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Stimulatory effects of simple sugars, honey, and selected defined minerals on growth vigor and production of inhibitory effects against selected Pathogens by a probiotic Lactobacillus acidophilus strain: An in vitro Study

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Abstract: **Probiotics are live microorganisms that confer identifiable health benefits to the host when introduced or when they multiply in the body in sufficient amounts. Lactobacilli species are the commonest microorganisms used as probiotics but their efficacy is highly unpredictable. However, existing data indicate that carrier-food enhance the benefit of probiotics. The current in vitro study investigated possible effect of dietary nutrients on the growth characteristics of *Lactobacillus acidophilus* NCFM strain and how such nutrients influence antimicrobial activities against enteric pathogens. This study examined the effects of selected sugars (fructose, glucose, sucrose, lactose, and galactose) as well as honey and defined minerals (Mega Plus®capsule) on growth based on net changes in optical density, OD. The study also investigated the antimicrobial activities of cell-free supernatant (CFS) recovered from this probiotic against selected pathogenic species– *Escherichia coli* ATCC 35401, *Salmonella typhi* ATCC 700931, and *Enterococcus faecalis* ATCC 19433. The findings, which were derived through regression, indicated that the growth of *L. acidophius* was affected by the following linear regression coefficients (Δgrowth / Δsugar concentration): -honey (1.1613), glucose (0.48937), fructose (0.38247), sucrose (0.29346), lactose (0.23102), and galactose (0.18771). Related effect with inverse linear regression coefficients (i.e., pathogen growth reduction) were generated from the antimicrobial potential of CFS extracted against the selected pathogens. Sugar concentration was directly proportional to the overall optical density (OD) of L. acidophilus. The addition of minerals enhanced growth by approximately 2.8-fold and antimicrobial effects of CFS by approximately 2.6-fold.**

Keywords: **Regression coefficient, Bacteriocins, stimulatory effect, mineral supplementation, cell-free-supernatant (CFS), Optical density (OD).**

I. INTRODUCTION

The concept of probiotics was first suggested by Elie Metchnikoff in the early 20th century[1]. Different types of probiotics approved for use today include Lactobacillus, Bifidobacterium, and Saccharomyces[2,3,4,5]. Today, Lactic acid bacteria (LAB) are the commonest microorganisms used as probiotics[6,7]. In vitro antibacterial activity of probiotics was initially investigated using agar spot method[8,9]. Such experiments have shown that most LAB strains produce active metabolites with antagonistic properties against potential enteric pathogens (bacteria and yeast) in the media[8,10,11]. Some of these pathogens including *Salmonella typhimurium*, *Escherichia coli*, *Enterococcus feacalis*, *Clostridium difficile*, and *Candida albicans* appear to be susceptible to LAB antimicrobial activity[12,13,14,15]. However, the efficacy of most probiotic strains is highly unpredictable.

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Nutrient supply is suggested to play roe positive role in the proliferation of probiotics and also negatively on the harmful intestinal microbiota. Based on existing data, nutrient supply plays an important role in the achievement of beneficial effect of probiotics[1,16,17]. In order enhance gut colonization, gut probiotics such as LAB are usually embedded or mixed with nutrients referred to as carrier food[18,19,20]. The carrier food is thought to help the probiotic to adapt to the host conditions such as extreme gut conditions and may also promote preferential growth of the probiotic in the intestines[4,21,22,24]. Milk and fermented milk products have been identified as ideal carrier-food for lactic acid bacteria because of their high contents of lactose that is a known promoter of the growth of lactic acid and bacteriocins producers, both of which are inhibitory to many pathogens[18,19,25,26]. However, the existing research information does not indicate how nutritional manipulation can be adopted to enhance the efficacy of probiotics against pathogens both in the *in vitro* or *in vivo* settings. This study investigated the effect of selected dietary nutrients on in vitro growth patterns of *Lactobacillus acidophilus*, a known probiotic, and the effect of such nutrients on production of inhibitory metabolites against selected pathogens Escherichia coli ATCC 35401, Salmonella typhi ATCC 700931, and Enterococcus faecalis ATCC 19433. This study examined the effects of selected sugars (fructose, glucose, sucrose, lactose, galactose) as well as honey and defined minerals supplements (Mega Plus® capsule) on growth and also investigated the antimicrobial activities of cell-free supernatant (CFS) recovered from this probiotic against selected pathogenic species.

