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ANTI-NEURODEGENERATIVE ACTIVITY OF THE PROBIOTIC STRAIN LACTOBACILLUS ACIDOPHILUS

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Abstract

Introduction: *Neurodegenerative disorders are characterized by gradual loss of selectively susceptible populations of neurons. Primary clinical features (e.g., dementia, parkinsonism, or motor neuron disease), anatomic distribution of neurodegeneration (e.g., frontotemporal degenerations, extrapyramidal disorders, or spinocerebellar degenerations), or primary molecular abnormality can all be used to classify neurodegenerative diseases. The frequency of neurodegenerative disorders gets increased every year as the population in the world gets older. Lack of effectiveness in currently available drugs and severe side effects lead to the necessity for development of novel drugs from natural sources.*

Present study focuses on evaluating the anti-neurodegenerative activity of a probiotic Lactobacillus acidophilus.

Material and methods: *Probiotic characterisation assay such as tolerance to NaCl, phenol and bile salts were evaluated. Antibiotic sensitivity test five standard antibiotics were performed. Acetyl cholinesterase inhibition and tyrosinase inhibition assay were carried out at five different concentrations. From the analysis, Lactobacillus acidophilus was found to be tolerant to NaCl, phenol and bile salts.*

Results: *The Lactobacillus acidophilus strain was found to be sensitive to all the tested antibiotics. Significant acetyl cholinesterase and tyrosinase was observed at 100 µL of crude extracts 69.29 ± 3.25% and 53.18 ± 2.89%.*

Conclusion: *Lactobacillus acidophilus as a probiotic supplement can be utilized for treatment and prevention of neurodegenerative disorders.*

Keywords: *acetylcholinesterase inhibition, Lactobacillus acidophilus, neurodegenerative disorders, tyrosinase inhibition.*

Introduction

In contrast to select static neuronal loss caused by metabolic or toxic illnesses, neurodegenerative disorders are characterized by gradual loss of selectively susceptible populations of neurons. Primary clinical features (e.g., dementia, parkinsonism, or motor neuron disease), anatomic distribution of neurodegeneration (e.g., frontotemporal degenerations, extrapyramidal disorders, or spinocerebellar degenerations), or primary molecular abnormality can all be used to classify neurodegenerative diseases. Parkinson's disease, Alzheimer's disease, amyloidoses, tauopathies, -synucle-

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inopathies, and TDP-43 proteinopathies are the most frequent neurodegenerative illnesses [*Dugger B, Dickson D, 2017*]. Proteins with aberrant conformational characteristics are seen in these diseases [*Myszczynska M et al., 2020*].

The pathogenic processes behind neurodegenerative illnesses have gotten a lot of attention lately, due to the ageing population, where neurodegenerative diseases are becoming a more common cause of impairment. Ischemia, neurodegenerative illnesses, immune-mediated disorders, infections, and trauma all cause immunological activation in the central nervous system [*Hu J, Wang X, 2022*]. It's possible that it contributes to neuronal damage. However, not all immune responses in the central nervous system are harmful; in fact, they often help repair and regeneration. Microglia, for example, clean debris following myelin injury, and if this process is slowed, delayed regeneration ensues [*Soto C, Pritzkow S, 2018*]. Immune activation is also important in preventing neurotropic viral infections and removing necrotic cells after ischemia. As a result, microglia have a dual function in neurodegeneration: they both cause harm and protect brain homeostasis. T cells, in addition to microglia, can help with recovery in neurodegenerative diseases [*Myszczynska M et al., 2020*], while the particular mechanisms for this positive effect of T cells are unknown. Complex interactions have been discovered by detailed investigations of neuroimmune interactions at both the cellular and molecular levels, indicating that immune cells release both neurotoxic and neuroprotective chemicals [*Fu H et al., 2018*]. As the pathophysiology of many of these diseases is unclear, one must investigate the involvement of environmental variables in these disorders [*Adams J et al., 2020*].

