International Food Research Journal 24(2): 900-903 (April 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Short Communication

Nisin production conditions optimization and its effect on *Bacillus cereus* and *Listeria monocytogenes*

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Article history

Received: 3 October 2015
Received in revised form: 22 March 2016
Accepted: 15 April 2016

Keywords

Lactococcus lactis
Nisin
Listeria monocytogenes
Bacillus cereus

Abstract

Lactococcus lactis isolate B18, has been selected according to its effect on listeria monocytogenes and Bacillus cereus and were respectively (5-4) mm as inhibition diameters by halls method after pH modification, nisin gene has been recognized by PCR technique, best production medium has been selected and was M17 broth besides to 3% lactose and 3% yeast extract, then production conditions have been optimized and were for listeria monocytogenes and *Bacillus cereus* respectively (pH 6, temperature 34°C, Incubation time 54 hrs, and inoculation size 1.5 ml), (pH 5.5, temperature 31°C, Incubation time 54 hrs, and inoculation size 1.5 ml). The inhibition diameter became 12 mm against listeria monocytogenes, the inhibition diameter became 9 mm against *Bacillus cereus*. Over all nisin effect on *listeria monocytogenes* affected by temperature, Incubation time, and inoculation size. and its effect on *Bacillus cereus* affected by pH, temperature, Incubation time, and inoculation size.

Introduction

In recent years health concern consumers, so that they are looking for natural foods without chemical preservatives which will fit in their healthy lifestyle. The increasing consumption of precooked food, temperature abuse, and the import of raw foods from developing countries are among the main causes of this situation. Biopreservation refers to extended shelf life and enhanced safety of foods using microorganisms or their metabolites (Ross *et al*., 2002). Lactic acid bacteria (LAB) are often inhibitory to other microorganisms and this is the basis of their ability to affect the keeping quality and safety of many food products. Factors which contribute inhibition are organic acids hydrogen peroxide, low pH, ethanol, nutrient depletion, and bacteriocin production (Adams and Nicolaides, 1997). In fermented foods, LAB displays numerous antimicrobial activities. This is mainly due to the production of antimicrobial metabolites including organic acids, bacteriocins and antifungal peptides. Bacteriocin generally show their antimicrobial action by interfering with the cell wall or the membrane of target organisms, either by inhibiting cell wall biosynthesis or causing pore formation, resulting in death (Sullivan *et al.*, 2002). Nisin is the only bacteriocin used as a food preservative and is therefore acceptable as a selective agent upon transformation of food-grade plasmids. The nisin immunity gene, nisI, from a nisin-producing Lactococcus lactis strain has been exploited as a selection marker in a food-grade vector (Takala and Saris, 2002). It is a naturally occurring antimicrobial peptide and was discovered in 1928 (Monteville and Chen, 1998), considered a Group A lantibiotic because it has a linear structure rather than a circular structure like Group B lantibiotics. It is comprised of 34 amino acid residues and has a molecular mass of 3510 Daltons. It has 5 internal ring structures (Rings A-E) formed by disulfide bridges that are contributed by lanthionine and B-methyl lanthionine (Klaenhammer 1993). Nisin has been proven to inhibit the growth of *Bacillus, Bifidobacrium, Brochothrix, Clostridium, Corynebacterium, Enterobacter, numerous Lactobacillus, Listeria, Micrococcus, Pediococcus, Staphylococcus*, and some Actinomyceae (Millette *et al*., 2004).

Bacteria related food poisoning is the most common. More than 90 percent of the cases of food poisoning each year are caused by *Staphylococcus aureus, Salmonella, Clostridium perfringens, Campylobacter, Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus,* Entero-pathogenic *Escherichia coli* and *Shigella* spp. These bacteria could found in many raw foods and cause food poisoning. (Jalalpour, 2012). Because the of food poisoning caused by pathogens, such as *Bacillus cereus* and *listeria monocytogenes*, this research aimed to Optimize Nisin production conditions, and

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study its inhibition effect on *Bacillus cereus* and *listeria monocytogenes*.

Materials and Methods

Selecting the best isolate according to its effect on Bacillus cereus *and* listeria monocytogenes

Lactococcus lactis has been isolated by using M17 media (MERCK-Germany), added to it glucose sugar 0.5% (Abts. *et al*., 2011). And after isolating *Lactococcus lactis* from food samples, biochemical assays have been done in former research, best isolate has been selected according to its inhibition effect on *Bacillus cereus* 7464 and *listeria monocytogenes* 11994 from BioBall Company – Australia by using halls method (Tagg. and McGiven, 1971).