II. MATERIAL AND METHODS

Preliminary Test

Preliminary test was done with a view to visualizing the general growth and antimicrobial potential of *L. acidophilus* NCFM under standard anaerobic microbial cultural conditions on Man Ragosa and Sharpe (MRS) agar based on modified colony overlay as previously described by Barbosa *et al* (2005) in which S. Typhi ATCC 700931 was inoculated on top for susceptibility test.

Growth of bacteria on Different Nutrients

The *L. acidophilus* NCFM were resuscitated on MRS agar. One colony was selected and used for all experiments to enhance physiologic homogeneity. The selected colony was used to prepare an inoculum of 10^4 CFU that was inoculated in culture bottles containing 25g/l buffered peptone (base proteins) supplemented with varying (0.05%-2.0%) concentrations of carbon sources: sucrose, lactose, glucose, fructose, galactose, or honey as indicated on Table 1 below. Two sets of samples were prepared for each sugar. The first set without minerals and the second set with defined minerals supplementation.

The defined minerals samples tests contained 8.0 g/L of minerals Mega Plus® (1.6g) capsule content – a total of 5 capsule per liter of media. Each capsule contained Zinc Sulfate (0.15mg), Dicalcium Phosphate (190.0mg), Potassium Iodide (0.0015mg), Potassium sulfate (1.0mg), manganese sulfate (0.01mg), magnesium sulfate (1.0mg), copper sulfate (0.01mg), iron sulfate (5.0mg), nicotinamide (8.0mg), calcium pantothenate (1.0mg), ergocalciferon (0.006mg), ascorbic acid (33.0mg), cyanocobalamine (0.001mg), pyridoxine (1.34mg), riboflavin (1.0mg), thiamine (1.0mg), and retinylpalmitate (0.6mg). The samples were prepared as shown on Table 1 below:

Table 1: Tabular illustration of the experimental design indicating the sample range, intervals, repeat experiments. The intervals were used for Correlation and Regression Analysis of Effects of Sugars (carbohydrates); the repeats were used to conduct Independent Samples t-Test (Case-Control) Analysis of Effect of Minerals

	Set 1: Without Minerals				Set 2: With Minerals				Total
	Range [Sugars (g/L)]	Intervals	Repeats	**Total sub samples S1**	Range [Sugars (g/L)]	Intervals	Repeats	**Total sub-samples S2**	**Sample Size**
Glucose	0 - 20	25	10	**250**	0 – 20	25	10	**250**	**500**
Fructose	0 – 20	25	10	**250**	0 – 20	25	10	**250**	**500**
Galactose	0 – 20	25	10	**250**	0 – 20	25	10	**250**	**500**
Sucrose	0 – 20	25	10	**250**	0 – 20	25	10	**250**	**500**
Lactose	0 – 20	25	10	**250**	0 – 20	25	10	**250**	**500**
Honey	0 – 20	25	10	**250**	0 – 20	25	10	**250**	**500**
Totals				**1500**				**1500**	**3000**

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Correlation and Regression Analysis of Effects of Sugars

Each culture bottle was incubated at 37°C for 48 hours in anaerobic condition. The initial OD was recorded. After incubation, the bacterial OD was measured using a spectrophotometer (600nm). Dilutions were made due to avoid the challenges associated with high-density growth that could hinder measurements. In order to enhance accuracy of the spectrophotometer reading, the bottles were shaken to homogenize the contents and 2 ml of the sample were immediately drawn using a sterile pipette into a clean tube to measure OD. The net changes in OD (initial OD before incubation – Final OD after incubation) were recorded on spreadsheets. The remaining contents of culture bottles were immediately processed to extract cell-free supernatants for antimicrobial tests. The ODs recorded were used to calculate / generate the regression equation that depicted the effect of sugars on growth/metabolism of *L acidophilus* as follows:

$$Y = M(x) + C$$

Where:

Y = Represent the net change in Optical density of *L. acidophilus* (OD)

M = Represents Control [growth/metabolic] coefficient of the experiment (linear regression); $Control\ Coefficient = \frac{[change\ in\ OD]}{[change\ in\ sugar\ concentration]}$

x = Represents the specific sugar concentration (conc.) added in the experiment sample

M(x)= Represent change in growth/ change in sugar concentration at specified concentration of sugar; (**M** = regression coefficient; **x** = sugar concentration, 0<x>20 g/L)

C = Represents the Y-intercept; at [x = 0], which is OD in protein (peptone) – where the specific sugar concentration (x) is at 0 g/L sugar (also represents the control). Note in one set peptone is supplemented with defined minerals whereas in another set the peptone is not supplemented with defined minerals.

