bifidum(BB)	0hr	24hrs	48hrs	72hrs	0hr	24hrs	48hrs	72hrs
	BB-C				BB-T			
Consortium(Co)	0hr	24hrs	48hrs	72hrs	0hr	24hrs	48hrs	72hrs
	Con-C				Con-T			

Table 1: Experimental design for the study on the effect of 4-NQO on test organisms.

Degradation study of 4-NQO by the test organisms

The test organisms of *Lactobacillus plantarum* (LP), *Lactobacillus casei* (LC), *Bifidobacterium bifidum* (BB) individually and as consortium (Con) were grown in Tryptone Soy Broth at 35°C in the presence of 200 ppm 4-NQO. The tests for further understanding and ease will be referred to as LP-T (*L. plantarum* with 4NQO), LC-T (*L. casei* with 4-NQO), BB-T (*B. bifidum* with 4NQO) and Con-T (Consortia of cultures with 4NQO) as indicated in Table 1, and the control (4-NQO without culture). For each test (LP, LC, BB and Con-NQO), 4 sets of 8ml broth (TSB) were prepared and the same were made up to 10ml with filter sterilized 2ml of stock solution of 4-NQO in each.

100µl of bacterial cultures (BB, LC, LP, and Con), were separately inoculated accordingly to each tube. 01 set from each of the tests (LP, LC, BB and Con-NQO) were processed for analysis immediately after inoculation to estimate the initial concentration of 4-NQO in the broth, the same to be read as 0hrs reading or the initial concentration. The tests were then incubated at 35°C. At specified time intervals of 24, 48 and 72hrs, one tube from each set was drawn to estimate the concentration of 4-NQO by HPLC at that time interval. The tests draw for HPLC was centrifuged at 4000 rpm at 4°C for 20 min. The cell-free supernatant (CFS) was then filtered by using a 0.45µ filter and subjected to HPLC analysis.

Estimation of 4-NQO by HPLC

The Chromatographic separation of HCA's was achieved using the reverse phase of Agilent 1290 infinity quaternary LC system with Agilent Poroshell 120 EC- C18, 4.6x150 mm column. The injection volume was 10 µl and samples were injected by autosampler. Methanol and water were used as mobile phase for analysis. A linear gradient at 1.5ml per minute was produced from 10% or 20% methanol in water to 90% or 100% methanol using the Variable Wavelength Detector (VWD) which was set at a range of 360nm.

Results

Effect of 4-NQO on test organisms

The effect of 4-NQO was studied on the selected test organism individually (LC, LP and BB) and as consortia. The effect studied was on the growth of the test organisms, individually and as consortia both, by estimating the cell concentration, represented as Log10 values of CFU/ml. Standard culture plating technique was used to estimate the cell concentration (CFU/ml) [13]. The cell concentrations were estimated at the start of the study (0 hrs) and the value obtained represented as initial concentration. Thereafter, the cell concentrations of each of the test organisms (individually and as consortia) were estimated at specified time intervals (24, 48 and 72 hrs). The estimated results are as shown in Table 2.

Growth of test organisms measured as cell concentration presented in Log10 Values of CFU/ml							
Test Organisms	**Initial Concentration**	**Control at time intervals without 4 -NQO**			**Test at intervals with 4- NQO**		
	0 hr	**24hrs**	**48hrs**	**72 hrs**	**24hrs**	**48hrs**	**72hrs**
L. casei (LC)	6.25	8.04	8.89	9.04	8.17	8.81	9.02
L. plantarum (LP)	6.17	8.45	8.1 3	8.24	8.21	8.17	7.3
B. bifidum (BB)	7.66	9.81	9.77	9.64	9.67	9.57	9.57
Consortium (Con)	10.16	11.31	11.31	11.23	11.17	11.13	11.15

Table 2: Effect of 4NQO on the growth of Test organisms.

