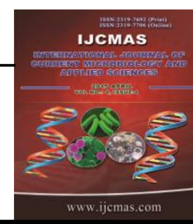
Int.J.Curr.Microbiol.App.Sci (2015) 4(4): 1082-1096

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 4 (2015) pp. 1082-1096**
http://www.ijcmas.com



Original Research Article

The Plackett-Burman Design to Evaluate Significant Media Components for Antimicrobial Production of *Lactobacillus rhamnosus*

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A B S T R A C T

The use of antibiotics to control fish disease is one of the constraints in tilapia farming. Hence, the use of probiotic as alternative strategies has received much more attention. This study was undertaken to evaluate the ability of *Lactobacillus rhamnosus* to producte bacteriocin as biological control agent, improve fish growth and immune responses. The objective of this study was to enhance production of bacteriocin, produced by *Lactobacillus rhamnous*, cultivation conditions and optimized medium composition. The Plackett Burman (PB) experimental design was effective in searching for the significant variables that influence bacteriocin production. From PB Design, four factors peptone, yeast extract, glucose and initial pH were found to be significant factors and had positive effect on bacteriocin production. The results showed that the final concentration of medium optimized with Plackett–Burman was (in g/l): glucose,50; yeast extract,5; peptone 15; PH 6.8. Under optimized medium, the average bacteriocin yield reached 10200 AU/ml. Bacteriocin production in a cost effective medium might facilitate industrial scale production of lactic acid bacteriocin and their use as a natural food biopreservative.

Keywords

Lactobacillus rhamnous, Bacteriocin production, media optimization and Plackett-Burman design

Introduction

In recent years bacterial antibiotic resistance has been considered a problem due to the extensive use of classical antibiotics in treatment of human and animal diseases (yoneyama and Katsumata,2006). As a consequence, multiple resistant strains appeared and spread causing difficulties and the restricted use of antibiotics as growth promoters. So, the continued development of new classes of antimicrobial agents has become of increasing importance for medicine (Kumar and Schweiser, 2005 and Fisher *et al*., 2005). In order to control their abusive use in food and feed products, one plausible alternative is the application of some bacterial peptides as antimicrobial substances in place of antibiotics of human application. Among them, Bacteriocins produced by lactic acid bacteria have attracted increasing attention, since they are active in a nanomolar range and have no toxicity. Bacteriocins are ribosomally

synthesized proteinaceous compound lethal to bacteria other than the producing strain. Bacteriocins are proteins or complexed proteins which are biologically active with antimicrobial action against other bacteria, principally closely related species. They are produced by bacteria and are normally not termed antibiotics in order to avoid confusion and concern with therapeutic antibiotics, which can potentially illicit allergic reactions in humans and other medical problems (Deraz *et al.*, 2005) Bacteriocins are commonly divided into four groups (Ennahar *et al.*, 2000 and Oscáriz and Pisabarro, 2001). They are I) Lantibiotics; II) small hydrophobic heat-stable peptides (< 13,000 Da); III) large heat-labile proteins (> 30,000 Da) and IV) complex bacteriocins showing the complex molecule of protein with lipid and/or carbohydrate.

Number of experimental factors known to influence the production of bacteriocins, where as optimization of media composition is one of the important parameter for enhancement of bacteriocin production. The effects of media composition and culture conditions on production of bacteriocins have already been reported (Biswas *et al.*, 1991; De Vuyst and Vandame., 1992; Parente and Hill., 1992). Conventional methods for optimization of medium and culture conditions involves may lead to unreliable and wrong conclusions and also extremely time consuming and expensive. Now a days the approach has shifted to statistical methods which offer several advantages over conventional methods in being rapid and reliable, helps understanding the interactions among the nutrients at various concentrations and reduces the total number of experiments tremendously resulting in saving time and material (Li *et al.*, 2001). The reported use of Response Surface Method and Plackett-Burman statistical designs in optimization and for studying effects of various parameters is gaining immense importance for the production of bacteriocins (Kumar and Srivastava., 2010; Patil *et al.*, 2011; Selvaraj *et al.*, 2012). In present study the Plackett-Burman design was used to determine the significance of various media ingredients and growth conditions for the production of bacteriocin by *Lactobacilli* isolated from different depth of El-Khadra lake.

Materials and Methods

Sample collection and Isolation of Lactobacillus

Water samples were collected from El-Khadra Lake at different depth of the lake water, these samples were transported to the laboratory immediately using cool box (4°C) and tested directly. LAB were isolated from water samples by adding 10 ml of samples and mixed with 90 ml of normal saline solution (8.5 gm NaCl/ l) and homogenizing for 2 min (Babji and Murthy, 2000). Serial dilutions up to 10^7 were prepared and appropriate dilutions were plated de Man Rogosa and Sharpe (De Man *et al.*, 1960) agar plates by pour plate method (Awan and Rahman, 2005). MRS Plates were incubated at 37^0C for 48 hours an-aerobically. Morphologically distinct and well isolated colonies were picked and transferred to new MRS agar plates by streaking. Finally, pure colonies were obtained.

