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RESEARCH ARTICLE

# Effect of UV and Microwave Irradiations on *Lactobacillus fermentum*

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## Abstract

*Lactobacillus fermentum* has been identified as a potential probiotic. In the present study, *L. fermentum* MTCC 903 and an isolate from curd sample were subjected to UV and microwave irradiation for different time periods. Changes were observed in their growth. The number of colonies reduced but, their size increased after UV and microwave irradiations. UV treated *L. fermentum* can be used for increasing the rate of fermentation in lazy-milks because a high degree of sugar fermentation was observed in the UV exposed cells, indicating an increased rate of acid production in the MRS broth. A change in the VP Test was also observed indicating high acetonin production. A change in the protein profile with respect to the number of bands and their molecular weights was observed in UV treated strains but no change was observed after exposure to microwave. *L. fermentum* shows antibacterial properties. Interestingly it was also found that after exposure to UV and microwave irradiations *L. fermentum* displayed an increased antibacterial property against *Escherichia coli*.

**Keywords :** Biochemical, Lazy milk, Microwave irradiation, Proteomic analysis, UV irradiation

## Introduction

Lactic acid bacteria possess several interesting properties of great economic importance such as lactose utilization, proteinase activity, bacteriophage defense mechanism, bacteriocin production. Therefore, research is now being focused on the improvement and stabilization of these industrially important features. The ultimate aim is to bring them all together in one or more starter culture with practical use. In developing countries simple biotechnological techniques like mutagenesis may be adopted in order to enhance food and goods productivity. Mutation has its harmful and beneficial effects (Allan and Greenwood, 2001; Voet *et al*, 1999). There are numerous documented cases where beneficial mutations with survival advantages have arisen in a population. Such beneficial mutations occur frequently among viruses, bacteria and higher organisms as well (Brown and Wichman, 2010). The effects include increase in enzyme activity of mutant strain of *Leuconostoc messenteroides* to about 2.5 fold higher than normal (Kamal *et al*, 2003). Evolution of a single clonal line of beer yeast cells with mutations in permease and phosphatase enzymes increases beer production when yeast cells are grown in a chemostat with limited phosphate (Francis and Hansche, 1972; 1973). Mutagenesis has been used in the selection and improvement of lactic acid bacteria starter culture by Harlander (1992) and Sudi *et al,* (2008) on *Lactobacillus bulgaricus* and *Streptococcus thermophilus.*

## Materials and Method

### Bacterial strains

Standard strain of *L. fermentum* was procured from MTCC, Chandigarh, India and indigenous strain of *L. fermentum* was isolated from curd which was identified biochemically and molecularly by 16S rRNA gene sequencing (Singh and Prakash, 2010)

### Chemicals and reagents

Chemicals and reagents used were procured from Sigma Aldrich (U.S.A), E. Merck (India), Bangalore Genei (India), SRL (India), Himedia (India), Qualigens (India).

The bacterial strains were revived in MRS (de Mann Rogosa Sharpe) broth. The revived bacterial strains were streaked on the specific medium MRS agar. The enriched cultures were further double enriched in MRS broth.

### Confirmation of *L. fermentum* pure culture

The double enriched bacterial cultures were examined by Gram staining, spore formation and catalase production. The cell morphology and colony characteristics on MRS agar were studied. Gram staining and biochemical tests were performed according to Laboratory Manual in General Microbiology (Benson, 2001) and laboratory manual by Sambrook *et al*, (1989).

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### Treatment with UV and Microwave irradiations

*L. fermentum* was exposed to Ultra Voilet (UV) irradiation (Sudi *et al,* 2008) with different periods of time (15, 30, 60, 120, 240 seconds). These time periods were selected based on the experiments performed by Voskanyan (1990) because higher duration of UV exposure kills the bacteria but lower time period exposure is enough to cause mutations. *L. fermentum* was exposed to microwave irradiation (Wei *et al,* 2009) under a microwave power of 800 W with different period of time (10, 20 and 30 sec).