Effect of 4-NQO on *L. casei*

The growth of *L. casei* was minimally affected by 4-NQO. The variation in the Log10 values of cell concentration of LC as tested with 4-NQO as compared to the control was negligible. While the Log 10 value was 6.25 as the initial concentration at 0 hrs, the growth pattern of LC as control were 8.04, 8.89, and 9.04 at 24, 48 and 72 hrs, respectively, and the growth pattern of LC as test (with 4-NQO) was correspondingly similar with 8.17, 8.81 and 9.02 at 24, 48 and 72hrs respectively. It is to be noted that the variation in Log10 values from the control to the test was not more than 0.7 estimated at any time interval during the test.

Effect of 4-NQO on *L. plantarum*

Observations similar to that of *L. casei,* were made with *L. plantarum* though, the log concentration varied to a notable level close to 1.0, after 72hrs of incubation. The log concentration value of LP as initial concentration was 6.17. LP as control showed a growth pattern with log concentrations of 8.45, 8.13 and 8.24 at 24, 48 and 72hrs, whereas as test the log concentrations were 8.21, 8.17 and 7.3 at 24, 48 and 72hrs, respectively.

Effect of 4-NQO on *B. bifidum*

The growth pattern of *Bifidobacterium bifidum* in the presence of 4-Nitroquinoline-1- oxide (4-NQO) also demonstrated a consistent trend of initial growth followed by a gradual decline with both control and the test, with no significant variation in the cell concentrations between the two. The log concentration value of BB as initial concentration was 7.66. BB as control showed a growth pattern with log concentrations of 9.81, 9.77 and 9.64 at 24, 48 and 72hrs, whereas as test the log concentrations were 9.67, 9.57 and 9.57 at 24, 48 and 72hrs, respectively.

Effect of 4-NQO on consortium

The results of the study of effect of 4-NQO on the cultures collectively as consortia was in concurrence to observations made individually on the selected strains LC, LP and B-B. The growth pattern of the consortium in the presence of 4 Nitroquinoline-1-oxide remained marginally affected as compared to the growth in control. The variation from control to the test observed periodically at 24, 48 and 72hrs were 0.14, 0.18 and 0.10. The growth in number of cells, both in control and test appeared to show no progression. The reasons for the same could be attributed to the co-culture conditions where competition, altruism or antibiosis expressed amongst the strains could mark the progression of each other, a subject that needs attention though is beyond the scope and object of the experiments conducted. Figure 1 depicts the variation in the growth pattern of individual cultures and as consortia as control and as tests (with 4-NQO).

Estimation of 4-NQO by high-performance liquid chromatography (HPLC)

While the effect of the carcinogen 4-NQO on the probiotic strains was studied on one hand, analysis was also carried out simultaneously to estimate the concentration of 4-NQO periodically, to study its degradation by the test organisms, and the same is discussed in the succeeding paragraphs. Individually and as a consortia, the concentration of 4-NQO was estimated by HPLC in the presence of test organisms, at specific time intervals as shown in Figure 2 (A-E) as chromatograms. Table shows the estimated values of 4-NQO in parts per million (ppm).

