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Development of an optimised growth strategy for intensive propagation, lactic acid and bacteriocin production of selected strains of *Lactobacilli* genus

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Abstract **-Lactobacilli belong to the group of lactic acid bacteria (LAB), widely used in the food industry nowadays. These microorganisms have several distinguishing abilities such as the production of lactic acid, enzymes such as β-galactosidase and natural antimicrobial substances called bacteriocins. They are mainly used as a natural acidifier for the inoculation of bulk quantities of milk and vegetables in order to produce a variety of fermented products. As such, large quantities of their biomass and the end products of their metabolism are necessary. The possibility of producing these substances in mass quantities will be investigated through several techniques. The selected Lactobacilli, L.plantarum NCIMB 8014, L.casei NCIMB 11970, L.lactis NCIMB 8586 were grown into simple batch cultures without pH control where their physicochemical needs were determined. Through the determination of the optimum nutritional conditions for the propagation of the Lactobacilli, an optimised medium for growth occurred.**

The optimum pH conditions for the growth of the bacilli were determined as well as parameters such as cellular yield coefficient, substrate and starter inoculum concentration and lactic acid rate and production. The metabolism of the Lactobacilli was determined as homofermentative, mainly producing lactic acid. The efficiency of the medium combining al the optimised parameters, enhancing the productivity biomass and therefore bacteriocins production from the strains, was tested on a 2L STR reactor operated batchwise with continuous pH control. A simple liquid turbidometric method was developed to test the bacteriocin productivity of the selected bacteria. The activity and potency of the bacteriocin produced was tested against L.delbruckii subsp.lactis NCIMB 8117.

Index Terms**-Doubling time, Growth rate, Lactic acid, Nisin**

I. Introduction

Lactobacilli are a bacterial group belonging into the genre of Lactic Acid Bacteria (LAB). Their metabolic end products such as lactic acid, acetic acid, protein structure antimicrobial compounds called bacteriocins and enzymes are widely applied as food preservatives in the contemporary food industry.

LAB in the form of starter cultures can be used to enhance the natural ripening of milk and plant origin products, such as butter, cheese, olives and cucumbers. Furthermore, their metabolic end products can be used as natural preservatives and antimicrobial agents against contamination and food spoilage occurring during or after the fermentation process. (2)

Lactobacilli distinctive ability is to decompose complex carbohydrate sources into simpler forms and synthesise mainly lactic acid. Their use as natural acid- producer bioreactors has been widely investigated throughout the recent years, in an effort to replace the production of lactic acid from petrol and other carbon sources. (4)

Furthermore, the enzymes produced from LAB have been attractively attended because these bacteria are normally considered safe so the enzymes derived from them might be used with no need of extensive purification and there are little or no adverse effects on fermented products (1)

Due to the previously referred reasons the need for bulk quantities of biomass and their end products is steadily augmenting. The research over the methods of production of enzymes, lactic acid and bacteriocins, their activity, their chemical characterisation and their extraction and their applications has to be reinforced.(5) In this work an attempt to develop a simplified nutrient medium of low cost which will reinforce the *Lactobacilli* growth , lactic acid and bacteriocin production has been made. Although *Lactobacilli* are widely applied in modern food industry their potential as natural anticontaminants has not been deeply exploited. As previously referred, numerous *Lactobacilli* strains are producing antimicrobial compounds called bacteriocins. These substances are divided into three major groups. The majority of the discovered substances belong into the first group named Lantibiotics. Lantibiotics are mainly active against bacteria of the same genus and they are produced during growth of the bacilli, especially during late exponential phase. They obtain a low molecular weight at about 3.000- 4.500 d approximately. (9)(15) Lantibiotics are thermostable molecules, can withstand acidic conditions occurring during growth and they can be degraded by the enzymes existing in the gastrointestinal tract. They are considered safe for consumption from human beings, having no toxic effect on human health. (13)Their use as natural antimicrobial preservatives has been proposed throughout the recent years. Several methods for their introduction into food have been examined in a constant effort to replace the chemical and in general artificial methods of food preservation due to high health risks resulting from their usage. The most common and widely exploited worldwide lantibiotic is Nisin, which is commercially available. Its use as a natural preservative has

been approved in the USA and in Europe. (11) Due to the previously referred reasons the need for bulk quantities of biomass and bacteriocins is steadily rising. The research over the methods of production of bacteriocins, their activity, their chemical characterisation and their extraction and their application has to be reinforced. In this work, a simplified liquid turbidometric method was fabricated so to facilitate the detection of these substances in nutrient broth.

II. MATERIALS AND METHODS

A. Materials

The yeast extract, peptone, glucose, sodium acetate, trisodium citrate, NaOH, $MgSO_4$, $MnSO_4$, were bought from Sigma-Aldrich Chemicals, UK.