### Characterization of UV and microwaves exposed bacterial strains

The cell growth, colony characteristics, Gram staining, spore staining, all the biochemical tests (Voges-Proskauer, catalase, gas production from glucose, hot loop, sugar fermentation tests) and protein profile by SDS-PAGE were studied. Whole cell protein was extracted following the methods of Dutoit *et al*, (2001). Bands were analyzed by UN SCAN IT 6.0 Gel compare software.

### Effect of heat on the growth of *L. fermentum*

Specific heat controls were kept to check whether the changes that occurred were due to UV or microwave irradiation or due to heat.

### Antimicrobial activity of UV and microwaves exposed *L. fermentum* cells against pathogenic *E. coli*

Agar well diffusion method was used to study the antimicrobial property of UV and microwaves exposed *L. fermentum* cells against pathogenic *E. coli* (MTCC 723) using Muller Hinton Agar (MHA).

## Results and Discussion

Characteristic creamish white, distinct colonies on MRS agar were observed for the pure bacterial culture. All the experiments were conducted in triplicates.

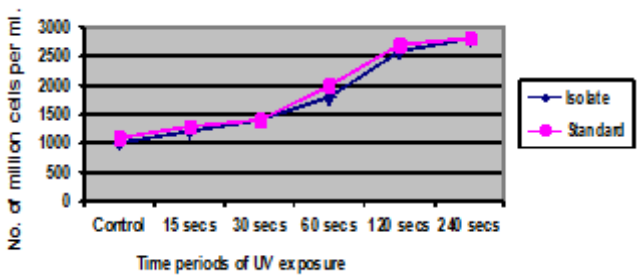


**Fig.1. Effect of UV on the growth of *L. fermentum* through McFarland's Nephelometric indices**

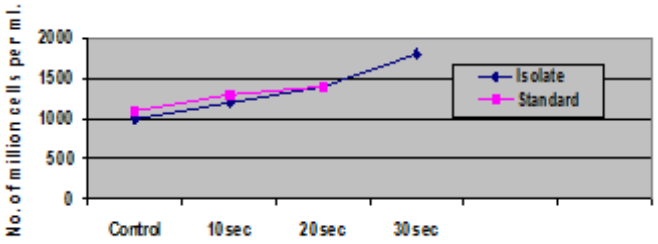


**Fig.2. Effect of Microwaves on the growth of *L. fermentum* through McFarland's Nephelometric indices**

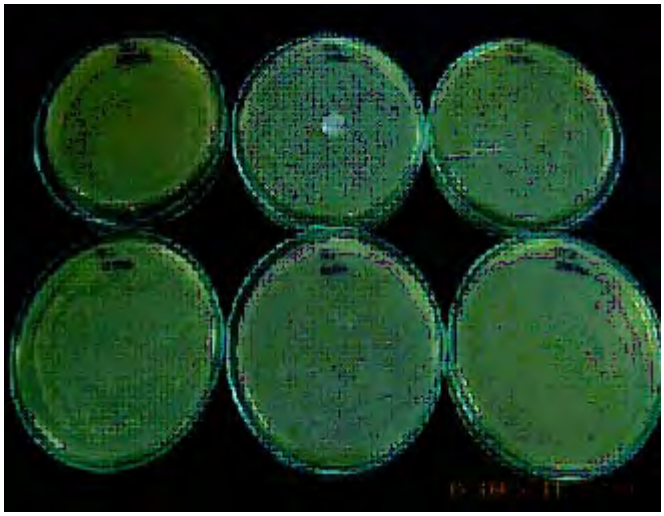

**Fig.3. UV exposure on *L. fermentum* Standard (MTCC 903)**

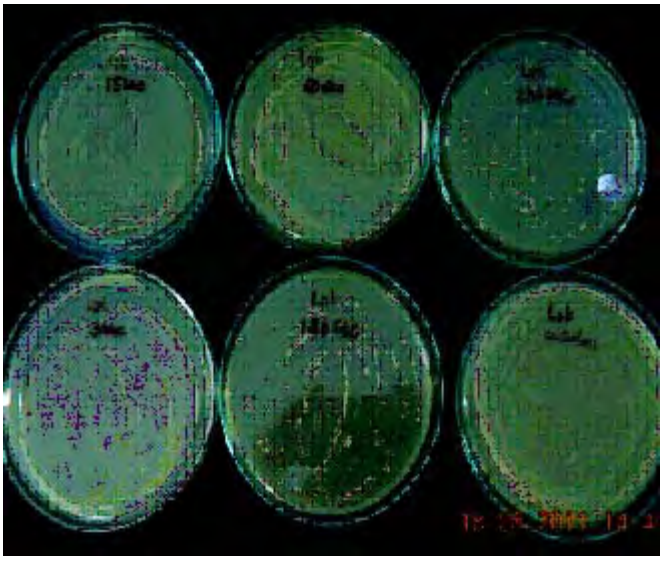

**Fig.4. UV exposure on *L. fermentum* Isolate**

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**Table 1. Gram Staining and Biochemical Characterization of UV exposed *L. fermentum***

| *L. fermentum* standard (MTCC 903) | Biochemical Tests | | | | | |
|---|---|---|---|---|---|---|
| | Gram staining | Spore formation | Voges- Proskauaer | Gas production | Catalase | Hot Loop |
| Control | + | – | – | – | – | – |
| 15 sec | + | – | – | – | – | – |