Identification of *Lactobacillus* species

Macroscopic appearance of all colonies was examined for cultural and morphological characteristics. Bacterial isolates were selected for biochemical test and 16S rRNA sequencing (Mandal *et al.*, 2008; Rouse *et al.*, 2008). Pure cultures were maintained in MRS broth at -20^0C with 10% (v/v) glycerol.

Target pathogen

Collection of samples were examined for the presence of *E. coli*, *Salmonella Pseudomonas* and *Staphalococcus*. Microbiological analysis A Portion (10 ml) from each sample was extracted aseptically and homogenized with 90 ml sterile enrichment broth (lactose broth for *E. coli*, asparagine broth for *P.aeruginosa* and peptone water for *S. aureus*) and incubated at 37 °C for 24 hours.

Media and growth conditions for the isolation and identification of E. coli, the enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37 °C for 24 hours. Morphologically typical colonies producing metallic sheen were taken into nutrient broth for further identification. S. aureus was isolated by using the technique given by Baird Parker (1962).

Enriched samples were streaked on Baird Parker Agar (BPA) and the plate was incubated at 37 °C for 24–48 hours. Appearances of jet black colonies surrounded by white halo were considered to be presumptive *S. aureus*.

All the tubes that showed growth with greenish-blue pigment, or both fluorescence under UV light were subcultivated in acetamide agar for confirmation as *P. aeruginosa* (APHA, 1981). Bacterial isolates were selected for physiological and biochemical examination and 16s rRNA . Pure cultures were maintained in MRS broth at -20°C with 10% (v/v) glycerol.

Screening for bacteriocinogenic potential

The inhibitory activity of the Lactobacilli isolates was determined by Agar- well diffusion assays (Schillinger and Lucke, 1989). Crude bacteriocin was prepared by inoculating the cultures (used to screen for bacteriocinogenic potential) in MRS broth (10mL) and incubated at 30°C. After incubation it was centrifuged at 12000 rpm at 4°C for 30 min and supernatant was collected and filtered through 0.45µm pore size filters in order to eliminate any possibility of remaining cell/contamination. The cell free supernatant was referred as crude bacteriocin preparation (Muriana *et al.*, 1991). Crude bacteriocin was neutralized by 1N NaOH and final pH was set at 7. Nutrient agar plates were overlaid with 0.1mL of the indicator bacterial strains. Wells were cut into agar plates and 100µl of neutralized crude bacteriocin preparation was placed into each well. The plates were incubated at 37°C for overnight and zones of inhibition were measured in cm (Muriana *and* Klaenhammer., 1991; Schillinger *et al.*, 1991). Antimicrobial activity of the bacteriocin was expressed as arbitrary units (AU) per mL. One AU was defined as the reciprocal of the highest dilution showing a clear zone of growth inhibition (Van Reenen *et al.*, 1998).

Identification of the significant factors by Plackett–Burman design(PBD) :

The purpose of the first optimization step was to identify important ingredients of the culture medium. PB design was applied for screening of the significant variables that influence bacteriocin production. By this, the most significant component that affects the production of bacteriocin can be determined and will be used for media optimization. The design experiment for screening the most significant component of MRS media such as:

Effect of pH on bacteriocin production

To determine the effect of pH on bacteriocin production, four batches of 100 mL MRS broth was prepared and adjusted to

pH=5,6,7,8 respectively with 1 N HCl or 1 N NaOH, and then autoclaved. Each medium was inoculated (1 % v/v) with an overnight culture of bacteriocin producing organism. Plates were kept at cool temperature for 1 hr and then incubated at 37°C for 24hrs.

Effect of carbon sources on bacteriocin production

The effect of carbon sources on the production of bacteriocin was carried out using 2 % (w/v) glucose, lactose, sucrose, fructose and maltose as carbon sources. The sterilized medium was inoculated with an (1 % v/v) overnight culture of bacteriocin producing *Lactobacillus.* Plates were kept at cool temperature for 1 hr and then incubated at 37°C for 24hrs

Effect of nitrogen sources on bacteriocin production

Different nitrogen sources such as beef extract, yeast extract, peptone, tryptone, urea and ammonium chloride which were supplemented at 2 % (W/V) to study their effect on bacteriocin activity bybacteriocin producing organism *Lactobacillus.*

The medium was sterilized and inoculated (1 % v/v) with an overnight culture of bacteriocin producing organism *Lactobacillus spp.* Plates were kept at cool temperature for 1 hr and then incubated at 37°C for 24hrs

Effect of inorganic salts on bacteriocin production

Different inorganic salts such as $MnSO_4$, $ZnSO_4$, $CuSO_4$, $FeSO_4$, K_2HPO_4, and KH_2PO_4 which were supplemented at 2 % (w/v) to study their effect on bacteriocin activity by bacteriocin producing organism *Lactobacillus.* The medium was sterilized and inoculated (1 % v/v) with an overnight culture of bacteriocin producing organism *Lactobacillus spp*. Plates were kept at cool temperature for 1 hr and then incubated at 37°C for 24hrs.

