International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print) 2222-5234 (Online)
Vol. 1, No. 3, p. 39-44, 2011
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Antimicrobial interaction of *Lactococcus lactis* subsp. *lactis* against some pathogenic bacteria

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Received: 3 April 2011
Revised: 19 May 2011
Accepted: 21 May 2011

Key words: Antimicrobial compound, *Lactococcus lactis* subsp. *lactis*, bacteria.

Abstract

A research was conducted to determine the interaction of antimicrobial compound produced by Lactococcus lactis subsp. lactis at various parameters against Salmonella and Staphylococcus aureus. The results showed static effect of antimicrobial compound against the bacteria. Salmonella exhibited the highest inhibition zone of 20.00±1.23mm at selected Lactococcus lactis subsp. lactis at initial substrate pH and temperature, but at different time of culturing period. Staphylococcus aureus showed the highest inhibition zone of 20.00±2.13mm at initial substrate pH 7.0, with Lactococcus lactis subsp. lactis incubation temperature of 37°C at 168 hours. From the above findings, the optimum condition of antimicrobial compound production was 37°C of incubation temperature with pH 7 of initial substrate.

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Introduction

Lactococcus lactis subsp. *lactis* is a spherical-shaped, Gram-positive bacterium used extensively for industrial production of fermented milk, cheese, and yogurt. These are very important food supplies for many people. *Lactococcus lactis* subsp. *lactis* can be found in plants and within the digestive tract of cows. It is believed that in nature, *Lactococcus lactis* subsp. *lactis* stays dormant on plant surfaces ahead to be ingested along with the plant into animal gastrointestinal tract, where it becomes active and multiplies intensively (Alexander *et. al.*, 2001). In industrial sector, *Lactococcus lactis* subsp. *lactis* is preferred for making soft cheese. *Lactococcus lactis* subsp. *lactis* is recognized as the species with clinical significance for human and veterinary medicine (Facklam *et al.*, 1995). In humans, *Lactococcus lactis* subsp. *lactis* has been associated with endocarditis (Fefer *et. al.*, 1998) and has also been isolated from clinical samples of blood, skin lesions, and urine (Elliot *et al.*, 1991). Known as preferred probiotic, as well as a mixture of commercialized yogurt culture, *Lactococcus lactis* subsp. *lactis* is traditionally known probiotic with high potential of healing acute diarrhea, improving immune function and preventing infections. Infections such as stomach ache and related disease are generally associated with pathogenic bacteria such as *Salmonella* and *Staphylococcus aureus*. *Salmonella* is a genus of rod-shaped, Gram-negative, non-spore forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 μm, lengths from 2 to 5 μm, and flagella which flow in all directions (Yasin *et al.*, 1996). *Salmonella* infections are zoonotic (Kim *et al.*, 2008) and can be transferred between humans and animals. In most cases, infections are due to ingestion of contaminated food (John, 2002). Salmonellosis (gastroenteritis characterized by nausea, vomiting, and diarrhea) is the most common disease caused by the organisms. Abdominal cramping also may occur. Salmonellosis thus produces the symptoms that are commonly referred to as food poisoning (Michael et. al., 1988). *Staphylococcus aureus,* in most cases, shows different symptom of infections compared to *Salmonella*.

Staphylococcus aureus (literally known as "golden cluster seed" or “golden staph”) is a facultatively anaerobic and Gram-positive coccus (Franklin, 1998). The pathogen can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, and abscesses, to life-threatening diseases such as pneumonia, meningitis, chest pain, bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections.

The probiotic also known for its potential to inhibit growth of spoilage fungi in food and feed products. It can produce a number of antimicrobial substances. Some of them (e.g. lactic acid and reuterin), inhibit the filamentous fungi such as toxin-producing *Aspergillus* (Onilude, 2005) and it was found that the produced antifungal metabolites were pH-dependent (Cassandra *et al.*, 2004).

Due to the important of *Lactococcus lactis* subsp. *lactis* towards the development of human health, this research is dedicated to determine the interaction of antimicrobial compound produced by *Lactococcus lactis* subsp. *lactis* at various parameters against *Salmonella* and *Staphylococcus aureus*.

Materials and methods

Materials

The slant of *Lactococcus lactis* subsp. *lactis* was obtained from the Department of Microbiology, Faculty of Applied Sciences, UiTM, Shah Alam. The fermentation media employed in this study was GYP (Glucose-Yeast-Peptone) media respectively (Fujitoshi et. al., 2005) and compositions of both

media are shown in Table 1. The antimicrobial activities of the extracts were tested against *Salmonella* and *Staphylococcus aureus*. Nutrients Agar (NA) which was purchased from MERCK (Germany) was employed as microbiological media in the bioassay activity and all chemicals used in this study were of ANALAR grade.

Table 1. Composition of GYP medium.