Inoculum source

All the *Lactobacilli, Lactobacillus casei NCIMB 11970 Lactobacillus plantarum NCIMB 8014, Lactobacillus lactis NCIMB 8586* and the target strain *Lactobacillus delbruckii subsp. lactis NCIMB 8117* were provided in a lyophilised form by National Collection of Food and Marine bacteria(NCIMB) , Aberdeen , Scotland.

B. Growth Experiments

2.2.1 Preliminary Growth experiments

Pyrex glass pressure tubes sealed with butyl rubber stoppers and aluminium seals were used to test the effect of basal and optimum on *Lactobacilli* growth. The tubes were prepared under aseptic and anaerobic conditions. The media recipe for the basal medium is glucose 2% w/v, yeast extract 1.5% w/v, peptone 1% w/v, sodium acetate, 0.5% w/v, tri-sodium citrate 0.2%, KH_2PO_4 0.2% w/v, $MgSO_4$ 0.02% w/v , $MnSO_4$ 0.002% w/v and resazurin dye 0.0005% v/v. Each component was tested separately so to certify its influence on growth in a range of concentrations between 0% w/v to 4% w/v.

All the components were combined and an optimised medium was fabricated. The medium's recipe is glucose 2% w/v, yeast extract 2 w/v, sodium acetate, 1% w/v, tri-sodium citrate 1% w/v, KH_2PO_4 0.5 w/v and resazurin dye 0.0005% v/v.

2.2.2 Bench Device (Stirring Tank Reactor, STR)

A 2L Pyrex glass fermenter has been selected for the procedure. The fermenter was equipped with an hydrargiric thermometer for temperature control, a pH probe (Fischer Scientific, UK) for pH control, a magnetic stir bar for agitation**,** an glass aeration port, a sampling and inoculation port, a gas flow stainless steel port connected with a filter for gas sterilisation (Polyvent filter, 0.2μm, Whatman Filters, UK)**,** a port for alkali/acid feed and stainless steel coils for heat emission**.** All the ports were made of stainless steel and were connected with plastic tubes of several lengths. The working volume of the reactor was set at 1.5L. The pH probe was connected with a pH controller apparatus (Electrolab FerMac 260, UK) which was calibrated with suitable acidic and alkali solutions (pH 4 and pH 7) to adjust the pH range. The gas filter was connected with a gaseous nitrogen flask via rubber tubes and the flow was set up at 50 ng/ml. The alkali feed port was connected with a plastic bottle containing an alkali solution of 100 ml of NaOH 1M which was placed on an electronic scale (Ohaus portable advanced, Switzerland**)** o to measure the volume of alkali/acid used for pH maintenance. The coils were connected with a water bath (Grant Water bath, UK) and a pump (Watson Marlow Digital, 505S, UK) for continuous preservation of steady temperature. The fermenter was placed on a magnetic stirring plate (SM1, Stuart Scientific, UK) and was constantly stirred at 150 rpm as being anaerobic bacteria

C. Analytical methods

2.3.1 Measurement of cellular growth and biomass

The cellular growth was measured by placing the pressure tubes into a spectrophotometer fitted with a test tube holder (PU 8625 UV/VIS Philips, France) at 660 nm. The tube had a 1.8 cm. light path.

2.3.2 Measurement of Lactic acid amount and rate

Lactic acid productivity rate and the amount of lactic acid produced by each strain during the pH and temperature control fermentation are indirectly determined by the following theorem: 1 M of NaOH neutralises the effect of 1 M Lactic acid. According to this equation the amount of lactic acid produced is directly proportional to the amount of sodium hydroxide consumed during the fermentation process.

The rate of lactic acid produced is indirectly calculated by the following equation:

$$\frac{dp}{dt}(mM \quad / L / h) = \left(\frac{(Na) * FR * M.W.}{V} \right)$$

(Equation 1)

where Na is the moles of the alkali solution used, FR is the feeding rate of the alkali solution in the culture, M.W. is the molecular weight of lactic acid and V is the overall volume of the culture. (1)

2.3.3. Assay for Nisin and bacteriocin concentration and quantification

Lactobacillus delbruckii subsp.lactis 8117 was selected as the target strain. A consistent inoculum size was prepared. The amount of the bacteriocin produced by each under investigation strain was primarily defined on the samples taken during the pH and temperature controlled fermentations. Furthermore an analysis of the overall amount of bacteriocin produced was performed on the broths collected after the end of fermentation. The selected samples (pH fermentation at 6.5) were transferred into 10 ml conical plastic tubes (Fisherbrand, UK) and centrifuged (10.000 rpm for 15 min.) (Biofuge Stratos Sorall , Kendro Products, Germany) in order to remove completely the biomass. The clarified liquid was filtrated through a 0.2 μm pore size filter for sterilisation. The sterilised liquid's pH was adjusted at 6.0 to eliminate the antimicrobial effect of