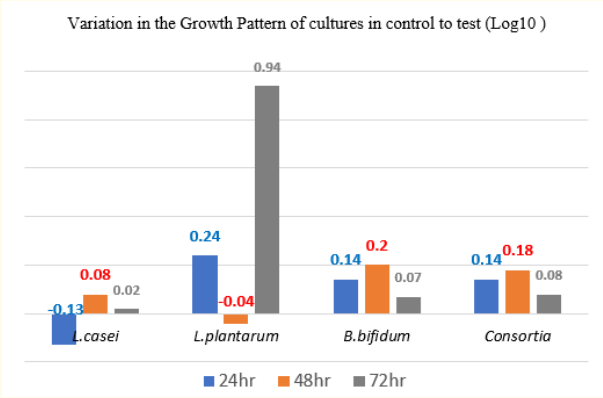
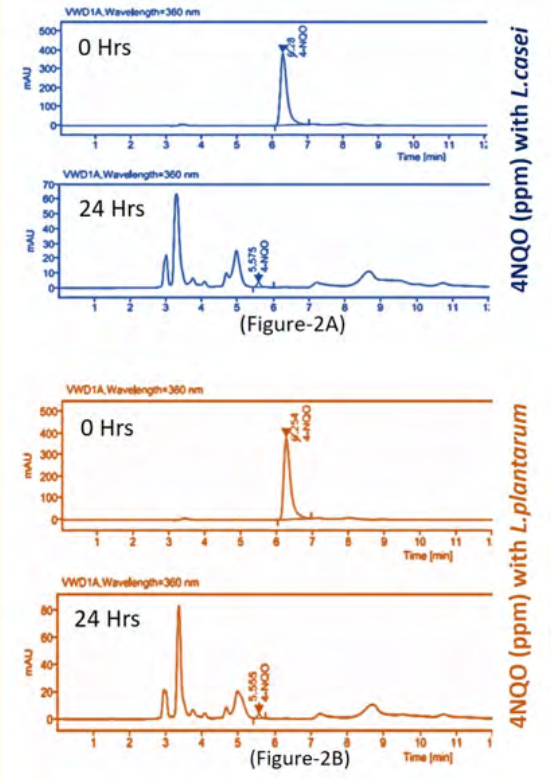
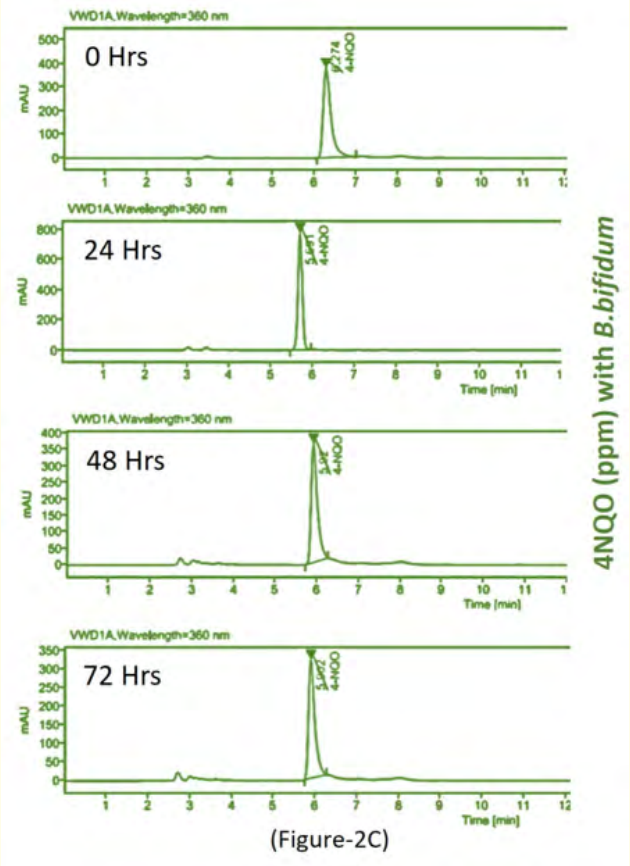
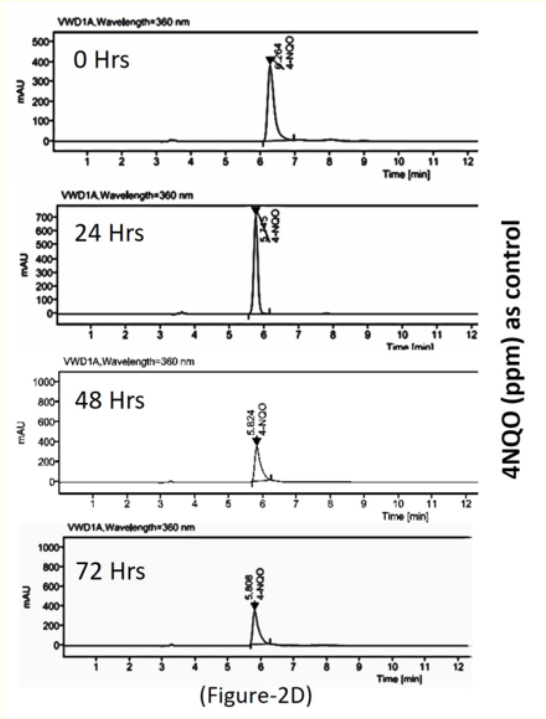