The frequency of neurodegenerative disorders varies greatly over the world. Overall, the standardized prevalence (all ages) per 100,000 in door-to-door surveys ranged from 57 to 230. This is higher than the prevalence observed with record-based studies. The increase of prevalence with age can be observed across the world although the absolute numbers differ [*Ahmadian-Moghadam H et al, 2020*].

Probiotics are live, non-pathogenic bacteria that are given to promote microbial balance, especially in the gastrointestinal system [*Williams N, 2010*]. They are controlled as nutritional additions and foods and are made up of yeast lactic acid bacteria or *Saccharomyces boulardii* like *Lactobacillus* and *Bifidobacterium* species. Probiotics work by reducing pathogenic organism colonisation and invasion, dropping intestinal pH, and changing the host immune response, among other things [*Tseng J et al., 2017*].

Probiotics' involvement in reducing antibiotic-associated diarrhoea, diarrhoea, irritable bowel syndrome, ulcerative colitis, *Clostridium difficile* infection, travellers' Crohn's disease, and vulvovaginal candidiasis need further investigation [*Albayram O et al., 2017*]. Probiotics are typically regarded safe and well tolerated, with the most common adverse effects being bloating and gas [*Goedert M et al., 2017*].

With increasing neurodegenerative disorders, lack of effectiveness in currently available drugs and severe side effects lead to the necessity for development of novel drugs from natural sources. Therefore, the present study focuses on evaluating the anti-neurodegenerative activity of a probiotic *Lactobacillus acidophilus* MTCC 10307. As there were several reports on biological properties of *Lactobacillus acidophilus*, this is the first study on anti-neurodegenerative property analysis.

Material and methods

***Procurement of strains*:** *Lactobacillus acidophilus* MTCC 10307 strains were procured from the microbial type culture collection centre. The lyophilised form of the strains was revived in MRS broth, cultured and utilised for further analysis as in figure 1 and 2.