hydrogen peroxide and lactic acid and then it was diluted with fresh medium. Into 25 ml of 0.02 M of HCl 25mg of Nisin are dispersed. This solution equals to 1000 IU/ml of Nisin. According to this formula the necessary quantities of solid Nisin were calculated to fabricate standard solution at the following concentrations: 0, 25, 50, 75, 85, 100, 110, 125, 150, 175, 200, 250, 500, 750, 1000, 1250, 1500, 1750, 2000 IU/ml. The solutions are preserved stable (up to 30 days) into 4°C. Into glass tubes containing 8 ml of optimised medium including metals ,so to ensure that any effect on growth of the tested microorganism results from the bacteriocin produced and not due to any other factors such as nutrient exhaustion of optimum anaerobic medium for the growth of the tested strain *L.delbruckii* (medium recipe: 2% w/v glucose, 2% w/v Y.E., 1% w/v sodium acetate, 1% w/v tri-sodium citrate, 0.5% w/v KH_2PO_4 , 0.05% w/v $MgSO_4$,0.005% w/v $MnSO_4$) 1 ml of the frozen inoculum of *L.delbruckii* and 1 ml of the supernatant resulting from pH control fermentations of differential concentration is added.

III. RESULTS AND DISCUSSION

A. The effect of standard and optimum medium on Lactobacilli growth

Thus, to achieve the optimum maximum growth rate and of the bacteria and enhance their productivity, the bacilli were inoculated in a medium of liquid form containing all the optimised parameters. (2% glucose, 1.5% yeast extract, .5%, KH_2PO_4 1% tri-sodium citrate, 1%sodium acetate).

There has been an effort to use the same parameters in the optimised medium for the inoculation and intensive propagation of all the chosen *Lactobacilli*., so to form a common economic simplified medium which will ameliorate distinctively the growth rates and the cellular yields of the bacteria.

The maximum growth rate of *L.casei* on the optimised medium was 0.24 h^{-1} and the doubling time reduced to 2.87 h. The final biomass concentration was 2.43 g/l. When compared to the basal medium where maximum growth rate was 0.16 h^{-1} a significant increase in the maximum growth rate was achieved. Similarly the final cell concentration of the fermentation has been raised from 1.19 g/l to 2.43 g/l. The maximum growth rate of *L.plantarum* on the optimised medium was 0.30 h^{-1} and the doubling time reduced to 2.30 h. The final biomass concentration was 2.61 g/l. When compared to the basal medium where maximum growth rate was 0.13 h^{-1} a significant high increase in the maximum growth rate was achieved. Similarly the final cell concentration of the fermentation has been raised from 1.32 g/l to 2.63 g/l. The maximum growth rate of *L.lactis* on the optimised medium was 0.22 h^{-1} and the doubling time reduced to 3.13 h. The final biomass concentration was 1.81 g/l. When compared to the basal medium where maximum growth rate was 0.07 h^{-1} a significant high increase in the maximum growth rate was achieved. Similarly the final cell concentration of the fermentation has been raised from 0.69 to 1.81. The optimised medium will be used as a medium for further investigation. (Fig 1. 2. 3.)