Therefore: $Y = M(x) + C$ is expressed in this study as:

$$OD = Control\ coeficient * (specific\ sugar\ conc.) + Growth\ in\ pure\ peptone$$

N/B: Control Coefficient (**M**) establishes the degree of control that a specific concentration of sugar exerts on flux and metabolite concentrations in a quantitative manner, thus replacing the intuitive, qualitative concept of rate limiting step. A linear regression is subject to this condition. The control coefficients have a few characteristics. The value of any flux control coefficient in a linear metabolic network is bounded between zero and one (i.e. correlation coefficient; R^2). Net change in Optical Density of bacterial broth culture was caused by cell density, effect of lactic acid produced into the media environment, and turbidity of metabolic by-product. The extent by which metabolic by-products were produced was limited to the number of live cells (i.e. bacterial growth) in the media. Therefore, growth was directly proportion to net change in optical density.

Independent Samples t-Test (Case-Control) Analysis of Effect of Minerals

As indicated in the columns of the experiment design table above (Table 1), a case-control experimental design was used to set of experiment samples supplemented with minerals (as case) and another set of experiment samples without minerals (as control) were run parallel/simultaneous to each other. The effect of minerals on the growth/metabolism was analyzed using independent sample t-test to determine the differences between control coefficients, Y-intercepts, and ODs of the two set of experiments (the mineral cases and controls without minerals) as illustrated on Table 1 above. The ODs recorded were used to calculate / generate the regression equations for the mineral/case set and the control set that depicted the effect on growth/metabolism of *L acidophilus* as based on the regression equation defined above.

Regression and Chi-Square Analysis of the Y-Intercept (Effect of Peptone)

The experiment where the value of x (concentration of sugar) was 0 g/L, the *L acidophilus* grew in media containing pure peptone, which represented the effect of pure proteins on growth/metabolism measure as OD. Peptone, a product of protein decomposition, is made through an incomplete hydrolysis process of proteins derived from beef, casein, powdered Milk,

gelatin, protein of soy, protein of silk, and fibrin among others. The composition of hydrolysate (peptone) would vary considerably from different sources of protein and varied hydrolytic conditions, and peptone is a complex blend of peptides. The molecular weights are between that of proteoses and peptides molecules (i.e., about 2000Da). Note that, the concentration of peptone was constant in all experiments. In this regard, in the regression equation defined for this study, C represents the Y-intercept; at [x = 0], which is the OD of protease digest proteins (peptone) only. The specific sugar concentration (x) is at 0 g/L sugar (also represents the constant or control or the starting point of each set of experiment. That is, in the case-control mineral experiment, the set of experiment at 0 g/L sugar concentration that was not supplemented with defined minerals represented the effect of protein on growth/metabolism and antimicrobial activity. In the regression equations generated, the Y-intercepts depict the physiology of *L. acidophilus* in the absence of all nutrients except the proteins/peptone.

Antimicrobials Tests for Cell Free Supernatants

The susceptibility of pathogens to Cell-Free Supernatant (CFS) produced by *L. acidophilus* from each of the experiment above were done on *E. coli* ATCC 35401, *S. typhi* ATCC 700931, and *E. faecalis* ATCC 19433. Approximately 75 µl of extracted CFS, was added to each well of the cell culture plates containing 20 µl of sterile buffered peptone broth, and inoculated with 5 µl of test pathogens to make 100 µl of cultured broth. The tests were done in multiples/repeats (i.e., n = 10) and a blank/negative control containing the broth with CFS but no pathogen. Positive control contained broth and pathogen but without CFS extract was inoculated with pathogen. The preparations were incubated at 37˚C (5% CO_2) for 12 hours. Thereafter, the optical density at 600 nm (OD600) was determined on spectrophotometer/ densitometer. The antimicrobial effect of the CFS against the test pathogens were calculated as follows:

$$Pathogen\ growth(\%) = \left(\frac{[OD\ of\ Positive\ Control - OD\ of\ CFS\ sample]}{[OD\ of\ Positive\ Control - OD\ of\ Negative\ Control]}\right) * 100$$

OR

$$Pathogen\ growth(\%) = \left(\frac{[OD\ of\ control - OD\ of\ CFS\ sample]}{\text{OD of control}}\right) * 100$$

Where:

OD of control = OD of positive control (broth + pathogen) – OD of negative control (broth + CFS)

OD of CFS sample = OD of test sample (broth + CFS + pathogen) – OD of negative control

Note that: OD of broth only = OD of broth + CFS; (as measured by this study)

This data was used to generate scatterplots of percentage growth of pathogen and used to interpret the antimicrobial effect of *L. acidophilus* CFS against the three selected pathogens.