Detect nisin gene by polymerase chain reaction technique:

DNA isolation has been done after bacterial cell growth at M17 broth at 37°C, as following: the culture was centrifuged at 7000 g for 10 min and re-suspended the cells in 5 mL of lysis buffer. Then Incubated in a 37°C water bath for 2 hrs, 500 µl of 10% SDS and 100 µl of 25 mg/mL proteinase K was added, then incubated in a 55°C water bath for 2 hrs, 2 mL of 5 M sodium chloride and 6 mL of chloroform–isoamyl alcohol then added, Incubation at room temperature for 30 min. 1 volume of 100% isopropanol was added, and washed with ice-cold 70% ethanol. Air-dry the DNA and re-suspended it in 600 µl of TE buffer (John and Alicia, 2001). For the polymerase chain reaction: 2 µl DNA isolated, 2.5 µl Reaction Buffer 10X, 3 µl $MgCl_2$, 0.5 µl dNTPs, 1 µl DNA polymerase, 1 µl primers, total size has been completed to 25 µl by distilled water. And nisin gene detection primers nucleated sequence.

Nisin F: CGGCTCTGATTAAATTCTGAAG.
Nisin R: GGATTAGCTAGTAGTAACTGTTC.

Thermal program for polymerase chain reaction

Thermal program for polymerase chain reaction started with preheating at 92°C for 10 m for on cycle then, Denaturation phase: 94°C for 54 Sec, Annealing phase: 50°C for 45 Sec, Extension phase: 72°C for 45 Sec, For 30 cycles, and final extension at 72°C for 10m. Electrophoresis has been done at Agarose gel 1.5% within EDTA-Tris (TE) solution, to Separate nucleic acid fragments. Samples are loaded into wells of an agarose gel and subjected to an electric field, 85 volt, 150 amber, then studied at gel documentary.

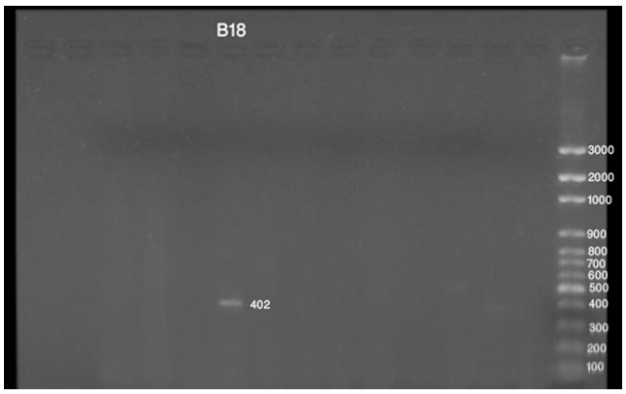


Figure 1. Electrophoresis for B18 isolate DNA with nisin primer

Choosing of production medium

Five media have been used to choose best medium for nisin production, M17 broth, M17 broth besides to 0.5% glucose, M17 broth besides to 3% lactose and 3% yeast extract, MRS, and MRS besides to 1% tween 80. After choosing the best broth for nisin production from *Lactococcus lactis* (M17 broth besides to 3% lactose and 3% yeast extract) production conditions have been optimized.

Optimization of production conditions (pH degree, temperature, incubation time, inoculation size):

Conditions have been selected for production of nisin from Lactococcus lactis according to proper degrees for bacterial growth and nisin production. To reach optimal conditions, four parameters have been selected, in each parameter five items have been studied with equal differences for each parameter, and they were: pH degree (4.5, 5, 5.5, 6, 6.5), temperature measured by siliceous centigrade (25, 28, 31, 34, 37), incubation time measured by hours (12, 24, 48, 72, 96) and inoculation size measured by milliliter (0.5, 1, 1.5, 2, 2.5). Then the mentioned above conditions studied by Statistical program.

Statistical analyses

Statistical program used was mini tab to optimize temperature, pH, incubation time, inoculation size. After choosing the best medium for nisin production.

Results and discussion

Lactococcus lactis isolation and selecting the best isolate according to its effect on *Bacillus cereus* and *listeria monocytogenes*. Best isolate B18 has been selected according to its effect on listeria monocytogenes and *Bacillus cereus* as inhibition diameters by halls method after pH modification. And the inhibition diameters were by average for *listeria monocytogenes* 5 mm and for *Bacillus cereus* 4 mm.