Based on the results of the above experiments, the selected carbon sources (glucose and lactose) and nitrogen sources (peptone and yeast extract) were further optimized together with other variables. The Plackett–Burman design was used in this stage

The Plackett–Burman experimental design (Plackett and Burman, 1944) based on the first-order model:

$$\mathbf{Y = \beta 0 + \Sigma\ \beta i\ Xi}$$

was used to screen the important variables that influence bacteriocin production. Where Y is the response (PGA yield), β0 is the model intercept and βi is the linear coefficient, and xi is the level of the independent variable. This model identifies the main parameters required for maximal bacteriocin production. Total number of trials to be carried out according to the Plackett–Burman is n+1, where n is number of variables (medium components). The experimental design with the name, symbol code, and actual level of the variables is shown in Tables 1 and 2. Each variable is represented at two levels, high and low, which are denoted by (+1) and (−1), respectively. Table 1 lists the factors under investigation as well as the levels of each factor used in the experimental design with the symbol code and actual level of the variables. The independent variables were screened in 7 combinations and actual level of the variables is shown in Table 2.

Table.1 Experimental variables at diferent levels used for the production of bacteriocin by *Lactobacillus rhamnous* using Plackett–Burman design

Variables	Codes	Units	Experimental values	
			Lower	Higher
Peptone	**P**	**g/l**	**1.5**	**15**
Yeast extract	**Y**	**g/l**	**0.5**	**5**
Glucose	**G**	**g/l**	**5**	**50**
Lactose	**L**	**g/l**	**5**	**50**
Incubation period	**I**	**hr**	**12**	**24**
pH	**PH**		**4.5**	**6.8**

Table.2 Eight-trial Plackett–Burman design matrix for seven variables with coded values along with observed results for screening of significant factors affecting bacteriocin production by *Lactobacillus rhamnous*

Trial	P (g/l)	Y (g/l)	L (g/l)	G (g/l)	A (g/l)	I (hr)	pH
T1	+1	+1	+1	-1	+1	-1	-1
T2	-1	+1	+1	+1	-1	+1	-1
T3	-1	-1	+1	+1	+1	-1	+1
T4	+1	-1	-1	+1	+1	+1	-1
T5	-1	+1	-1	-1	+1	+1	+1
T6	+1	-1	+1	-1	-1	+1	+1
T7	+1	+1	-1	+1	-1	-1	+1
T8	-1	-1	-1	-1	-1	-1	-1

Results and Discussion

Ten *Lactobacillus spp.* were isolated and identified from water of El-Khadra lake. The physiological and biochemical characteristics of the *Lactobacillus* isolates were studied and screened for the probiotic properties antimicrobial activity and their degree of inhibition against the entero pathogens.

Eight isolates were Gram-positive, non-motile, non-spore forming, catalase-negative rods, with the classical characteristicsof *Lactobacillus* (Kandler and Weiss, 1986). Among these, six of the strains were found to be bacteriocin producing *Lactobacillus* spp., when tested against the indicator strains, and the isolate L8 was found to be more potential and the results are shown in Tables (3&4).

Table.3 Biochemical tests for identification of lactobacilli

Code	Gram's test	Catalase test	Gas production from glucose
Lact1	G +ve, rod	(-)	(-)
Lact2	G +ve, rod	(-)	(-)
Lact3	G +ve, rod	(-)	(-)
Lact4	G +ve, rod	(-)	(-)
Lact5	G +ve, rod	(-)	(-)
Lact6	G -ve, rod	(-)	(-)
Lact7	G +ve, rod	(-)	(-)
Lact8	G +ve, rod	(-)	(-)
Lact 9	G-ve cocci	(-)	(+)
Lact 10	G+ve cocci	(+)	(+)

Based on the biochemical results which facilitated the choice of appropriate molecular methods for further species identification. Lact8 strains identified as *Lactobacillus rhamnosus* results shown in Table (5) and Fig(2)

Table.4 Primary screening for bacteriocinogenic activity of isolates

LAB isolate	**Mean diameter of zone of inhibition (cm)**					
	Aeromonas hydrophila	***E.coli***	***S.aureus***	***P.aerogenosa***	***Salmonella entricia***	**Cumulative of zone of inhibition (cm)**
Lact1	2.70	1.60	0.83	0.96	0.27	6.36
Lact2	1.19	2.91	1.3	1.5	0.5	7.40
Lact3	ND	ND	ND	ND	ND	ND
Lact4	0.80	2.30	0.86	0.66	0.6	5.22
Lact5	2	2.4	0.6	0.4	0.4	5.80
Lact6	2	2.8	1.35	1.16	0.6	7.91
Lact7	ND	ND	ND	ND	ND	ND
Lact8	3.4	5.2	1.23	0.8	0.6	11.23