Figure 1: Effect of 4-NQO on the growth of test organisms.

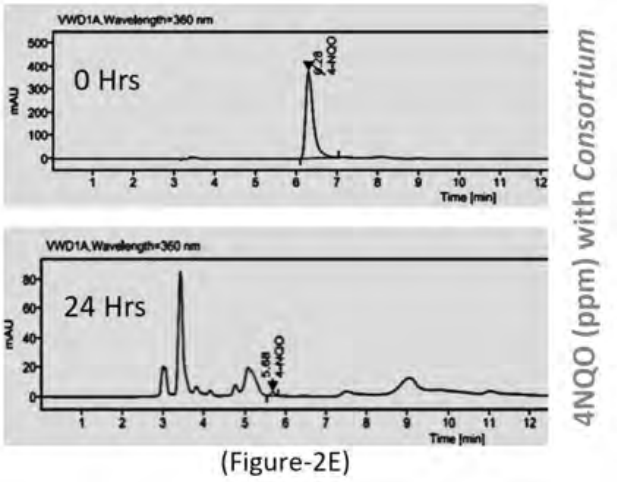




(Figure-2C)



(Figure-2D)



(Figure-2E)

Table-4.0 Estimated 4NQO Concentrations (in ppm) with test organisms and as control

	4NQO Control	4NQO + *L.casei*	4NQO + *L.plantarum*	4NQO + *B.bifidum*	4NQO + *Consortia*
0hrs	199	199	197	198	197.4
24hrs	198	< 1	< 1	197	< 1
48hrs	196	-	-	169	-
72hrs	194	-	-	149	-

Figure 2: (A-E): Chromatograms depicting the estimated concentration of 4-NQO as control and with the test organisms.

Degradation of 4-NQO by L. casei

As discussed in the earlier section and as shown in Figure 3a, 4-NQO had a negligible effect on the growth of *L. casei.* Evidently, the variation in growth, estimated as CFU/ml at specific time intervals (24, 48 & 72 hours) was linearly progressive both in control as well as test and with insignificant variation between them. Nevertheless, the concentration of 4-NQO (ppm) when estimated at the same specified time intervals in the presence of the test organism *L. casei* the concentration reduced considerably (close to nil) within 24hrs, while the control with 4-NQO (only) in broth, showed marginal reduction over time (Figure 3b).

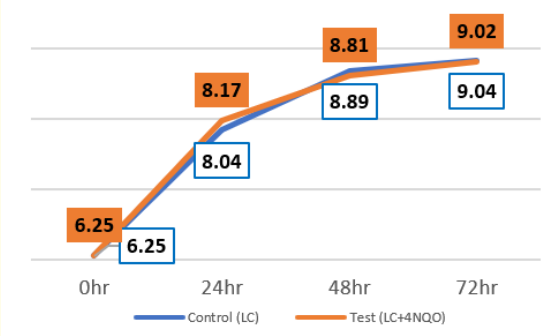


Figure 3a: The growth pattern of *L. casei* in control (absence of 4-NQO) and test (presence of 4-NQO).