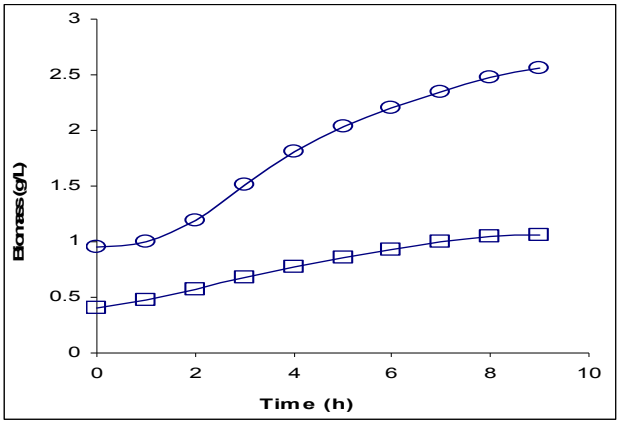

Fig 1 Growth of *L.casei* on basal(□)and optimised (○) media

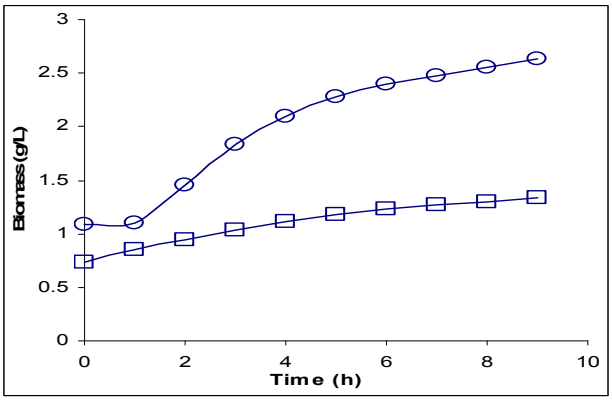

Fig 2 Growth of *L.plantarum* on basal (□) and optimised (○) media

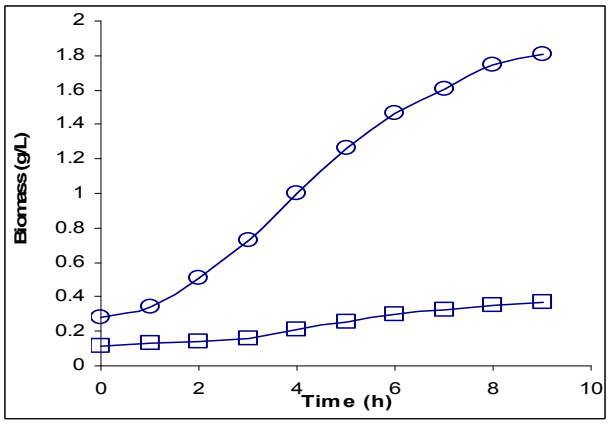

Fig 3 Growth of *L.lactis* on basal (□)and optimised (○) media

B. Growth of Lactobacilli on a STR

In order to obtain a better maximum growth rate and higher growth yields and improved productivity a pH controlled STR system was developed. As to investigate the influence of pH over growth in terms of growth rate, doubling time and product and biomass yields the system was operated with a continuous pH control maintenance system. The influence of pH was tested in a range of highly acidic (4) to neutral (7) pH. All the process was performed in batch mode. The optimised medium was used. (3)

The results of the experiments are shown in Fig 4, 5and 6

and Table 1. There is a strong correlation between the pH and the growth of the bacilli. The maximum growth rate was enhanced when the culture was controlled at pH 5.5, 6.5 and 7. Maintenance of pH on a steady state throughout the 10 h fermentation process was combined with the use of the optimised liquid medium gave highest biomass yields and maximum growth rates as compare to the uncontrolled pH growth systems. It can be also observed that on acidic pH values of 4 and 4.5, the growth of the bacilli is strongly inhibited. The amount of lactic acid produced by the bacilli was identified as being equal to the amount of NaOH used for pH maintenance. Over the 10 h fermentation the pH 5, 5.5 and 6 the bacilli were still growing as they had slower maximum growth rates and long lag periods prior to growth.

Samples were measured on an hourly basis and they were analysed for biomass, pH and in some occasions the glucose and the end product were also analysed. The effect of reduced pH is strong where no growth was observed at pH 4 and pH 4.5.The optimum pH was 6.5 in the conditions studied for *Lactobacillus casei NCIMB 11970 Lactobacillus plantarum NCIMB 8014, Lactobacillus lactis NCIMB 8586.*

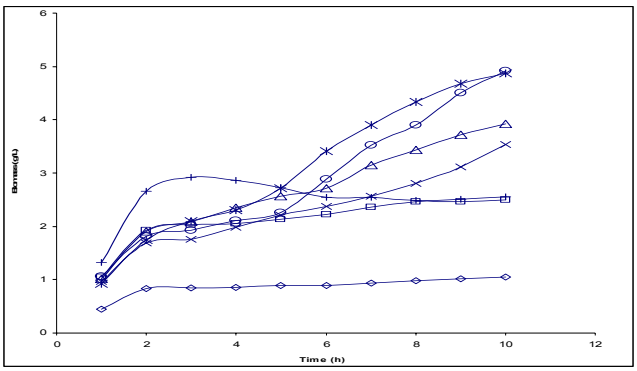

Fig 4 Growth of *L.casei* on Different pH range in a 2L STR, Growth(◊) on pH 4, Growth (□) on pH 4.5, Growth (Δ) on pH 5 Growth (x) on pH 5.5, Growth (*) on pH 6, Growth (○) on pH 6.5, Growth on pH 7(◊)

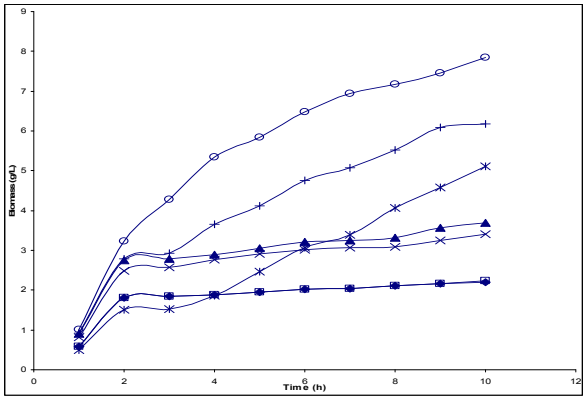

Fig 5 Growth of *L.plantarum* on Different pH range in a 2L STR, Growth(◊) on pH 4, Growth (□) on pH 4.5, Growth (Δ) on pH 5 Growth (x) on pH 5.5, Growth (*) on pH 6, Growth (○) on pH 6.5, Growth on pH 7(◊)