ND: not detected under the experimental conditions

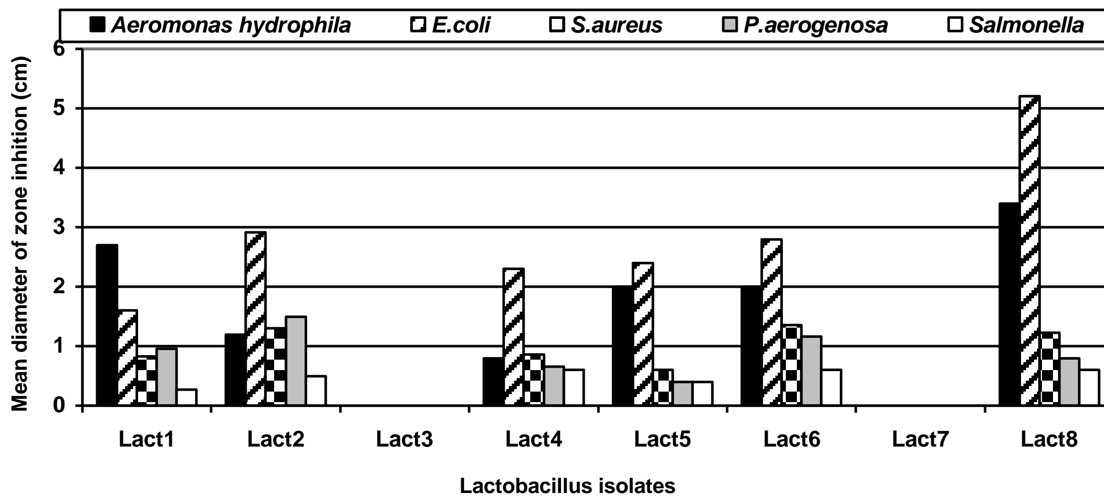

Fig.1 Inhibition of the selected bacterial fish pathogens by different *Lactobacilli* isolates.

Table.5 Biochemical tests for *Lactobacillus rhamnosus*

Biochemical test	Reaction
Gram reaction	G +ve
Motility	Non-motile
Catalase	−
NH_3 from Arginine	−
Acid from glucose	+
Gas from glucose	−
Acid from sugar	
Mannitol	+
Lactose	+
Sucrose	+
L-Arabinose	+
Glycerol	−
Glactose	−
Maltose	+
Gluconate	+

G+ve:Gram positive + **:**Positive results -: negative results

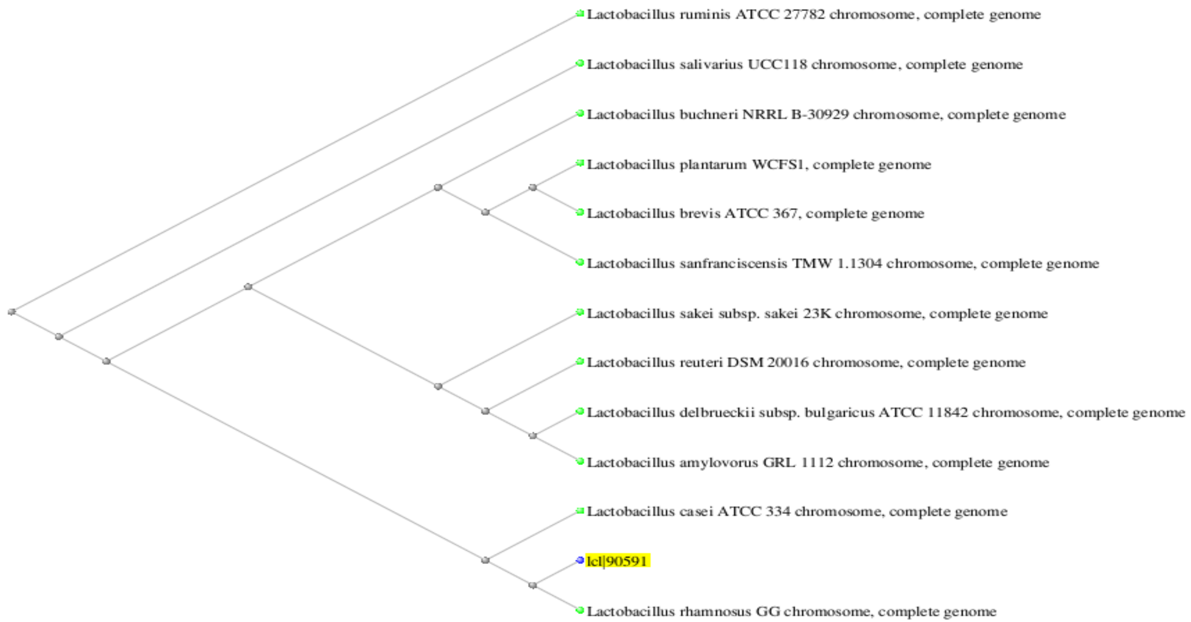

Fig.2 Phylogenetic dendrogram showing taxonomic positions of *Lactobacillus rhamnous*. type strains based on the 16s rRNA partial sequences.