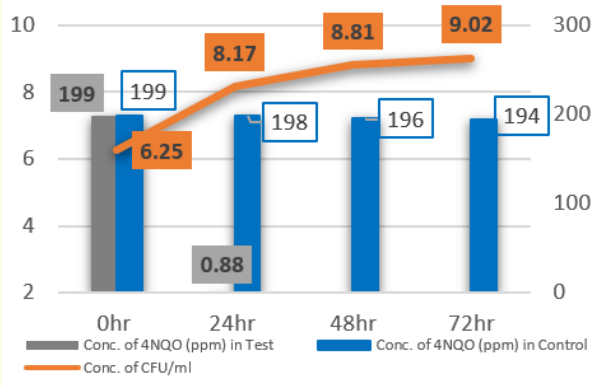


Figure 3b: Concentration of 4-NQO in Control and Test vis-a-vis growth pattern of *L. casei* in the presence of 4-NQO.

Degradation of 4-NQO by *L. plantarum*

Similar to that of *L. casei*, the growth of *L. plantarum* as tested in the presence of 4-NQO was progressive till 48hrs of incubation. The growth pattern of the test to the control showed insignificant variation, but at 72hrs the cell concentration of *L. plantarum* in the test declined significantly as compared to the control (Figure 4a), the Log 10 values of the test and control being 7.3 and 8.24, respectively. Correspondingly, the concentration of 4-NQO reduced from 197 ppm to less than 1 ppm within 24hrs of incubation with the test organism *L. plantarum* (Figure 4b).

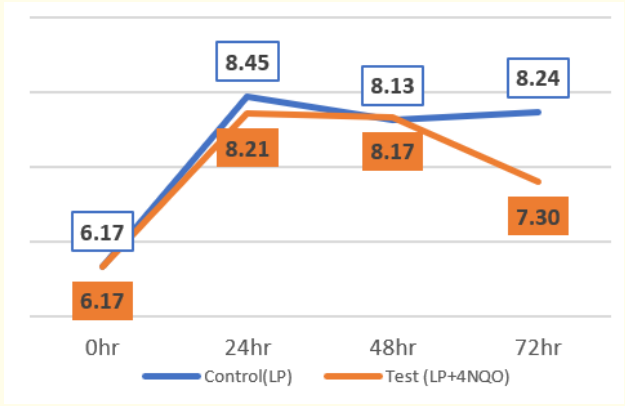


Figure 4a: The growth pattern of *L. plantarum* in control (absence of 4-NQO) and Test (presence of 4-NQO).

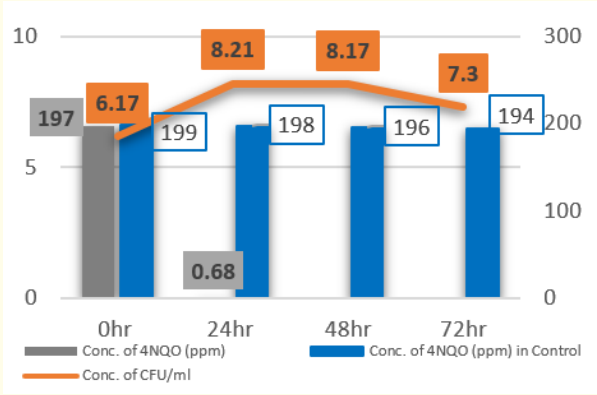


Figure 4b: Concentration of 4-NQO in Control and Test vis-a-vis growth pattern of *L. plantarum* in the presence of 4NQO.

Degradation of 4-NQO by *B. bifidum*

The growth of *B. bifidum* as a test organism with 4-NQO was not significantly affected. The trend of the growth pattern of *B. bifidum* both as test and control were similar with minor variations. The growth in test was marginally reduced as compared to the control (Figure 5a). Unlike the cases with the previous two test organisms (*L. casei* and *L. plantarum*), 4-NQO in culture broth of *B. bifidum* did not show marked reduction in concentration despite incubation for a period of 72hrs. The concentrations of 4-NQO at 24, 48 and 72 hrs were 198, 196 and 194 ppm, respectively (Figure 5b).