Table 1: Lactic acid production on different pH conditions on an STR

Selected Strains	**pH**	***Rate of Lactic acid Produced (mM/L/h)***	***Total amount of Lactic acid produced (mM)***	**Selected Strains**	**pH**	***Rate of Lactic acid produced (mM/L/h)***	**Selected Strains**	**pH**	***Rate of Lactic acid produced (mM/L/h)***	***Total amount of Lactic acid produced (mM)***
L.casei	4	9.94	50	*L.plantarum*	4	1.09	*L.lactis*	4	4.9	23
	4.5	8.45	30		4.5	1.24		4.5	2.8	8
	5	125.2	500		5	5.73		5	7.2	37
	5.5	181.32	650		5.5	8.89		5.5	127.99	487
	6.0	158.31	715		6.0	136.2		6	165.33	543
	6.5	239.98	900		6.5	161.5		6.5	183.23	563
	7	2.82	4		7	133.52		7	140.71	474.4

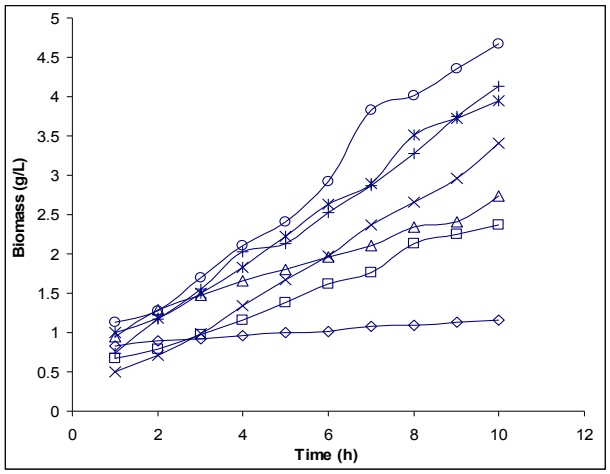

Fig 6 Growth of *L.lactis* on Different pH range in a 2L STR, Growth(◊) on pH 4, Growth (□) on pH 4.5, Growth (Δ) on pH 5 Growth (x) on pH 5.5, Growth (*) on pH 6, Growth (○) on pH 6.5, Growth on pH 7(◊)

C. Testing the activity of the commercially available Nisin against the Selected Lactobacilli strain L.delbruckii subsp.lactis 8117

3.3.1 Selection and growth strategy of the target strain L.delbruckii subsp.lactis 8117

As previously stated, the Lantibiotics produced from the Lactic Acid Bacteria (LAB) are mainly active against bacteria of the same genre *L.delbruckii subsp. lactis 8117* was selected as the target strain to test the activity of the produced bacteriocins and the commercially available Nisin. The selected strain was grown on a suitable enriched medium for growth. This was selected so avoid false result due to glucose exhaustion.

When grown on enriched, medium the strain had *L.delbruckii* has a maximum growth rate of 0.30 h^{-1} and a doubling time of 2.30 h are achieved. The final biomass concentration is 1.21g/L. Furthermore, so to have consistent, accurate and credible results, during the bacteriocin and Nisin testing activity assays, the inoculum of *L.delbruckii* had to be consistent. A cryopreservation method was selected to achieve this result. The growth on the enriched medium of the strain is demonstrated graphically below. When grown on optimized medium the strain had *L.delbruckii* has a maximum growth rate of 0.32 h^{-1} and a doubling time of 2.15 h are achieved. The final biomass concentration is 1.40 g/L. When the biomass of the strain was reaching 1.50 g/L a solution of glycerol 50% v/v was inserted in a 1:1 dilution and the samples of the inoculum were stored in -70°C.

3.3.2. Testing the activity of Nisin against the target strain

Primarily the effect of commercially available Nisin was tested. (2.5% purified Nisin from *Lactococcus lactis,* Sigma) The effect was tested against the selected *Lactobacilli* target strain *L.delbruckii subsp.lactis NCIMB 8117* in a quantity range of 0 IU/ml to 2000 IU/ml.

In complete absence of Nisin *L.delbruckii* grown on enriched optimized medium (Figure 7) has a μ max of 0.30 h^{-1} and a doubling time of 2.30 h. The final biomass concentration after a 10 h static incubation achieved was 1.67 g/L. Though when 2000 IU/ml were added in the growth medium no growth of the bacillus was observed. (Fig 8)