Table.6 Biochemical characterization of *E. coli*, *P.aeruginosa*, *S. aureus* and *Sallmonella entricia*

Biochemical test	*E.coli*	*P.aeruginosa*	*S. aureus*	*Sallmonella entricia*
Gram reaction	-ve	-ve	+ve	-ve
Motility	Motile	Motile	Non-motile	Motile
Catalase	+	-	+	+
H2S production	-	+	-	+
Simmon's citrate	-	+	-	-
Indole production	+	+	+	-
Nitrate reduction	+	+	+	+
Methyl red	+	-	+	+
Voges- Proskauer	-	-	+	-
Urease	-	-	-	-
Lactose fermention	+	-	-	-
Acid from sugar				
Glucose	+	+	+	+
Mannitol	+	+	+	+
Lactose	+	+	-	-
Sucrose	+	+	+	-

Morphologically typical colonies of *Pseudomonas aeruginosa*were identified by Gram's staining.Catalase reaction, Methyl red test, Voges-Proskauer test, Nitrate reduction, fermentation of sugars (Table 8) and Fig (5)

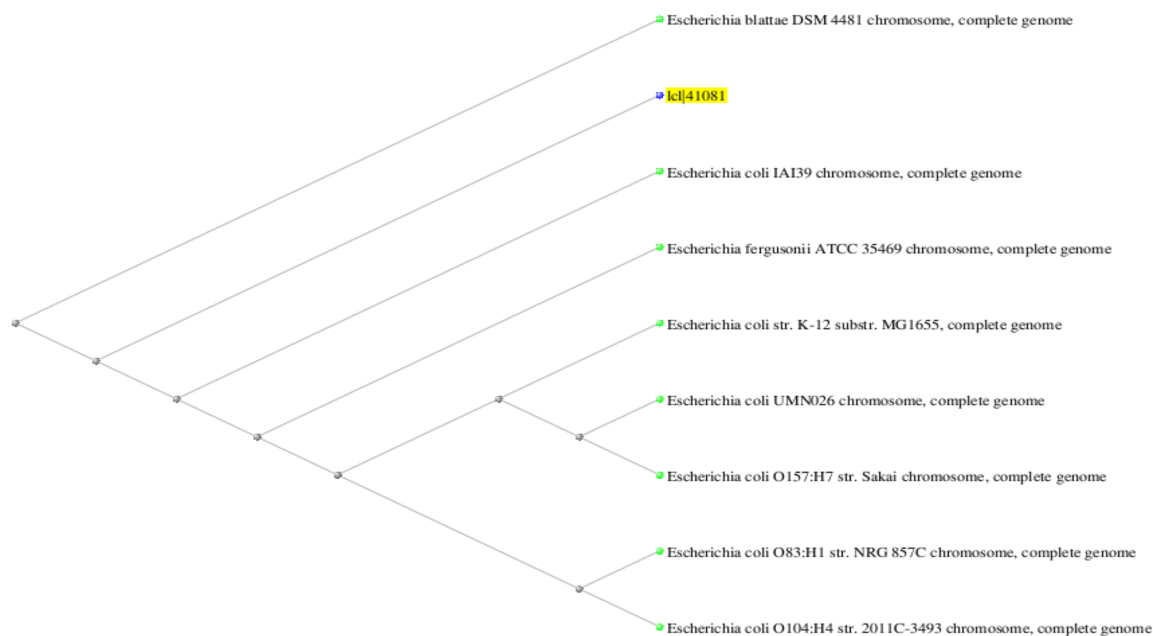

Figure.3 Phylogenetic dendrogram showing taxonomic positions of *E.coli*. type strains based on the 16s rRNA partial sequence

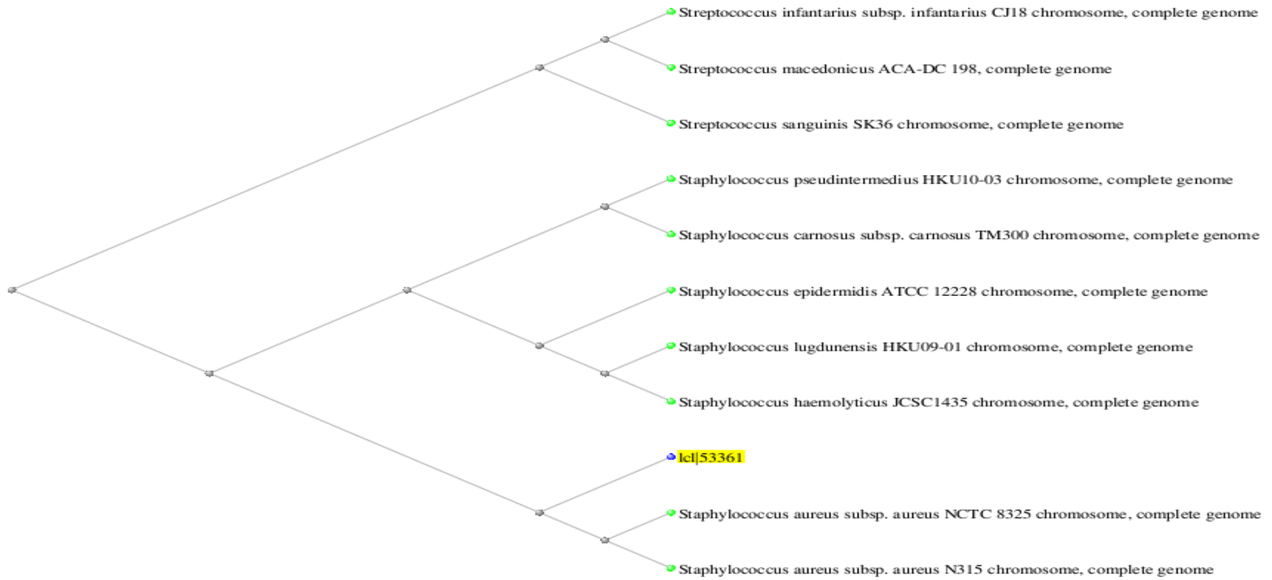

Figure.4 Phylogenetic dendrogram showing taxonomic positions of *Staphylococcus aureus*. type strains based on the 16s rRNA partial sequences