III. RESULT

The findings of correlation and regression analyses indicated that there was a difference in the OD trends (i.e., the growth and/or metabolic pattern) of *L. acidophilus* and the nature of antimicrobial activity of CFS as influenced by the different sugars. Additionally, the findings of independent samples t-test that compared samples without defined minerals (as control experiment) verse those with defined minerals (as mineral experiment) indicated that there was a significant difference in the OD trends (i.e., the growth and/or metabolic pattern) of *L. acidophilus* and the nature of antimicrobial activity of CFS as influenced by the addition of defined minerals. In this regard, the choice of sugar (or carbon source) and the addition or absence of defined minerals determines the extend of probiotic colonization and associated antimicrobial effect in the in vitro environment. The comprehensive details of how these outcomes were derived feature in the results and discussion sections below. Sugar concentration was found to be directly proportional to the final OD of *L. acidophilus* and addition of various mineral enhanced the growth and the “antimicrobial potency” of the derived metabolites at least 2 folds.

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Analysis of Effects of Nutrients (Sugars and Minerals) on Growth (Biomass)

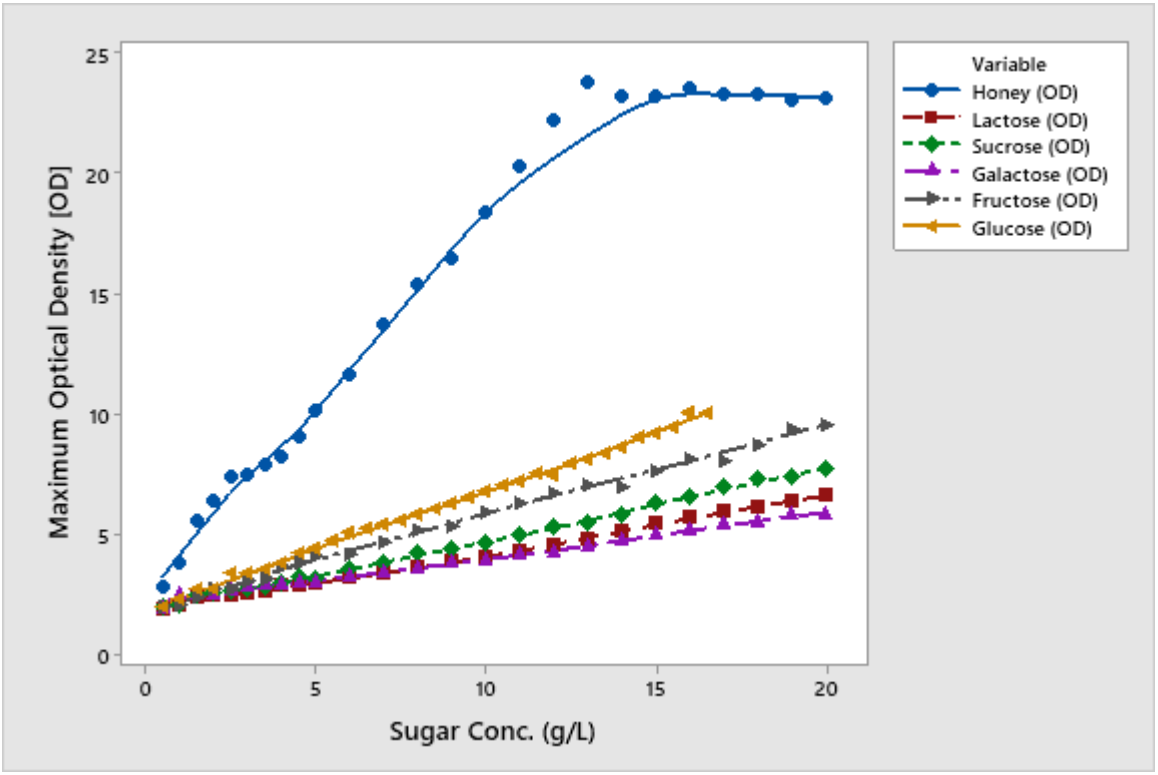
The regression equation (Y = Mx + C) used to test the effect of sugars on growth indicated that sugar concentration was directly proportional to the final OD of *L. acidophilus* as indicated in the Table 2 below:

Table 2: List of Regression equations depicting the Stimulatory effects of simple sugars, honey, and selected defined minerals supplementations on growth vigor of probiotic Lactobacillus acidophilus

	Regression Equations	
Sugar Type	***Set without Minerals***	***Set with Minerals***
Fructose	OD = 1.9248 + 0.38247 Fructose(g/L)	OD = 2.140 + 1.1824 Fructose+(g/L)
Glucose	OD = 1.8977 + 0.48937 Glucose (g/L)	OD = 3.671 + 1.2221 Glucose+ (g/L)
Galactose	OD = 2.1102 + 0.18771 Galactose (g/L)	OD = 2.1265 + 0.49963 Galactose+ (g/L)
Sucrose	OD = 1.8294 + 0.29346 Sucrose(g/L)	OD = 1.9748 + 1.03164 Sucrose+ (g/L)
Lactose	OD = 1.9175 + 0.23102 Lactose (g/L)	OD = 1.9704 + 0.48872 Lactose+ (g/L)
Honey	OD = 4.584 + 1.1613 Honey (g/L)	OD = 3.3897 + 1.16967 Honey+ (g/L)

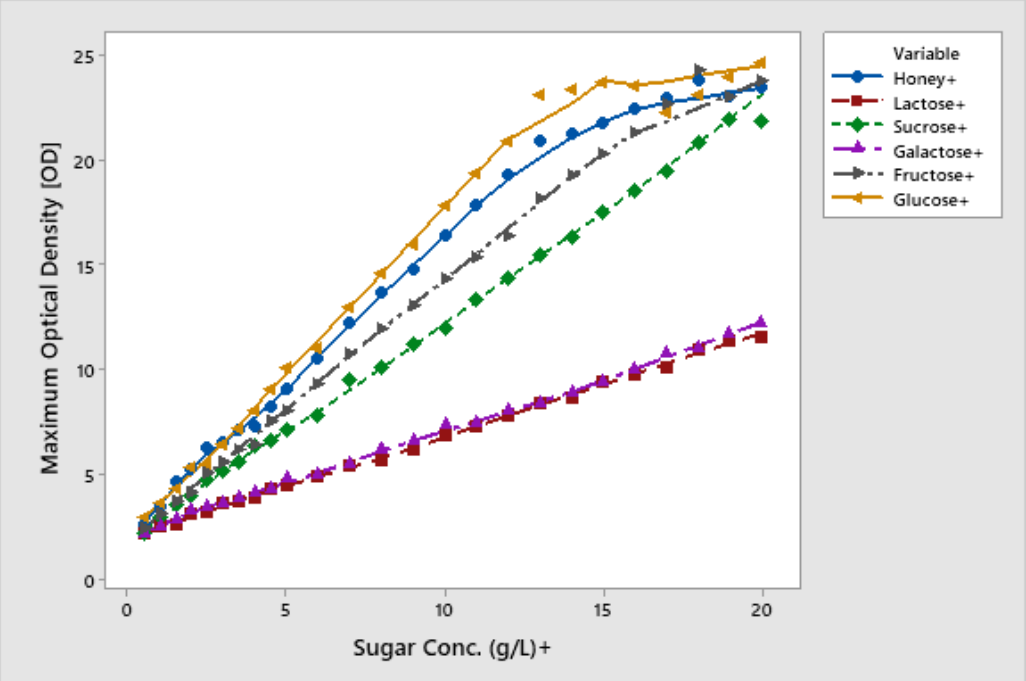
Each of the equation above is represented graphically in Figure 1 (without defined minerals supplementation) and Figure 2 (with defined minerals supplementation) below. Note that the OD represent the average optical density readings of 10 repeats (– refer to research design in Table 1)

Figure 1: The regression plots of the effect of each of the sugar on the growth of *L. acidophilus* in the broth without mineral supplementation. Growth increased with increase in sugar concentration. Glucose had the highest effect and was followed by fructose, sucrose, lactose, and galactose in that order as indicated by the slope or regression coefficients in Table 1 above. The effect of honey is shown in **Figure 2** below.