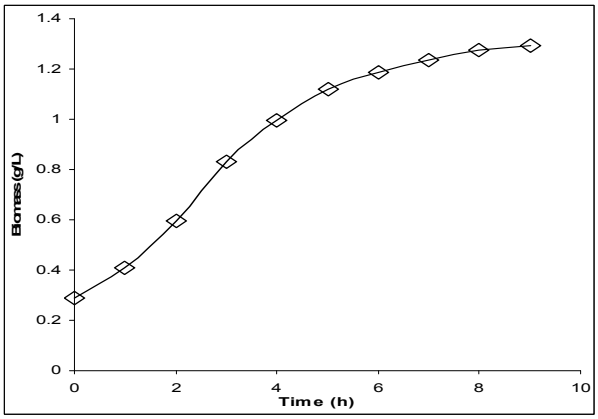

Fig 7: Growth of *L.delbruckii* on 0 IU/ml of Nisin

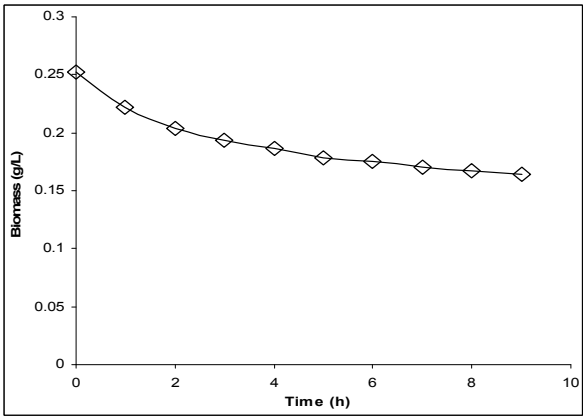

Fig 8: Growth of *L.delbruckii* on 2000 IU/ml of Nisin

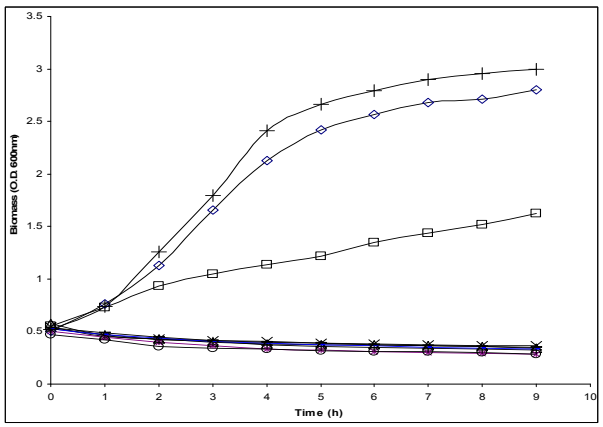

Fig 9: Growth of *L.delbruckii* under different Nisin concentrations, Growth(+) on 0 IU/ml Growth(◊) on 50 IU/ml, Growth (□) on 150 IU/ml, Growth (Δ) on 250 IU/ml, Growth (x) on 500 IU/ml, Growth (*) 1000 IU/ml, Growth (○)on 1250 IU/ml, Growth(-) on 1500 IU/ml, Growth(+) on 1750 IU/ml

As the maximum growth rate and doubling time have been examined for *L.delbruckii* grown on the suitable optimized medium and it has been certified that Nisin in a certain concentration has a bacteriostatic effect (Section 3.3.2.) over its growth, further research had to be done so to

examine the exact extent of this effect, against the microbial strains on different concentrations. These results could serve as a response model so to evaluate the activity and the amount of bacteriocins produced by the selected *Lactobacilli*.

The effect of Nisin in a range of 50IU/ml to 1750 IU/ml was investigated and it is described in Figure 9. The effect has been evaluated in terms of doubling time and maximum growth rate of the bacterium. In a concentration of 50 IU/ml growth of *L.delbruckii* remains relatively unaffected, the highest maximum growth rate being 0.31 h^{-1} and a doubling time of 2.22 h. The final biomass concentration is 1.23 g/L. For a concentration of 150 IU/ml *L.delbruckii* growth is strongly affected, the highest maximum growth rate being 0.10 h^{-1} and a doubling time of 6.9 h. The final biomass concentration is 0.79 g/L. On a Nisin concentration though of 250 IU/ml and above no growth of the target strain is observed. As it can be assumed from the previously referred results Nisin has a strong bacteriostatic effect on a range of concentrations above 250 IU/ml.

Further research has been made to investigate the effect of the substance on smaller concentrations that 250 IU/ml. The selected range which was further investigated was 0 IU/ml to 200 IU/ml. The activity of Nisin was evaluated in terms of maximum growth rate and doubling time. In Figure 10 below the effect of Nisin in a range of 0 IU/ml to 200 IU/ml is described. In a concentration of 25 IU/ml to 75 IU/ml the growth of *L.delbruckii* remains unaffected, the highest maximum growth rate being 0.34 h^{-1} with a doubling time of 2.03 h. and a final biomass concentration is 1.21 g/L, for a concentration of 25 IU/ml. *L.delbruckii* growth is relatively affected on a range between 50 IU/ml to 150 IU/ml where the growth is slower. In a concentration of 175 IU/ml no growth is observed. The limiting concentration for growth is 175 IU/ml of Nisin incorporated in the liquid growth medium.