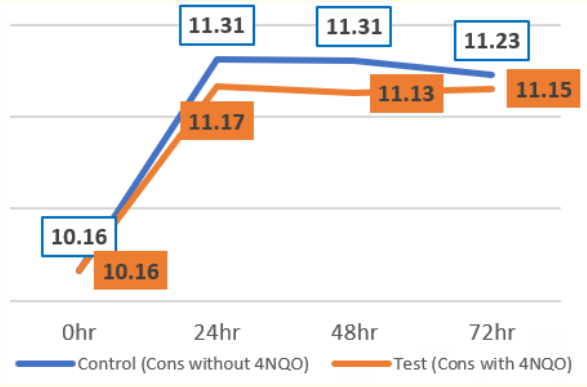


Figure 5a: The growth pattern of *B. bifidum* in control (absence of 4-NQO) and Test (presence of 4-NQO).

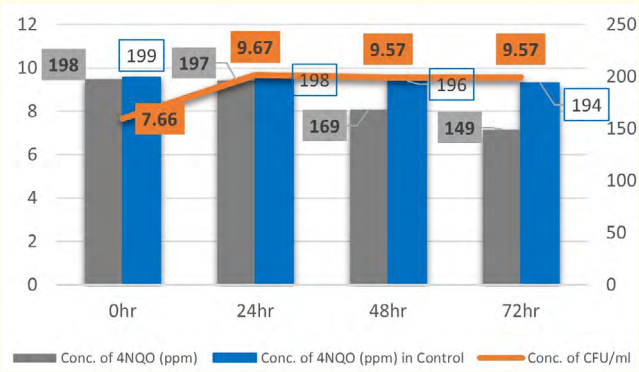


Figure 5b: Concentration of 4-NQO in Control and Test vis-a-vis growth pattern of *B. bifidum* in the presence of 4-NQO.

Degradation of 4-NQO by Consortia of cultures *L. casei*, *L. plantarum* and *B. bifidum*

The growth of the consortia cultures estimated collectively remained unaffected by 4-NQO throughout the period of observation. The growth pattern was typical to both the test and the control with insignificant reduction in the test as compared to the control after 24hrs of incubation (Figure 6A). The concentration of 4-NQO on the other hand reduced from above 190 ppm to less than 0.64 ppm with a period of 24hrs in the test with the consortia, while the concentration of 4-NQO in control broth (without cultures) did not show any significant reduction. These results were comparable to that of *L. casei* and *L. plantarum* as tests with 4-NQO (Figure 6B).

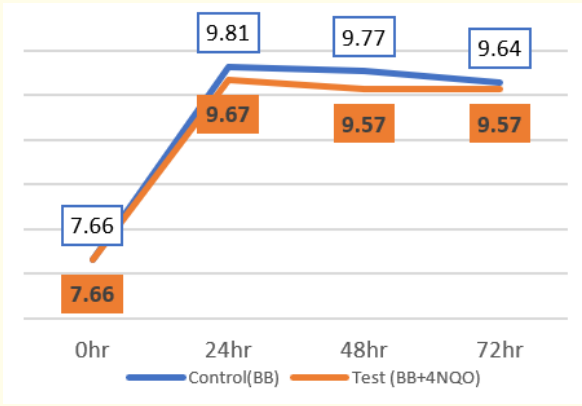


Figure 6a: The growth pattern of Consortia (LP+LC+BB) in control (absence of 4-NQO) and Test (presence of 4-NQO).

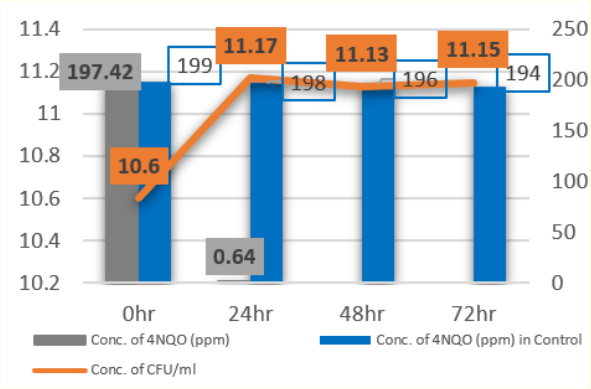


Figure 6b: Concentration of 4-NQO in Control and Test vis-a-vis growth pattern of Consortia (LP+LC+BB) in the presence of 4-NQO.