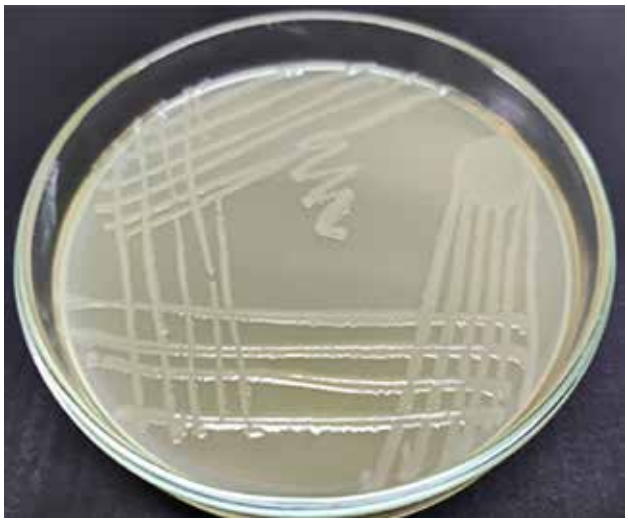

Figure 1. *Lactobacillus acidophilus MTCC 10307 used in the study*

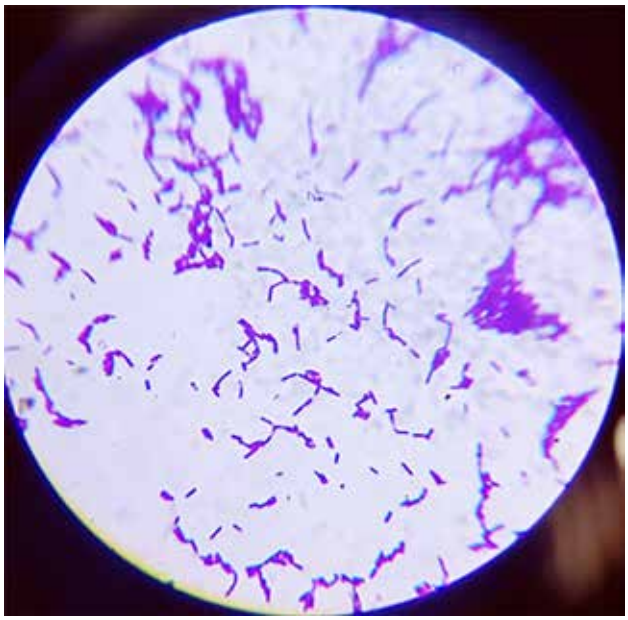

Figure 2. *Microscopic observation of Lactobacillus acidophilus MTCC 10307*

Probiotic characterisation assay: Tolerance to NaCl and Phenol: The isolated bacteria (100 µL) were inoculated into the MRS broth of different sets and incubated for 14 *h* at 37°C. the first set of the MRS broth was supplemented with sodium chloride (NaCl) as 2%, 4%, 6%, 8% and 10% (w/v, 99.5% purity, M.W. 58.44). The second set as added with phenol (Sigma-Aldrich, St. Louis, USA, M.W. 94.11) in different concentrations as 0.2%, 0.4%, and 0.6%. From every culture later incubation for 24 *h*, optical density at 600 *nm* was calculated. Control cultures were grown in normal MRS broth.

Tolerance to Bile Salts: *Lactobacillus acidophilus* overnight cultures in MRS broth were pelleted by centrifugation at 16,200 *g* for 1 *minute*, resuspended in PBS pH 7.4, and pelleted once more. Final numbers of $8x10^7$ bacteria cells per ml were added to the PBS solution with 0.3% and 0.5% bile salts (a mixture of 50% cholic acid sodium salt, w/v, Sigma-Aldrich, and 50% deoxycholic acid sodium salt, 0.1 *g/mL* of solubility in water, Sigma-Aldrich). The culture was incubated for 3 hours at 37°C. The serially diluted bacterial samples were also inoculated onto the MRS agar in replica and incubated anaerobically for a period of 24 hours at 37°C.

Antibiotic sensitivity test: Antibiotic sensitivity test of the *Lactobacillus acidophilus* MTCC 10307 strains were evaluated against five standard antibiotics (tetracycline, amoxicillin, ampicillin, methicillin and streptomycin). Muller-Hinton agar was prepared, and the test organism was streaked all over the plate. Antibiotic discs were procured from Hi-Media and placed on the agar surface. Plates were incubated for 24 hrs at 37°C. Inhibitory zones were measured in *mm*.

Anti-neurodegenerative activity:

Acetyl cholinesterase inhibition: Briefly, by introducing140 µL of 20 µL of DTNB solution, sodium phosphate buffer (0.1 *mol*/L, pH 7), different concentration (20 µL, 40 µL, 60 µL, 80 µL and 100 µL) of crude supernatants (grown for 48 *hrs*) and 20 µL of acetylcholinesterase solution (5 *Um/L*) in Tris-HCl buffer (20 *mmol/L*, pH 7.5), the reaction mixture (S) was prepared. As a negative control (C), sample-free mixture was used and galantamine as a positive control, though the sample colour control had buffer as a replacement for of enzyme. Every treatment was done in triplicate. Utilising ELISA reader at a wavelength of 412 *nm*, the absorbance was calculated.

Tyrosinase inhibition assay: By introducing 80 µL of sodium phosphate buffer (40 µL of tyrosinase solution (46 U/*L*) ,0.1 *mol/L*, pH 7), and different concentration (20 µL, 40 µL, 60 µL, 80 µL and 100 µL) of crude supernatants, the reaction mixture (S) was set. As a negative control (*C*), the sample-free mixture was utilised and kojic acid as a positive control, though the sample colour control confined buffer as an alternative of enzyme. The absorbance was calculated utilising ELISA reader at a wavelength of 475 *nm*, later the adding of 40 µL of L-DOPA buffer solution and incubation for 30 minutes at 25°C. Respectively, treatment was performed in triplicate.