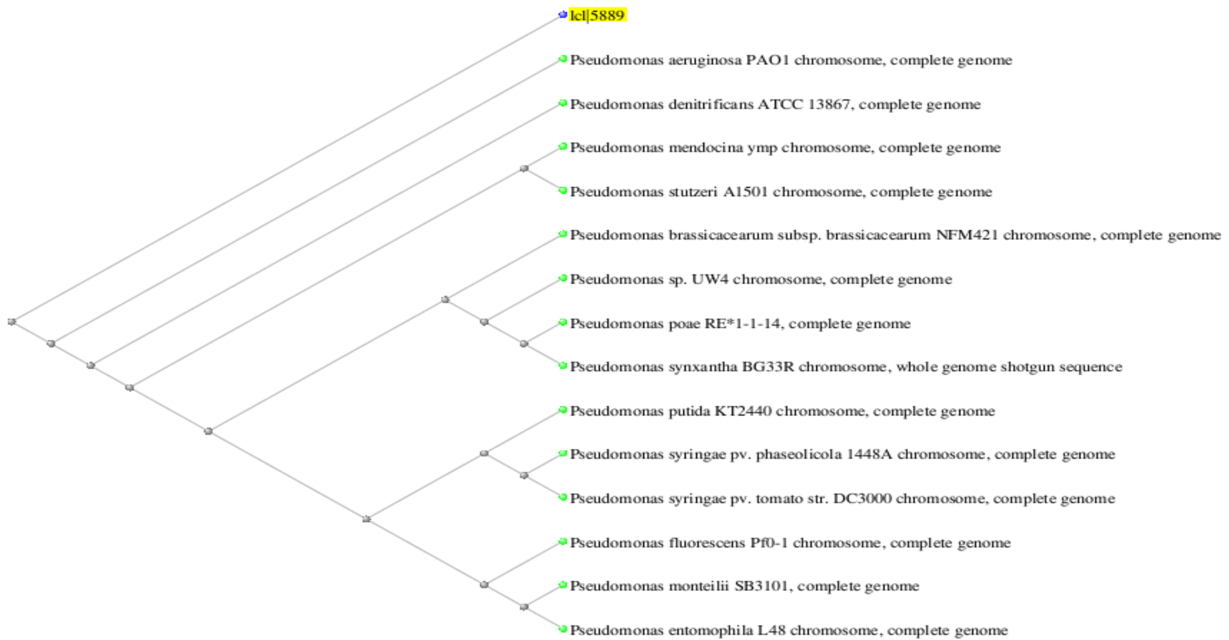

Figure.5 Phylogenetic dendrogram showing taxonomic positions of *Pseudomonas aeruginosa*. type strains based on the 16s rRNA partial sequences