| 30 sec | + | – | – | – | – | – |
| 60 sec | + | – | – | – | – | – |
| 120 sec | + | – | – | – | – | – |
| 240 sec | + | – | + | + | – | – |
| ***L. fermentum* Isolate** | | | | | | |
| Control | + | – | – | – | – | – |
| 15 sec | + | – | – | – | – | – |
| 30 sec | + | – | – | – | – | – |
| 60 sec | + | – | – | – | – | – |
| 120 sec | + | – | – | – | – | – |
| 240 sec | + | – | + | + | – | – |

**Table 2. Sugar Fermentation Tests of UV exposed *L. fermentum***

| *L. fermentum* Standard | Sugars | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glucose | Sucrose | Galactose | Lactose | Raffinose | Maltose | Mannose | Cellobiose | Melibose | Melizitose | Arabinose | Xylose |
| Control | + | + | + | + | + | + | + | + | + | + | + | + |
| 15 sec | + | + | + | + | + | + | + | + | + | + | + | + |
| 30 sec | + | + | + | + | + | + | ++ | + | + | + | + | + |
| 60 sec | ++ | ++ | + | + | + | + | +++ | +++ | + | + | + | ++ |
| 120 sec | ++ | ++ | ++ | + | + | + | +++ | +++ | ++ | + | ++ | ++ |
| 240 sec | ++ | ++ | +++ | + | + | ++ | +++ | +++ | ++ | + | ++ | +++ |
| *L. fermentum* Isolate | | | | | | | | | | | | |
| Control | + | + | + | + | + | + | + | + | + | + | + | + |
| 15 sec | + | + | + | + | + | + | + | + | + | + | + | ++ |
| 30 sec | + | + | + | ++ | + | + | +++ | +++ | + | ++ | + | ++ |
| 60 sec | ++ | + | ++ | ++ | + | + | +++ | +++ | + | ++ | ++ | +++ |

**+ = Positive, ++ = Moderately Positive, +++ = Strongly Positive**

## Study of UV exposed *L. fermentum* cells

### *Growth*

The number and size of the colonies were affected. There was no change for 15 sec and 30 sec exposures. The growth started decreasing after 60 sec UV exposure and was remarkably less for 120 and 240 seconds of exposure. On comparison with the nephelometric series (million cells per ml) same results were obtained with respect to the nephelometric indices (Fig.1, 3 and 4)

### *Gram staining, spore staining*

All the strains were found to be Gram positive, non-spore forming and bacilli (Table 1).