Utilising the subsequent equation, the inhibition fraction (I) for acetyl cholinesterase and tyrosinase inhibition was calculated and the results are accessible as mean ± standard error:

$$I\% = [C - S/C] \times 100$$

Results

Probiotic characterization assay

Tolerance to NaCl: Lactobacillus acidophilus tolerance to NaCl was assessed by monitoring growth in MRS broth supplemented with various doses of NaCl. The strain was tolerant at 10% of NaCl with a growth of 35.54± 2.78% as noticeable in figure 3. 56.73 ± 2.66% and 78.38 ± 3.24% growth was observed at 8% and 6% NaCl concen-

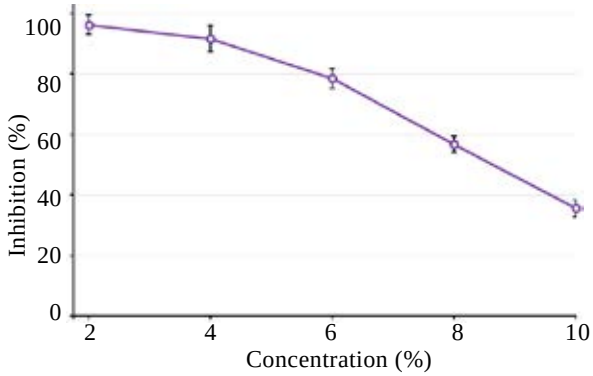

FIGURE 3. *Tolerance to NaCl*

tration. 91.66 ± 4.47% and 96.21 ± 3.16% growth was observed at 4% and 2% NaCl.

Tolerance to bile salts: Bile salts are necessary that facilitate digestion and assimilation of fat and fat-soluble vitamins in the digestion process. Two concentrations of bile salts were used, and significant growth was observed 98.24 ± 3.24% growth at 0.15% bile salt and 96.68 ± 2.82% growth at 0.30% bile salt concentration.

Tolerance to phenol: Three concentrations (0.2%, 0.4% and 0.6%) of phenol were used to determine the tolerance of *Lactobacillus acidophilus.* 64.79 ± 5.51% growth was observed at 0.2% phenol; 28.33 ± 3.42% and 22.57 ± 2.93% growth was observed at 0.4% and 0.6% phenol concentrations as shown in figure 4.

Antibiotic susceptibility test (Antibiotic sensitivity test): The Antibiotic sensitivity test was carried out against five antibiotics (tetracycline, amoxicillin, ampicillin, methicillin and streptomycin). The *Lactobacillus acidophilus* was found to be sensitive to all the antibiotics. Table shows the inhibitory zones of *Lactobacillus acidophilus* against tested antibiotics.

Anti-neurodegenerative assay

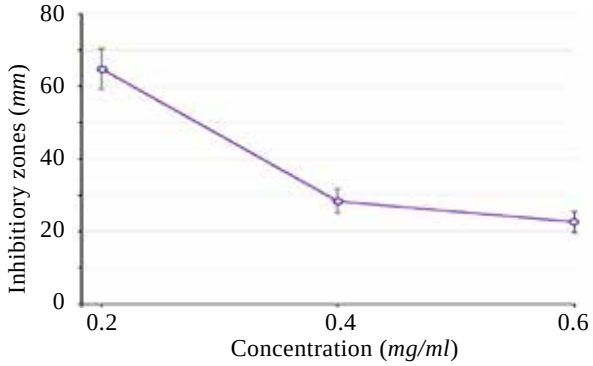

FIGURE 4. *Tolerance to phenol*

TABLE 1.

Antibiotic sensitivity test

Sl. No	Drug	Zone of inhibition (mm)
1	Tetracycline	34
2	Amoxicillin	30
3	Ampicillin	32
4	Methicillin	11
5	streptomycin	26

Acetylcholinesterase inhibition analysis: Five different concentrations (20 µL, 40 µL, 60 µL, 80 µL and 100 µL) of the crude extracts were examined for acetylcholinesterase inhibition. 20 µL and 40 µL of crude extracts showed 7.16 ± 1.15% and 21.76 ± 1.62% of inhibition; 60 µL, 80 µL and 100 µL extracts showed 45.34 ± 2.78%, 67.47 ± 1.38% and 69.29 ± 3.25% of inhibition as presented in figure 5 A.