In an effort to obtain an accurate quantification curve on Nisin activity against the selected strain a range of concentrations between 75 IU/ml to 150 IU/ml was selected. The effect of Nisin was evaluated in terms of maximum growth rate and doubling time. As it can be assumed by Figure 11, even in low concentrations Nisin influences the growth of the selected target strain by extending the logarithmic phase of growth. When higher concentrations are incorporated in the growth medium the effect is clearly bacteriostatic. All the numerical values in terms of doubling time and maximum growth rate of Nisin against the target strain are tabulated in table below (Table 2)

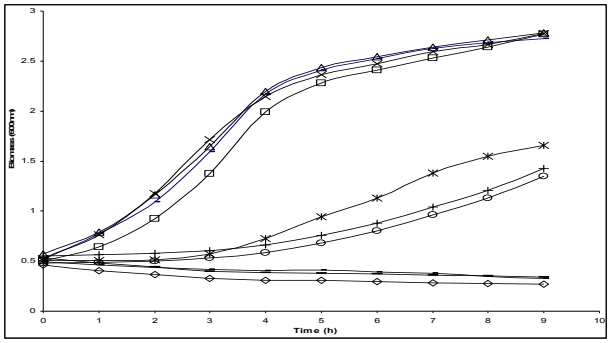

Fig 10: Growth of *L.delbruckii* under different Nisin concentrations, Growth(/) on 0 IU/ml, Growth (□) on 25 IU/ml, Growth (Δ) on 50 IU/ml, Growth (x) on 75 IU/ml, Growth (*) 100 IU/ml, Growth (○)on 125 IU/ml, Growth(+) on 150 IU/ml, Growth(-) on 175 IU/ml , Growth(-) on 200 IU/ml

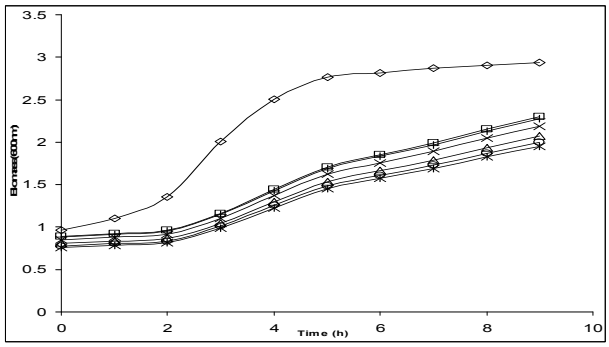

Fig 11: Growth of *L.delbruckii* under different Nisin concentrations, Growth(◊) on 0 IU/ml, Growth (□) on 75 IU/ml, Growth (Δ) on 85 IU/ml, Growth (x) on 95 IU/ml, Growth (*) 110 IU/ml, Growth (○)on 125 IU/ml, Growth(/) on 150 IU/ml

Based on the numerical values of the maximum growth rates achieved in the previous series of experiments during the growth of the target strain *L.delbruckii* under the influence of several Nisin concentrations a dose response curve was achieved.

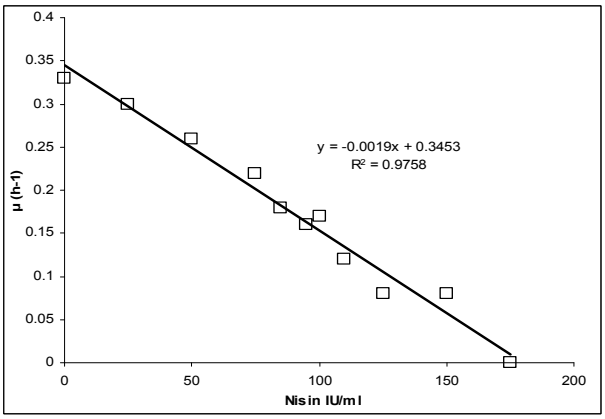

Fig 12: Dose response curve for Nisin

Table 2: Numerical Values of the effect of Nisin against the target strain

Selected target strain	Nisin Concentration (IU/ml)	Doubling Time (td, h)	Maximum growth rate (μ max,	Final Biomass Concentration (g/L)	Selected target strain	Nisin Concentration (IU/ml)	Doubling Time (td, h)	Maximum growth rate (μ max,	Final Biomass Concentration (g/L)

			h^{-1})					h^{-1})	
L.delbruckii	0	2.30	0.30	1.67	*L.delbruckii*	175	No growth	No growth	0.25
	25	2.03	0.34	1.21		200	No growth	No growth	0.25
	50	2.22	0.31	1.22		225	No growth	No growth	0.5
	75	2.30	0.30	1.22		250	No growth	No growth	0.5
	85	2.88	0.24	0.74		500	No growth	No growth	0.5
	95	3.63	0.19	1.00		1000	No growth	No growth	0.5
	100	3.83	0.18	1.00		1250	No growth	No growth	0.5
	110	5.75	0.12	0.870		1500	No growth	No growth	0.5
	125	6.9	0.10	0.890		1750	No growth	No growth	0.5
	150	6.90	0.11	0.65		2000	No growth	No growth	0.5