Discussion

Research paradigm on the potential degradation of carcinogens by probiotics is still evolving, and needs to be understood. While there have been reports associating the intake of dairy products and a reduced occurrence of colorectal cancer, there have been studies suggesting that certain probiotics may play a role in the degradation or detoxification of carcinogens [14]. For instance, the ability of *Lactobacillus rhamnosus* GG to detoxify Aflatoxin B1 [15], detoxification of Heterocyclic Aromatic Amines (HAAs - HAAs are carcinogenic compounds formed during high-temperature cooking of meat and fish) by *Lactobacillus casei* and *Lactobacillus*

plantarum [16,17]. Similarly, metabolism of Polycyclic Aromatic Hydrocarbons (PAHs) by *Lactobacillus* and *Bifidobacterium* [18], have been reported lately. It is important to note that although these studies suggest a potential role for certain probiotics in degrading or detoxifying carcinogens, the potency of specific strains or species of probiotics and their efficacy remains unexplored.

In this study, probiotic species, particularly, *L. casei*, *L. plantarum,* and *B. bifidum,* have been screened for their potency to degrade 4- NQO, along with the possible counter effect of 4-NQO on the organism in terms of the growth and propagation. The results indicate that the concentration of 4-NQO was reduced by *L. casei* and *L. plantarum*, within 24hrs. The concentration of 4-NQO (in ppm) was reduced to less than 1ppm which was initially 200 ppm. However, the same was not observed with the *B. bifidum*, wherein there was a gradual concentration (4-NQO) reduction over a period of 72hrs, the reduced concentration was less compared to the control though, it was not as comparable to that of the other two *Lactobacillus spp*. Similarly, 4-NQO was degraded with a consortium containing all three test organisms with the same efficacy as *L. casei* and *L. plantarum*.

It is also of relevance to infer that the carcinogen did not show any effect on the growth of the test organisms as observed in the study. These observations are indicative of carcinogen (4-NQO) degradation by the test organisms, *L. casei* and *L. plantarum*. This observation is important for the mitigation of cancer that are mediated via foods. One of the major causes of food-mediated cancers (Colorectal cancer for instance) are food-derived carcinogens. Food-derived carcinogens can originate from various sources, including natural processes, food processing techniques, and environmental contamination, however, one of the main causes is the high-temperature processing of meat and meat products. High-temperature cooking methods, such as grilling, frying, roasting, and smoking, can lead to the formation of carcinogenic compounds. For example, the Maillard reaction, which occurs when proteins and carbohydrates are heated together, can produce heterocyclic aromatic amines (HAAs) and polycyclic aromatic hydrocarbons (PAHs), both of which are known carcinogens. The risk of cancer increases multifold with consumption of heat-processed meat owing to 2 reasons, namely, i) the formation of the carcinogens, while processing of food, ii) meat having to take a longer time for digestion, the exposure time of the food-derived carcinogen in the digestive tract is higher.

Conclusion

While regulatory agencies and food manufacturers continuously work to minimize the levels of carcinogens in food and establish safety standards to protect consumers, a safer way to address the concern would be to screen probiotic species, which otherwise not only confer health benefits, to detoxify or degrade the food-derived carcinogens in the gut, if consumed as diet or as supplement. Given that hypothetically, our observations are intriguing and encouraging, more understanding on the influence of gut microbiota composition and other factors impacting the probiotics on carcinogen degradation is required. Also, further research is needed to establish the exact mechanisms involved and to validate these findings.