### *Biochemical tests*

Changes were observed for 240 sec UV exposed cells in Voges- Proskauer, gas production from glucose, and sugar fermentation tests. An instant color change was observed in sugar fermentation tests after 240 seconds exposure (Table 1 and 2).

**Table 3. Molecular weights (3.5 to 20.5 Kd) of UV exposed *L. fermentum***

| Lanes → Bands↓ | Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6 | Lane 7 | Lane 8 | Lane 9 | Lane 10 | Lane 11 | Lane 12 | Lane 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Band I | 23.42 | 20.18 | 19.38 | 15.33 | 10.38 | 5.11 | 20.5* | 28.32 | 24.31 | 10.44 | 10.17 | 10.17 | 8.12 |
| Band II | 11.16 | 8.01 | 4.2 | 4.2 | 5.11 | 4.08 | 10.73 | 10.6 | 10.31 | 5.36 | 5.33 | 5.41 | 5.05 |
| Band III | 9.83 | 7.92 | 5.5 | 4.47 | 2.68 | 2.68 | 5.84 | 5.33 | 5.26 | 4.12 | 4.19 | 2.41 | 2.43 |
| Band IV | 4.77 | 2.96 | 2.15 | 2.14 | 2.15 | 2.3 | 3.5* | 2.15 | 2.36 | 2.43 | 2.38 | 2.1 | 2.42 |
| Band V | 3.46 | 3.46 | 3.48 | 3.9 | 5.1 | 2.8 | | 2.1 | | | | | |

**Lane 1: *L. fermentum* standard** control; **Lane 2: *L. fermentum* standard for** 15 sec; **Lane 3: *L. fermentum* standard for** 30 sec; **Lane 4: *L. fermentum* standard for** 60 sec; **Lane 5: *L. fermentum* standard for** 120 sec; **Lane 6: *L. fermentum* standard for** 240 sec; **Lane 7: Protein molecular marker** 3.5 to 20.5 kd; **Lane 8: *L. fermentum* Isolate** control; **Lane 9: *L. fermentum* Isolate for** 15 sec; **Lane 10: *L. fermentum* Isolate for** 30 sec; **Lane 11: *L. fermentum* Isolate for** 60 sec; **Lane 12: *L. fermentum* Isolate for** 120 sec; **Lane 13: *L. fermentum* Isolate for** 240 sec

**Table 4. Effect of Antibacterial property of UV exposed *L. fermentum* against pathogenic *E.coli***

| Time periods(sec) | Zone of inhibition of UV treated *L. fermentum* Standard 903(MTCC) against *E.coli* (in mm) | Zone of inhibition of UV treated *L. fermentum* isolate against *E.coli* (in mm) |
|---|---|---|
| Control | 8 | 9 |
| 15 | 8 | 9 |
| 30 | 10 | 10 |
| 60 | 11 | 10 |
| 120 | 11 | 11 |
| 240 | 11 | 11 |

*SDS-PAGE*

The molecular weights of the bands as obtained by UN SCAN IT 6.0 Gel compare software are given in Table 3 and Fig.9.

*Antimicrobial activity*

There was an increase in zone of inhibition with increase in length of UV exposure (Table 4, Fig.8(a))

**Study of Microwave exposed *L. fermentum* cells**

*Growth*

The number and size of the colonies were affected. There was no growth observed after 20 seconds in standard and after 30 sec in isolate. The growth started decreasing and sizes of colonies were increased with increase in length of time. On comparison with the nephelometric series (million cells per ml) same results were obtained with respect to the nephelometric indices (Fig.2, 5, and 6)

*Gram staining, spore staining*

All the strains were found to be Gram positive, non-spore forming and bacilli (Table 5).