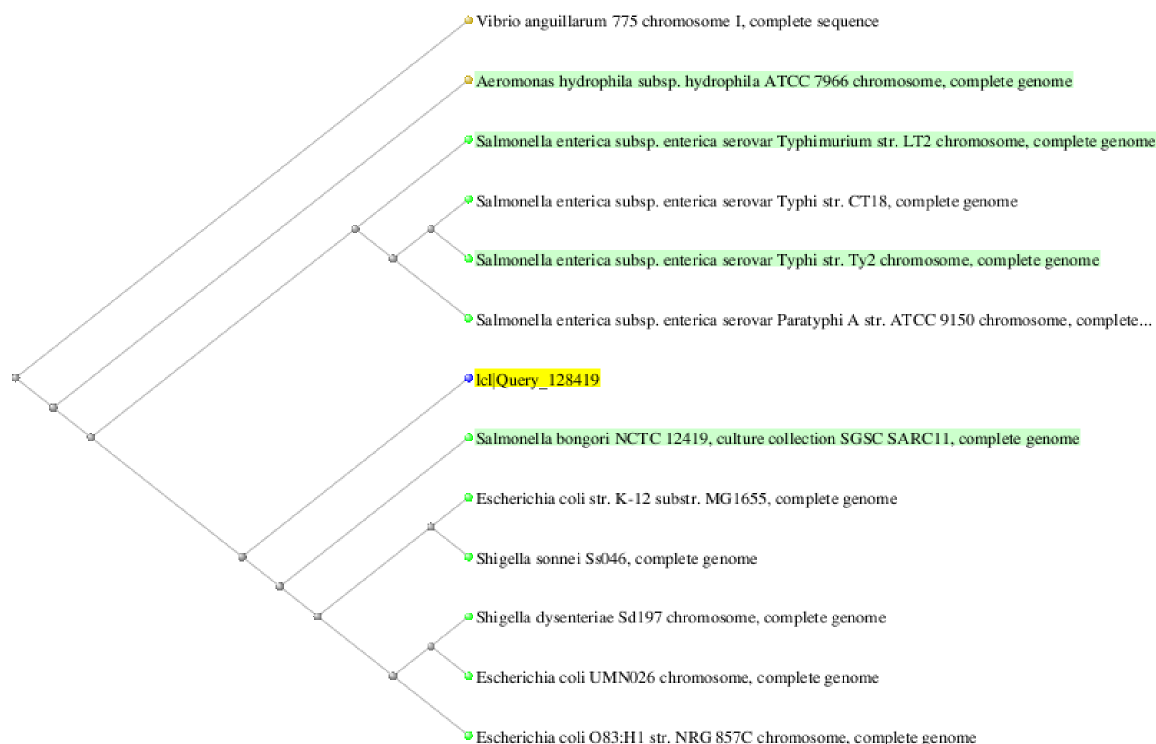

Figure.6 Phylogenetic dendrogram showing taxonomic positions of *Salmonella entricia P.aeruginosa*. type strains based on the 16s rRNA partial sequences

Physiological, biochemical and molecular examination of target organisms

Four to five suspected colonies from each bacterial plate were picked, cultured and then identified by the various biochemical tests.

Biochemical tests were performed to confirm *E. coli and salmonella* using Gram staining, Catalase test, Indole, Methyl red, Voges- Proskauer test, Nitrate reduction, Urease production, Simon citrate agar, and various sugar fermentation tests (Table 6) and Fig(3).

Confirmation of the genus, *Staphylococcus* was done by Gram staining and various biochemical tests including Catalase test, Oxidase test, Indole, Methyl red, Voges-Proskauer test, Nitrate reduction, acid from different sugars, and haemolysis on Sheep Blood Agar (S.B.A.) following the method of Cruickshank (1970), while the species, *S aureus* was confirmed by Coagulase test as described by Monica (1991) (Table 8 and Fig 4)

Bacteriocins or peptide antibiotics produced by *Lactobacilli* are gaining more importance in biopreservation of various packaged and unpackaged foods particularly because of less/no adverse effects to the consumers. Furthermore, the use of mixture of various bacteriocin as a combination for biopreservation could have major role in enhancing food safety. In present study, attempt has been made to identify the significant media components and culture conditions influencing production of bacteriocin. The evaluation is done with the help of Plackett-Burman statistical design.The growth of bacteria and accumulation of cellular metabolites are

strongly influenced by growth environment and medium composition such as carbon sources, nitrogen sources, growth factors, and inorganic salts. Search for the major factors and their optimization for biotechnological processes including multi-variables is difficult. The traditional 'one-factor-at-a-time approach' that was used in medium optimization to obtain high yields of the desired metabolites disregards the complex interactions among various physicochemical parameters (Wang and Liu, 2008).

Determination of suitable nutrient sources

In preliminary step of optimization, nine nitrogen sources, ten carbon sources, temperature, and PH were separately added into the flasks with the effluent (40% v/v) as basal medium. The results obtained in these experiments were shown in Table 9. It is obvious that peptone, yeast extract, glucose, lactose,$MnSO_4$, K2HPO_4, KH_2PO_4 and PH 6.5 and 7 show comparatively greater positive effect on the bacteriocin production. Among the carbon sources, arabinose gave the negative effect. In the presence of peptone or yeast extract as the only nitrogen source, or a combination of peptone and meat extract, bacteriocin was produced. A combination of peptone and yeast extract yielded 2400 AU/OD.

Growth in the presence of meat extract as the only nitrogen source, or a combination of meat extract and yeast extract (1:1), resulted in approximately reduction of bacteriocin production. Concluded from these results, the key nitrogen source needed for optimal bacteriocin production is a combination of yeast extract and peptone. Bacteriocin was produced at 3000 AU/OD in MRS broth at an initial pH of 6.5(Table 10). Approximately reduction in bacteriocin activity (1400 AU/OD) was recorded in the same medium adjusted to an initial pH of 4.5 (Table 9). The end pH of both cultures was 3.8 (Table 10). Concluded from these results, production of bacteriocin was stimulated at an initial medium pH of 6.5.

Supplementation with inorganic salts used for bacteriocin production was also investigated as seen from Table (10), $MnSO_4$, $ZnSO_4$, $FeSO_4$, $CuSO_4$, K_2HPO_4 and KH_2PO_4 significantly stimulated bacteriocin production. It can be interpreted that $MnSO_4$, K_2HPO_4 and KH_2PO_4 are considered as macronutrients in bacterium nutrition whereas iron copper and zinc are viewed as micronutrients.