Tyrosinase inhibition assay: The crude extracts were examined for tyrosinase inhibition at five different concentrations (20 µL, 40 µL, 60 µL, 80 µL and 100 µL). 20 µL and 40 µL of crude extracts showed 6.72 ± 2.41% and 15.37 ± 2.82% of inhibition; 60 µL, 80 µL and 100 µL extracts showed 36.34 ± 1.53%, 49.97 ± 1.46% and 53.18 ± 2.89% of inhibition and the graph is represented by figure 5B.

DISCUSSION

Lactobacillus acidophilus belongs to the lactic acid bacteria family, which is distinguished by the production of lactic acid as the single or primary end result of carbohydrate metabolism. *Lactobacilli* are naturally present or added purposely to raw milk and dairy products for instance yoghurts, cheese,

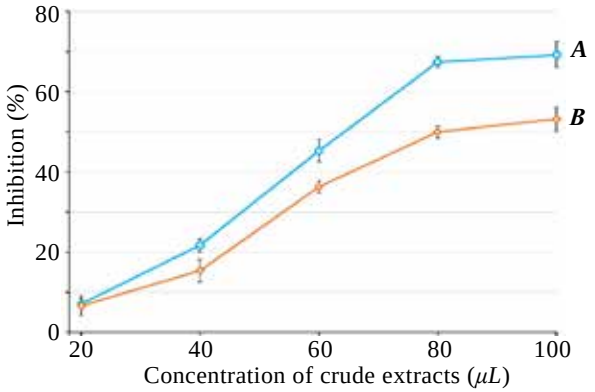

FIGURE 5. *Acetyl cholinesterase (A) and Tyrosinase (B) inhibition analysis*

and fermented milks because of their health assistances to consumers [*Peng W et al., 2021*]. The nutritional and therapeutic advantages of lactic acid bacteria include vitamin synthesis, immunomodulation, a decrease in the threat of diarrhea, mutagenic activity, and a drop in blood cholesterol [*Zhao W et al., 2020*] and also it produces several inhibitory components, including as organic acids, hydrogen peroxides, ammonia, bacteriocins, and diacetyl, which have antibacterial effect against food-borne microbes. The generation of lactic acid and hydrogen peroxide by *Lactobacillus acidophilus* may also subsidize to its antibacterial effectiveness against other microorganisms [*de Faria Banos A et al., 2018*]. As a result of the production of bacteriocins, *Lactobacillus acidophilus* has potential probiotic benefits in the human environment; they take precedence over other microorganisms in the gut [*Di Cerbo A et al., 2016*].

Anwar A. Abdulla (2014) investigated that *Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus pyogenes, Proteus vulgaris, Staphylococcus aureus*, and *Aeromonas hydrophila* had the maximum inhibitory activity against *Staphylococcus epidermidis* and *E. coli*, the activity was lower. This suggests that *Lactobacillus acidophilus* has antimicrobial properties. This research supports the findings of the current study.

According to Moura et al. (2016), the antioxidant activity of the probiotic dairy dessert ranged from 86.7 ± 1.5 % (1st day) to 72.4 ±2.0 % (15th day) inhibition of the 2,2 -diphenyl-1-picrylhydrazyl radical, whereas the conventional dairy dessert exhibited 28.9 ± 0.6 % (1st day) to 21.1 ±0.5 % (15th day) inhibition of the same radical. The development of organic acids and short peptides from milk proteins, which are recognized scavengers of free radicals in vitro, might explain the alteration between the traditional and probiotic desserts. *Lactobacillus acidophilus* has antioxidant properties in terms of the present investigation.

Conclusion

The anti-neurodegenerative activity of the probiotic strain *Lactobacillus acidophilus* was evaluated in the present study. The probiotic properties were also examined. From the analysis, it was shown that *Lactobacillus acidophilus* possess significant acetylcholine esterase and tyrosinase inhibition. Since *Lactobacillus acidophilus* has obtained generally regarded as safe status, further studies are required to investigate the anti-neurodegenerative property *in vivo*.

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