*Biochemical tests*

No changes were observed (Table 5 and 6).

*SDS-PAGE*

The molecular weights of the bands as obtained by UN SCAN IT 6.0 Gel compare software are given in Table 7 and Fig.10.

*Antimicrobial activity*

There was an increase in zone of inhibition with increase in microwave exposure for 20sec (Table 8, Fig.8(b)).

**Study of effect of heat on the growth of *L. fermentum***

There was no change observed in growth due to heat (Fig.7).

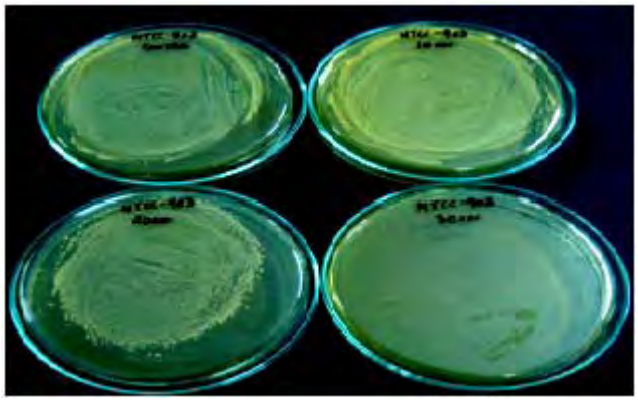


Fig.5. Microwave exposure on *L. fermentum* standard

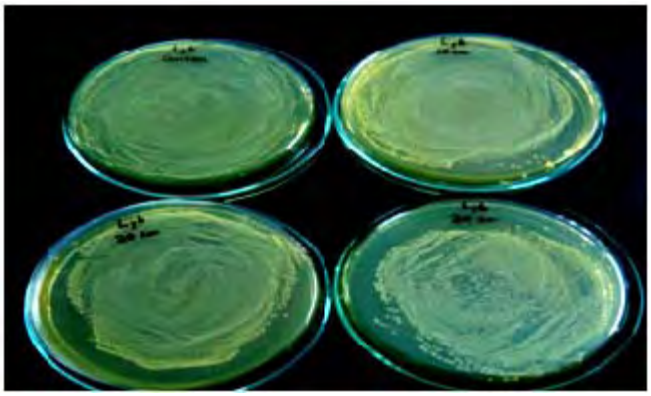


Fig.6. *L. fermentum* standard and *L. fermentum* isolate

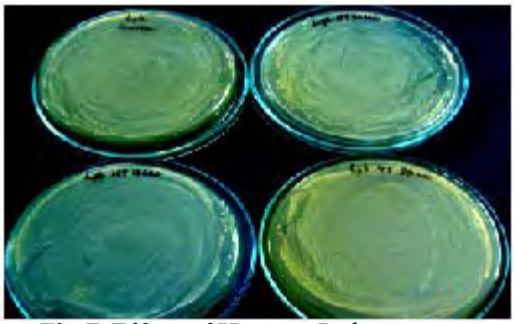
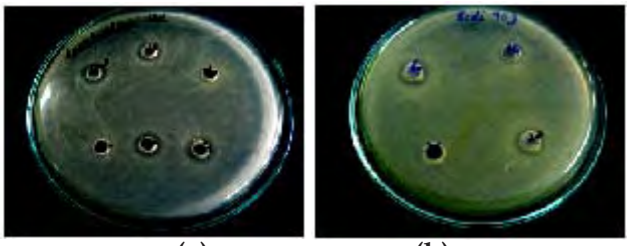


Fig.7. Effect of Heat on *L. fermentum*



(a) (b)

Fig.8. Effect of (a) UV irradiation (b) microwave irradiation on *L. fermentum* antibacterial property against pathogenic *E.coli*