D. Bacteriocin production on the STR

The crude extracts of the bacterial cultures deriving from the *Lactobacilli* grown on a 2L STR with optimized medium were tested. At the end of each set of the pH and temperature controlled fermentation performed by the *Lactobacilli,* 10 ml samples were collected and tested against the target strain. Under the influence of *L.casei* treated supernatant *L.delbruckii* has a maximum growth rate of 0.16 h^{-1} and a doubling time of 4.31 h are achieved. The final biomass concentration is 1.01g/L. When tested against the treated supernatant of *L.plantarum L.delbruckii* has a maximum growth rate of 0.16 h^{-1} and a doubling time of 4.31 h are achieved. The final biomass concentration is 1.20g/L. When tested against *L.lactis* though *L.delbruckii* has a maximum growth rate of 0.14 h^{-1} and a doubling time of 4.92 h is achieved. The final biomass concentration is 1.24g/L. (Fig 13, 14, 15)

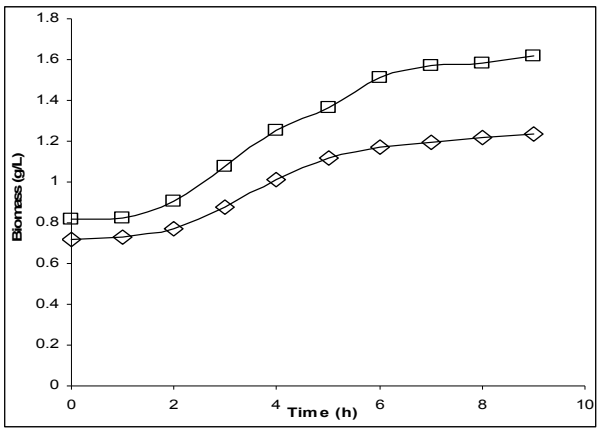

Fig 13: Growth of *L.delbruckii* (□) & *L.delbruckii* (◊) under *L.casei* supernatant

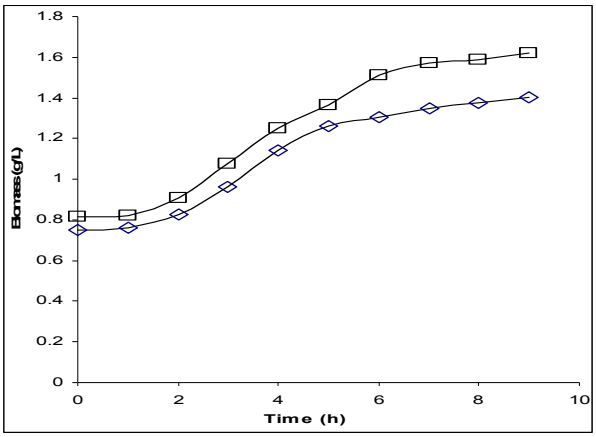

Fig 14: Growth of *L.delbruckii* (□) & *L.delbruckii* (◊) under *L.plantarum* supernatant

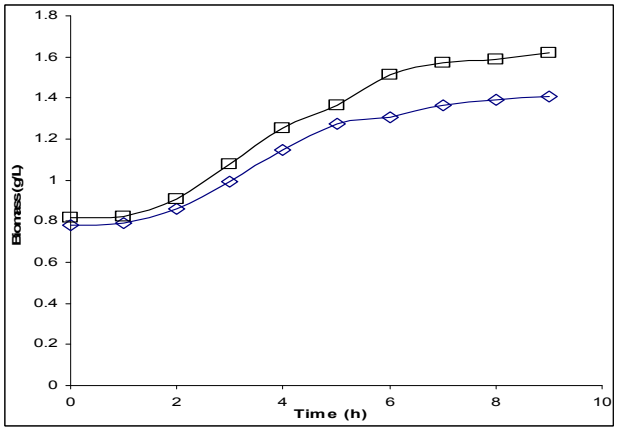

Fig 15: Growth of *L.delbruckii* (□) & *L.delbruckii* (◊) under *L.lactis* supernatant

IV. CONCLUSIONS

In this work, a new growth strategy to enhance lactic acid production and the growth of *Lactobacilli* was studied. Significant changes were notified when the optimized medium was used on the growth of *Lactobacilli*. Optimized pH conditions also reinforced the cellular growth and the productivity of lactic acid, though the medium was proven to promote sufficiently the production of bacteriocins from the selected strains. The sensitivity of the selected target strain, *L.delbruckii subsp.lactis* against the commercially available bacteriocin, Nisin was certified; the strain was tested against the bacteriocins produced by the selected *Lactobacilli*. According to the growth response curve fabricated *L.casei* is producing 103 IU/ml, *L.plantarum* 105 IU/ml and *L.lactis* 138 IU/m. Using the turbidometric liquid assay the limitations of the solid methods are surpassed . Furthermore the method is simple, reproducible and has a low cost. Further research though, should be performed to develop extraction techniques for lactic acid and bacteriocins and test further their production on the nutrient media.

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