Acknowledgments

The authors would like to acknowledge the support and cooperation extended by Heads of the Departments of Agriculture Sciences and Home Sciences, Dayalbagh Educational Institute and Dr. Nemai Chandra Ghosh, Head of Central Lab, Patanjali Ayurved Ltd (Unit 3), Patanjali Food and Herbal Park Pvt Ltd. Vill- Padartha, Laksar Road, Haridwar-249404 (Uttarakhand), India.

Bibliography

1. American Cancer Society. Colorectal Cancer Facts and Figures 2020-2022. Atlanta: American Cancer Society; (2020).

2. Sesink Aloys LA., *et al*. "Red meat and colon cancer: dietary haem-induced colonic cytotoxicity and epithelial hyperproliferation are inhibited by calcium". *Carcinogenesis* 22.10 (2001): 1653-1659.

3. Chao Ann., *et al*. "Meat consumption and risk of colorectal cancer". *Jama* 293.2 (2005): 172-182.

4. Cross Amanda J., *et al*. "A large prospective study of meat consumption and colorectal cancer risk: an investigation of potential mechanisms underlying this association". *Cancer Research* 70.6 (2010): 2406-2414.

5. Smith JS., *et al*. "Effect of marinades on the formation of heterocyclic amines in grilled beef steaks". *Journal of FOOD SCIENCE* 73.6 (2008): T100-T10.

6. Sugimura Takashi., *et al*. "Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish". *Cancer Science* 95.4 (2004): 290-299.

7. Cheng Ka-Wing., *et al*. "Heterocyclic amines: chemistry and health". *Molecular Nutrition and Food Research* 50.12 (2006): 1150-1170.

8. Melo Armindo., *et al*. "Effect of beer/red wine marinades on the formation of heterocyclic aromatic amines in pan-fried beef". *Journal of Agricultural and Food Chemistry* 56.22 (2008): 10625-10632.

9. De Vries Maaike C., *et al*. "Lactobacillus plantarum—survival, functional and potential probiotic properties in the human intestinal tract". *International Dairy Journal* 16.9 (2006): 1018-1028.

10. Salminen S., *et al*. "Functional food science and gastrointestinal physiology and function". *British Journal of Nutrition* 80.S1 (1998): S147-S171.

11. Orrhage K M., *et al*. "Effects of lactic acid bacteria on the uptake and distribution of the food mutagen Trp-P-2 in mice". *Scandinavian Journal of Gastroenterology* 37.2 (2002): 215-221.

12. Bocci Alessandro., *et al*. "In vitro inhibition of 4-nitroquinoline-1-oxide genotoxicity by probiotic Lactobacillus rhamnosus IMC501". *Journal of Microbiology and Biotechnology* 25.10 (2015): 1680-1686.

13. Collins E B. "Enumeration of Lactobacillus acidophilus with the agar plate count". *Journal of Food Protection* 41.6 (1978): 439-442.

14. Clinton Steven K., *et al*. "The world cancer research fund/ American institute for cancer research third expert report on diet, nutrition, physical activity, and cancer: impact and future directions". *The Journal of Nutrition* 150.4 (2020): 663-671.

15. Vosough Parya Rahnama., *et al*. "In vitro effect of Lactobacillus rhamnosus GG on reduction of aflatoxin B1". *Nutrition and Food Science* 44.1 (2014): 32-40.

16. Khosravi-Darani., *et al*. "Detoxification of heterocyclic aromatic amines by probiotic to inhibit medical hazards". *Mini Reviews in Medicinal Chemistry* 19.15 (2019): 1196-1203.

17. Yousefi Mojtaba., *et al*. "Detoxification properties of microorganisms in foods". *Microbial Biotechnology in Food and Health*. Academic Press, (2021): 81-112.

18. Anwar Haseeb., *et al*. "Biodiversity of gut microbiota: Impact of various host and environmental factors". *BioMed Research International* 2021 (2021).