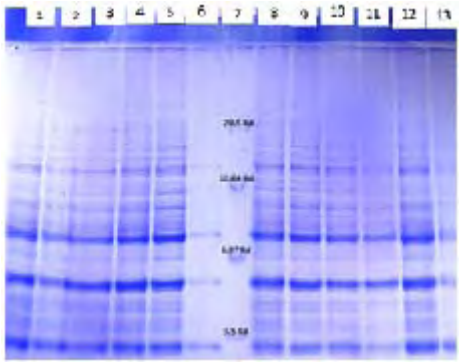
Table 5. Gram Staining and Biochemical Characterization of Microwaves exposed *L. fermentum*

| *L. fermentum* standard (MTCC 903) ↓ | Biochemical Tests | | | | | |
|---|---|---|---|---|---|---|
| | Gram staining | Spore formation | Voges-Proskauaer | Glucose Production | Catalase | Hot Loop |
| Control | + | – | – | – | – | – |
| 10 sec | + | – | – | – | – | – |
| 20 sec | + | – | – | – | – | – |
| *L. fermentum* Isolate ↓ | | | | | | |
| Control | + | – | – | – | – | – |
| 10 sec | + | – | – | – | – | – |
| 20 sec | + | – | – | – | – | – |
| 30 sec | + | – | – | – | – | – |

Table 6. Sugar Fermentation Test of Microwave exposed *L. fermentum*

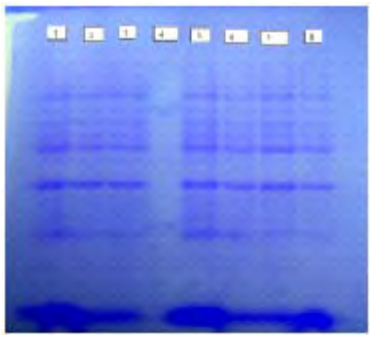
| *L. fermentum* sstandard | Sugars | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glucose | Sucrose | Galactose | Lactose | Raffinose | Mallose | Mannose | Cellobiose | Melibiose | Melizitose | Arabinose | Xylose |
| Control | + | + | + | + | + | + | + | + | + | + | + | + |
| 10 sec | + | + | + | + | + | + | + | + | + | + | + | + |
| 20 sec | + | + | + | + | + | + | + | + | + | + | + | + |
| *L. fermentum* Isolate | | | | | | | | | | | | |
| Control | + | + | + | + | + | + | + | + | + | + | + | + |
| 10 sec | + | + | + | + | + | + | + | + | + | + | + | + |
| 20 sec | + | + | + | ++ | + | + | + | ++ | + | + | + | + |
| 30 sec | + | + | + | ++ | + | + | ++ | +++ | + | + | + | + |

+ = Positive, ++ = Moderately Positive, +++ = Strongly Positive



Lane 1: *L. fermentum* standard control; Lane 2: *L. fermentum* standard for 15 sec; Lane 3: *L. fermentum* standard for 30 sec; Lane 4: *L. fermentum* standard for 60 sec; Lane 5: *L. fermentum* standard for 120 sec; Lane 6: *L. fermentum* standard for 240 sec; Lane 7: Protein molecular marker 3.5 to 20.5 kd; Lane 8: *L. fermentum* Isolate control; Lane 9: *L. fermentum* Isolate for 15 sec; Lane 10: *L. fermentum* Isolate for 30 sec; Lane 11: *L. fermentum* Isolate for 60 sec; Lane 12: *L. fermentum* Isolate for 120 sec; Lane 13: *L. fermentum* Isolate for 240sec

**Fig.9. SDS-PAGE gel electrophoresis of UV exposed *L. fermentum***



Lane 1: *L. fermentum* standard; Lane 2: *L. fermentum* standard for 10 sec; Lane 3: *L. fermentum* standard for 20 sec; Lane4: Protein molecular marker 3.5 to 20.5 kD; Lane 5: *L. fermentum* Isolate; Lane 6: *L. fermentum* Isolate for 10 sec; Lane 7: *L. fermentum* for Isolate 20 sec; Lane 8: *L. fermentum* Isolate for 30 sec