Table 1. Estimated Coefficients Regression for *listeria monocytogenes*

Term	Regression Coefficients	Standard Error of Coefficients	T	P. Value
Constant	10.0167	0.6491	15.431	0.000
pH	0.6746	0.3747	1.800	0.090
Temperature	0.6081	0.3993	1.523	0.146
Time	-0.0599	0.3785	-0.158	0.876
Substrate	0.6613	0.3752	1.762	0.096
pH*pH	-0.4979	0.3092	-1.610	0.126
Temperature*Temperature	-0.8878	0.3074	-2.888	0.010
Time*Time	-0.9565	0.3468	-2.758	0.013
Substrate*Substrate	-0.9568	0.2946	-3.248	0.005
pH*Temperature	1.2526	0.7969	1.572	0.134
pH*Time	-0.7620	0.5843	-1.304	0.210
pH*Substrate	-0.0040	0.4775	-0.008	0.993
Temperature*Time	0.8630	0.7622	1.132	0.273
Temperature*Substrate	-0.1680	0.6281	-0.268	0.792

R-Sq = 66.1%

Response Optimizer plot:

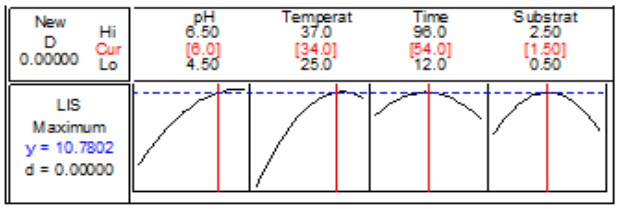
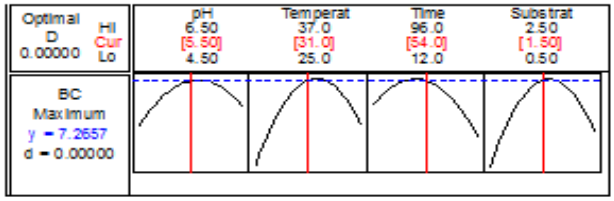


Table 2. Estimated Coefficients Regression for *Bacillus cereus*

Term	Regression Coefficients	Standard Error of Coefficients	T	P. Value
Constant	7.6060	0.5320	14.297	0.000
pH	0.3919	0.3071	1.276	0.219
Temperature	0.4043	0.3272	1.236	0.233
Time	-0.1171	0.3103	-0.377	0.711
Substrate	0.5130	0.3075	1.668	0.114
pH*pH	-0.5402	0.2534	-2.132	0.048
Temperature*Temperature	-0.9468	0.2519	-3.758	0.002
Time*Time	-0.9086	0.2843	-3.196	0.005
Substrate*Substrate	-1.0125	0.2415	-4.193	0.001
pH*Temperature	1.0056	0.6532	1.540	0.142
pH*Time	-0.6077	0.4789	-1.269	0.222
pH*Substrate	0.2154	0.3913	0.550	0.589
Temperature*Time	0.7576	0.6247	1.213	0.242
Temperature*Substrate	-0.1068	0.5148	-0.207	0.838

R-Sq = 71.5%

Response Optimizer plot:



Electrophoresis results for B18 isolate showed that this isolate has the nisin gene, Figure (1). Optimum production medium for nisin production was M17 broth besides 3% lactose and 3% yeast extract,

It has been noticed from table (1) that the production of bacteriocin nisin from B18 isolate and its effect on *listeria monocytogenes* affected by temperature, Incubation time, and inoculation size. And thus the inhibition diameter became 12 mm. The optimum parameters were pH 6, temperature 34°C, Incubation time 54 hrs, and inoculation size 1.5 ml.

It has been noticed from table (2) that the production of bacteriocin nisin from B18 isolate and its effect on *Bacillus cereus* affected by pH, temperature, Incubation time, and inoculation size. And thus the inhibition diameter became 9 mm. The optimum parameters were pH 5.5, temperature 31°C, Incubation time 54 hrs, and inoculation size 1.5 ml. The results disagree with scientist Mall results for the nisin production medium and temperature (Mall *et al.*, 2010). And agree with scientist Cheigh results for the nisin production medium but not for production conditions except for pH of nisin conditions against listeria (Cheigh, 2002).

Conclusions

The main inhibition factor against listeria monocytogenes and *Bacillus cereus* was nisin bacteriocin after pH modification. Optimization results on nisin production and its effect on l*isteria monocytogenes* and *Bacillus cereu*s were pH 5.5-6, temperature 31-34°C, incubation time 54 hrs, inoculation size 1.5ml. Most of the studied parameters affected nisin production and its effect on *listeria monocytogenes* and *Bacillus cereus*.

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