Plackett-Burman design

To investigate the effects of nutrient supplements on bacteriocin production of *Lactobacillus rhamnosus*, A Plackett-Burman design was carried out when the suitable nutrient supplements had been determined. As can be seen from Table (11) yeast extract, glucose, peptone and PH gave positive effect on bacteriocin production with level confidence range between (95-97 %). In previous research published by various groups (Oslon, 1993; Ogunbanwo *et al.*, 2003), glucose was found to be affecting the bacteriocin production by *Lactobacilli* when used in various concentrations. On the other side, peptone and meat extract both acts as nitrogen source, have various significance or no significance at all for both the isolates. In one study Todorov and Dicks (2009) found that the nitrogen sources like yeast extract, meat extract, peptone and tryptone, when used solely and in various combinations with each other, influence the bacteriocin production by various species of *Lactobacilli*. pH were found to be significant for the production of bacteriocin.

Table.9 Effect of nutrient components on biomass production and bacteriocin activity (AU/ml) by *Lactobacillus rhamnosus*

Treatment	Concentration	Final pH	Growth (OD 600 nm)	Bacteriocin activity AU/ml
Nitrogen sources				
Peptone	20	4.2	3.6	1200
Beef extract	20	4.15	2.65	800
Yeast extract	20	4.1	3.53	1200
Peptone + beef extract	12.5+7.5	4.16	3.85	1800
Peptone+Yeast extract	12.5+7.5	4.02	4.73	2400
Beef extract+yeast extract	12.5+7.5	4.07	4.45	1800
Beef extract+ yeast extract	10+10	4.07	4.81	1800
Urea	2.0	4.3	1.93	600
Amm.sulphate	4.5	4.4	1.51	400
Carbon sources				
Glucose	20	4.02	4.95	6400
Sucrose	20	4.1	4.89	3000
Glactose	20	4.4	4.67	1400
Mannitol	20	4.1	5.49	2400
Lactose	20	4.1	4.26	6400
Starch	20	4.3	2.89	1400
Glycerol	20	4.01	4.66	1800
Maltose	20	4.39	4.56	2400
Arabinose	20	5.5	1.55	ND
Fructose	20	4.19	3.72	2400
Inorganic salts				
$MnSO_4$	free	4.4	3.95	2400
$ZnSO_4$	0.2	4.5	3.56	600
$CuSO_4$	0.2	4.5	3.51	600
$FeSO_4$	0.2	4.47	2.59	600
K_2HPO_4	2	4.11	4.89	3000
KH_2PO_4	2	4.05	4.14	3000
PH				
4.5		3.8	3.42	1400
5.5		3.8	4.56	1800
6.5		3.8	4.81	3000
7		5.02	5.00	3000
8		6.01	2.91	1800

Table.10 Eight-trial Plackett–Burman design matrix for seven variables with coded values along with observed results for screening of significant factors affecting bacteriocin production by *Lactobacillus rhamnous.*

Trial	P (g/l)	Y (g/l)	L (g/l)	G (g/l)	A (g/l)	I (hr)	pH	Bacteriocin production (AU/ml)
T1	+1	+1	+1	-1	+1	-1	-1	6000
T2	-1	+1	+1	+1	-1	+1	-1	8600
T3	-1	-1	+1	+1	+1	-1	+1	9200
T4	+1	-1	-1	+1	+1	+1	-1	6400
T5	-1	+1	-1	-1	+1	+1	+1	4600
T6	+1	-1	+1	-1	-1	+1	+1	8200
T7	+1	+1	-1	+1	-1	-1	+1	10200
T8	-1	-1	-1	-1	-1	-1	-1	1800

Table.11 Statistical analysis of Plackett–Burman design results showing estimated effect

Variables	**Effect**	**Coefficient**	**Standard error**	***t*-value**	***P*-value**	**level Confidence %**
Intercept	-	3.550788	-	-	-	-
Peptone	3.88	1.43833	0.7289	3.81141	0.050	95
Yeast extract	4.88	2.08500	0.8215	-5.81822	0.025	97.5
Glucose	3.92	-1.88167	4792.	- 5.28594	0.034	97
Lactose	1.54	0.77167	0.46916	2.10234	0.248	85.5
Incubation period	2.6	1.41167	0.2519	2.91349	0.059	93
PH	3.54	-1.88167	0.29192	- 4.88594	0.034	97

However, in neither cases both of these were found to be significant. Patil *et al.,* (2011) found that pH and temperature have strong influence on bacteriocin production when compared to incubation time and any alteration in pH and temperature values has significant effect on production of bacteriocin. Oslon, (1993) observed that the dependence of bacteriocin production on pH may be due to the regulation of bacteriocin production genes expression by pH. Thus, variation in the significant media ingredients concentrations or physical culture parameters will have considerable influence on the amount of bacteriocin produced by microorganisms.

Lactobacillus rhamnosus was characterized as bacteriocin production independent producer strain. The use of statistical models to optimize culture medium components and conditions has increased in present-day biotechnology, due to its easy applicability, reliability and validity. In this study, it is evident that various process parameters like yeast extract and culture volume are the most significant factors influencing bacteriocin production. Under the optimized conditions, the maximum bacteriocin obtainedafter 12h of cultivation, when compared to the results obtained in basal production medium was (3000 AU/ml).

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