**Fig.10. SDS-PAGE gel electrophoresis of Microwaves exposed *L. fermentum***

**Table 7. Molecular Weight (3.5 to 20.5 Kd) of Microwave Exposed *L. fermentum***

| Lanes → Bands↓ | Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6 | Lane7 | Lane 8 |
|---|---|---|---|---|---|---|---|---|
| Band I | 23.42 | 23.18 | 23.8 | 20.5* | 28.32 | 28.31 | 28.3 | 28.1 |
| Band II | 11.16 | 11.11 | 11.06 | 10.73 | 10.6 | 10.51 | 10.6 | 10.4 |
| Band III | 9.83 | 9.80 | 9.78 | 5.84 | 5.33 | 5.31 | 5.29 | 5.32 |
| Band IV | 4.77 | 4.77 | 4.75 | 3.5* | 2.15 | 2.11 | 2.11 | 2.12 |
| Band V | 3.46 | 3.4 | 3.38 | | | | | |

Lane 1: *L. fermentum* standard; Lane 2: *L. fermentum* standard for 10 sec; Lane 3: *L. fermentum* standard for 20 sec; Lane4: Protein molecular marker 3.5 to 20.5 kD; Lane 5: *L. fermentum* Isolate; Lane 6: *L. fermentum* Isolate for 10 sec; Lane 7: *L. fermentum* for Isolate 20 sec; Lane 8: *L. fermentum* Isolate for 30 sec

**Table 8. Effect of Antibacterial Property of Microwaves Exposed *L. fermentum* Against Pathogenic *E.coli***

| Time periods (sec) | Zone of inhibition of Microwaves treated *L. fermentum* Standard 903(MTCC) against *E.coli* (in mm) | Zone of inhibition of Microwaves treated *L. fermentum* Isolate against *E.coli* (in mm) |
|---|---|---|
| Control | 8 | 9 |
| 10 sec | 10 | 9 |
| 20 sec | 11 | 10 |
| 30 sec | - | 11 |

The ability of the UV and microwave treated L. fermentum strains to grow on MRS agar medium and to remain catalase negative is an indication that the bacteria still retain biological activities of the control strain (Togo, 2002). The bacteria that are still Gram positive are presumed to have potentials of lactic acid bacteria. Lactic acid bacteria are responsible for the fermentation of milk (Brock and Madigan., 1991) and non-dairy materials (Steinkraus, 1996; Varnam, 2002). Bacterial strains showed ability to produce lactic acid in sugar broth using mannose, cellobiose, and xylose. The increased sugar utilization observed explains the increased acid production in some UV exposed cells and therefore suggests the use of these sugars as a supplement to increase the rate of milk fermentation. Similar work was done by Igyor (2005) where he used yeast extract supplementation in milk fermentation and Sudi *et al*, (2008) stated that UV- light mutagenesis on Lactobacillus bulgaricus and Streptococcus thermophilus suggests the use of fructose as a supplement to increase the rate of milk fermentation in "lazy – milk" which has a potential use as a starter culture. The property of high acetonin production after UV induced mutagenesis can be used to enhance the flavour and acid producing abilities of lactic acid bacteria. The varied protein profile obtained reflects a change, in the nucleotide sequence and advanced molecular techniques can be utilized to analyse the DNA and gene expression to get further insight to this study. Antibacterial nature of the treated bacterial strains was enhanced and therefore can be used as an effective control against pathogens. This finding can be constructively employed to induce changes and utilize these mutant forms of the bacteria in the fermentation industry to obtain different varieties of end products. The inoculum required for the production of fermented foods and drinks requiring *L. fermentum* can be modified as per the requirement of the particular end product.

## Acknowledgement

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