The regression coefficients of growth in the presence of each sugar are shown in Table 1, which suggests that carbohydrate supply is the limiting factor for growth. The sugar with the greatest stimulatory effect on growth based on OD was glucose followed (in decreasing order of significant) by glucose (0.48937), fructose (0.38247), sucrose (0.29346), lactose (0.23102), and then galactose (0.18771).

Figure 2: The regression plots of the effect of sugars on the growth of *L. acidophilus* in the broth with mineral supplementation. Growth increased with increase in sugar concentration. Glucose had the highest effect on growth (1.2221) and was followed by fructose (1.1824), honey (1.1613), sucrose (1.03164), galactose (0.49963), and lactose (0.48872) in that order as indicated by the slope or regression coefficients.



The regression curves with stationary phase occurred in experiments of honey, glucose, and fructose. The stationary in these cases indicated that increase in concentration of these sugars in the media could not increase the maximum OD for the experiment. This suggested that higher concentration of honey (at 17 g/L), glucose (at 15 g/L), fructose (at 18 g/L), and sucrose (at 19 g/L) that were supplemented with defined minerals led to the consumption of all the amino acids in peptone and thus represented the maximum level of growth that this concentration of peptone could support. In other words, peptone became a limiting nutrient at higher concentration of sugar and mineral supplementation. This inference was supported by Biuret test and Ninhydrin test for the presence of peptides and amino acids respectively[27,28,29,30]. The stationary phase scenario did not occur in experiments containing lactose and galactose. Additionally, after 48 hours of culture, the Biuret test and Ninhydrin test confirmed the presence of peptides and amino acids respectively. This indicated that even with the maximum concentration of sugar tested (at 20 g/L), the *L. acidophilus* reached maximum OD without depleting the proteins (peptone) in the media. In sets of experiments that were not supplemented with defined minerals, only honey reached stationery (at 17 g/L). This finding indicate that mineral supplementation did not affect the growth of L. acidophilus. It also indicates that honey contained substances that boosted the growth of L. acidophilus in media to an extent it consumed all the proteins (in peptone) in the media.

Table 3: Regression/Control coefficients (M) and Y-intercepts of the regression equation of each sugar. The comparison of Mx along the column determines the sugar that had the highest effect on growth. The comparison of Mx along the rows (i.e., Mx_1 against Mx_2) determined the effect of mineral supplementation as illustrated in **Table 2** below. Y-intercept is the control experiment whereas Correlation is the precision of the regression relationship between sugar concentration and the resultant growth of *L. acidophilus*

	Without minerals supplementation			**With minerals supplementation**		
Sugar in Media	Y-intercept [at sugar 0g/l]	Control Coefficient $M(x)_1$	Correlation (R^2)	Y-intercept [at sugar 0g/l]	Control Coefficients($x)_2$	Correlation (R^2)
Fructose	1.9248	0.38247	0.9949	2.140	1.1824	0.9932
Glucose	1.8977	0.48937	0.9977	3.671	1.2221	0.9434

Galactose	2.1102	0.18771	0.9931	2.1265	0.49963	0.9983
Sucrose	1.8294	0.29346	0.9969	1.9748	1.03164	0.9986
Lactose	1.9175	0.23102	0.9786	1.9704	0.48872	0.9988
Honey	4.584	1.1613	0.9194	3.3897	1.16967	0.9689

Effect of Defined Minerals on Growth of *L. acidophilus*

The synergistic effect minerals and sugars when added as supplements to growth media for *L. acidophilus* were analyzed. The effect of minerals significantly affected the growth (OD) of *L. acidophilus*, which was shown by significant shift in the regression coefficient by approximately 2.6 folds as shown in **Table 3** below.

Table 4: Minerals supplementation affected the control coefficients of *L. acidophilus* [colonization/OD] by comparing the regressions of sets of experiments without mineral supplementation (Mx_1) verses those with mineral supplementation (Mx_2). The calculations indicate that minerals supplementation increased the control coefficients in fructose (3.1-fold), glucose (2.5-fold), galactose (2.66-fold), sucrose (3.5-fold), and lactose (2.1-fold) but did not change that of honey (remained at 1-fold).

	Without mineral supplementation	**With mineral supplementation**	**Effect of mineral supplementation**	
Carbohydrate (sugar) supply	Regression/Control coefficient $M(x)_1$	Regression/Control coefficient $M(x)_2$	Effect = $M(x)_1/M(x)_2$	Effect of minerals
Fructose	0.38247	1.1824	3.091484	3.1
Glucose	0.48937	1.2221	2.497292	2.5
Galactose	0.18771	0.49963	2.661712	2.66
Sucrose	0.29346	1.03164	3.515437	3.5
Lactose	0.23102	0.48872	2.115488	2.1
Honey	1.1613	1.16967	1.0072	1.0
Average effect of minerals supplementation (on growth)			**2.7762**	**≈2.8 folds**

Antimicrobial Activity of Cell-Free Supernatants (CFS)

This analysis was based on the hypothesis that nutrients supplied to a probiotic organism (*L. acidophilus*) influence the range of metabolites produced by the probiotics. In this case, sugar and minerals supplied to *L. acidophilus* were analyzed based on the antimicrobial activity of the CFS produced. Note that the assumption in this study is that increase in sugar concentration increased the level of antimicrobial metabolites in CFS, which in turn increased the antimicrobial effect of CFS against the test pathogen. The regression equation (Y = Mx + C) or (Y = C + Mx) was used to test the effect of sugars on antimicrobial activity or production of antimicrobial metabolites in CFS, which was measured based on reduction of growth of test pathogens, where:

Y = percentage reduction in growth of the test pathogen relative to control (OD)

Mx = change in pathogen growth/ change in sugar concentration; (**M** = regression coefficient; **x** = sugar concentration, 0<x>20 g/L)

C = growth in samples without sugar (0 g/L sugar); thus, representing the control (Y-intercept)

The effects of minerals were determined by comparing the regression coefficients of samples without mineral supplementation with those with mineral supplementation as shown in Figure 3 below. The negative regression coefficient in the study of antimicrobial activity of the CFS of *L. acidophilus* indicates that increase in sugar concentration in L acidophilus' broth media increased the production antimicrobial effects. CFS from honey medium had the greatest antimicrobial effect. It was followed by mineralized glucose, fructose, and sucrose. The CFS from concentrations of Lactose and galactose showed lesser effects when compared to other sugars.

Effect of Defined Minerals on Antimicrobial Activity of *L. acidophilus*

The addition of minerals in the media increased the production of these antimicrobial products by average of 2.6 folds as illustrated on table below. These findings exhibited that the addition of defined minerals increased the regression coefficients of all sugars between 1.8-fold (lactose) and 3.0-fold (fructose). The stimulatory effect of minerals was lowest in the presence of lactose and highest in the presence of fructose.

Table 5: Effect of Minerals on the overall antimicrobial activity of CFS extracted from *L. acidophilus* media. The effect was determined by comparing the regression coefficients of sugars without mineral supplementation (Mx_1 column) and that of sugars with mineral supplementation (Mx_2 column).

Sugar	Pathogen	$M(x)_1$ [Regression coefficient]	$M(x)_2$ [Regression coefficient]	Effect: $\frac{M(x)_2}{M(x)_1}$	≈Folds increase: x[$M(x)_1$]
Fructose	*E. coli*	-3.5022	-9.81	2.801096	2.8-
	S. Typhi	-3.7832	-9.74	2.57454	2.5
	E. faecalis	-4.784	-14.602	3.052258	3
Glucose	*E. coli*	-4.3544	-11.334	2.602884	2.6
	S. typhi	-4.7711	-13.146	2.755339	2.8
	E. faecalis	-5.9072	-16.22	2.745802	2.7
Galactose	*E. coli*	-1.9123	-4.2561	2.225645	2.2
	S. typhi	-1.875	-4.7153	2.514827	2.5
	E. faecalis	-2.7328	-2.7328	2.300205	2.3
Sucrose	*E. coli*	-2.8161	-7.641	2.713327	2.7
	S. typhi	-3.0767	-9.247	3.005493	3
	E. faecalis	-3.7971	-12.57	3.310421	3.3
Lactose	*E. coli*	-2.201	-4.109	1.866879	1.8
	S. typhi	-2.329	-4.784	2.0541	2.1
	E. faecalis	-3.0109	-5.925	1.96785	2
Honey	*E. coli*	-11.316	-12.073	1.0669	1
	S. typhi	-13.778	-12.2	0.8854	0.9
	E. faecalis	-16.65	-18.685	1.1222	1.1
Average				**2.5660444**	**≈ 2.6-fold**

IV. DISCUSSION

The summation theorem illustrated by the findings of this research is that the flux control of nutrients was shared – i.e., when one control coefficient changed, other control coefficients changed as well. This means that the control coefficient is a system property rather than an enzyme-specific property. For instance, the findings demonstrated that the sugars tested influence growth and production of antimicrobial components by the probiotics, which was demonstrated by significant regression coefficients and distinct slopes on the graphs. The order of effects of these sugars from the highest to the lowest is as follows – glucose, fructose, sucrose, lactose, and galactose. It is clear from the graphs that monosaccharide performed better than disaccharides. For instance, regression coefficients of fructose (1.1824) and glucose (1.2221) indicated better effects than sucrose (1.03164) and lactose (0.48872). However, there was no significant difference between galactose and lactose that had regression coefficients of 0.49963 and 0.48872 respectively. Based on these results the LAB had better growth and enhanced production of pathogen inhibitory molecules when grown in media supplemented with sucrose compared to lactose.

The growth and production of antimicrobial effect of L. acidophilus was further stimulated by supplementation of the media with defined minerals. In this study, defined minerals increased growth by approximately 2.8-fold and production of antimicrobial effects of L acidophilus NCFM against E. coli, S. typhi and E. faecalis by approximately 2.6-fold. The effect was determined by comparing the regression coefficients of sugars without mineral supplementation and that of sugars with mineral supplementation. These findings correlate with that of Larsen (2007) who demonstrated that the presence of calcium enhances the growth conditions of stable cultures of L. acidophilus NCFM. These findings exhibited that the addition of

defined minerals increased the regression coefficients of all sugars between 1.8-fold (lactose) and 3.0-fold (fructose). The stimulatory effect of minerals was lowest in the presence of lactose and highest in the presence of fructose.

The findings of this study also showed that the use of honey as carbohydrate source without mineral supplementation significantly promotes the growth of L. acidophilus and production of antimicrobial effects of L acidophilus NCFM against E. coli, S. typhi and E. faecalis. When all the simple sugars were supplemented with sugars, their effects to growth, measured based on change in OD, were at least doubled and the respective antimicrobial effects their CFS were also doubled. However, the addition of defined minerals to media containing honey did not indicate any significant change as analyzed through t-test. This outcome can be tentatively or presumptively attributed to the fact that honey with minerals naturally contains minerals and thus supplementation with defined minerals when using honey as carbon source may not be necessary. This inference can be deduced from the coefficients of honey (1.1613) that reflected those of the best performing simple sugars – fructose (1.1824) and glucose (1.2221) – that had been supplemented with defined minerals. It is important to note that the findings of this study indicated that the regression coefficient of honey (1.1613) was significantly different those of the best performing simple sugars without defined minerals supplementation i.e. glucose (0.48937), fructose (0.38247), sucrose (0.29346), lactose (0.23102), and then galactose (0.18771).

Natural honey is a mixture of sugars and minerals. It is strongly believed that the minerals found naturally in honey caused it to have a stimulatory effect on growth of L. acidophilus. However, in this study, minerals supplementation also caused glucose, fructose, and sucrose to exhibit similar stimulatory effects exhibited by honey. These findings suggest that the probiotics properties of L. acidophilus can be enhanced through nutrient manipulation, which in the case of this study, sugar and minerals demonstrated this concept.

The regression equations for the effects of cell-free supernatants produced by L. acidophilus NCFM demonstrated that all the test pathogens were susceptible to antimicrobial effect of the LAB CFS. The negative regression coefficients further indicated that higher sugar concentration L. acidophilus increased the level of antimicrobial effect of the LAB and this stimulation was further enhanced by additions of minerals.

V. CONCLUSION

This study has demonstrated the in vitro effect of different nutrients on the growth and antimicrobial effects of Lactobacillus acidophilus. This study demonstrated that manipulation of nutrients (defined sugars and minerals) is critical for stimulating growth and probiotic properties of Lactobacillus acidophilus. The best source of carbon/carbohydrates based on the findings of this study is honey. Alternatively, fructose and glucose can be used as source of carbohydrates though the best results can be achieved by supplementing them with minerals, which have stimulatory effects on growth and antimicrobial activities of L. acidophilus against pathogens. This study demonstrated to the probiotic consumers that defined nutrition is critical attribute for their efficiency especially on growth, which involve colonization within the host, and the enhancement of the beneficial effects as depicted by Lactobacillus acidophilus NCFM.

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