Ingredients	g/L
Glucose	10.0
Yeast extract	10.0
Peptone	10.0
Sodium acetate	10.0
Salt solution	5.0

Methods

All media and glassware were sterilized by autoclavation at 121°C for 15 minutes in a floor standing vertical autoclave (TOMMY, U.S.A) and wet sterilized glassware was dried overnight in a cabinet dryer at 70°C. The GYP fermentation medium was prepared according to the composition stated in Table 1 and the initial substrate pH values of 5.5 and 7.0 were adjusted for the media using NaOH or HCl. Hence, a total of 4 fermentation media was prepared for the fermentation experiment (please refer to Table 2). A loop of *Lactococcus lactis* subsp. *lactis* was cultured anaerobically in 20ml GYP broths for four days until good growth was obtained. The fermentation experiments were performed according to the Table 2. All fermentation media were contained in 250ml Schott bottles and were incubated under anaerobic condition. All bottles were placed inside an incubator (Incucell MMM incubator) at 35°C and 37°C for 1-4 days respectively. 5ml samples were aseptically taken at 2 day intervals and commenced from day 5, day 7, day 9, day 11, day 13 and day 15 for sampling. The Ph changes of samples were determined using a Ph meter (Mettler Toledo) equipped with a combined Ph electrode. 0.5ml of samples (previously serial diluted) were poured onto the agar plates and lawned using sterile hockey stick. The samples were incubated anaerobically for 5days. The formation of bacteria colonies on the surface of the agar plate was counted and noted. All samples were clarified by centrifugation at 5000rpm for 5 minutes using an Eppendorf centrifuge (Heraeus, Germany). The method was followed by the preparation of Nutrient Agar at which 20g of nutrient agar powder (MERCK, Germany) was suspended in 1L of deionized water and mixed thoroughly. The solution was autoclaved at 121°C for 15 minutes. The sterilized molten agar suspensions were poured into pre sterilized plastic petri dishes and allowed to solidify inside the laminar flow in the presence of UV radiation for 30 minutes. The agar plates were incubated at room temperature. The bacterial test organisms were prepared by inoculating into Nutrient broth (NB). The inoculated broths were incubated aerobically using an orbital shaker (INNOVA 400) for 24hours at 150rpm and 30°C. After incubation, 0.5 ml of the stock culture of test organism was lawned to the respective surfaces. 4 wells were drilled into an agar by using sterilized cork barrel. In this method, 60µl of clarified samples were pipetted into the remaining agars well. All the bioassay plates were incubated at room temperature. After 3 days of incubation periods, all inhibition zones were measured in millimetre (mm). For determination of static/ cidal effects, a loop of each test organism which exhibited the biggest inhibition zone was taken from the clear zone and streaked onto new agar plates. The plates were incubated at room temperature to determine its static (inhibitory) or cidal (lethal) effects of the clarified antimicrobial compound in the fermentation samples.

Table 2. Types of fermentation media and temperature conditions for fermentation experiments.

	pH 5.5		pH 7	
	35°C	37°C	35°C	37°C
1	bottle	1 bottle	1 bottle	1 bottle

Results and discussion

Inhibition zones were successfully produced by *Lactococcus lactis* subsp. *lactis* cultured in GYP medium against *Salmonella* and *Staphylococcus aureus* (incubated at room temperature) respectively. The zones generated by the effect of antimicrobial compound on GYP medium against *Salmonella* at 35°C with initial substrate pH 5.5 and pH 7.0 were elaborated in the graph shown in Fig. 1.

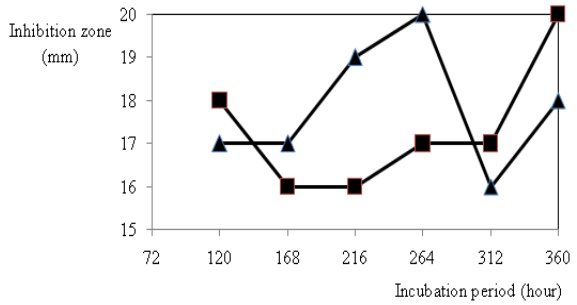

Fig. 1. Inhibition zones produced by *Lactococcus lactis* subsp. *lactis* against *Salmonella* with initial substrate pH 5.5 (▲) and pH 7.0 (■) incubated at 35°C.

Depicted from Figure 1, the highest inhibition zone generated was 20mm for respective pH 5.5 and 7.0 at different incubation period. The antimicrobial effect from *Lactococcus lactis* subsp. *lactis* with initial substrate pH 5.5 produced the highest inhibition zone at 264 hours of incubation period in comparison with 360 hours of incubation period for initial substrate pH of 7.0. It can be suggested, the optimal pH for producing antimicrobial compound from *Lactococcus lactis* subsp. *lactis* at 35°C was pH 5.5 because shorter incubation period is needed to produce antimicrobial compound with the ability to inhibit the growth of *Salmonella*.

Fig. 2 shows the inhibition zone produced by the probiotic against *Salmonella* with initial substrate pH 5.5 and 7.0 at 37°C. The highest inhibition zone generated was 20mm for respective pH 5.5 and 7.0 at the same time of incubation period. At 360 hours, *Lactococcus lactis* subsp. *lactis* produced the antimicrobial compound which has the high affinity to inhibit the growth of *Salmonella*. The antimicrobial effect from *Lactococcus lactis* subsp. *lactis* with initial substrate pH 5.5 and 7.0 show the overlapping at 3 point of incubation period, namely 168, 264 and 360 hours.

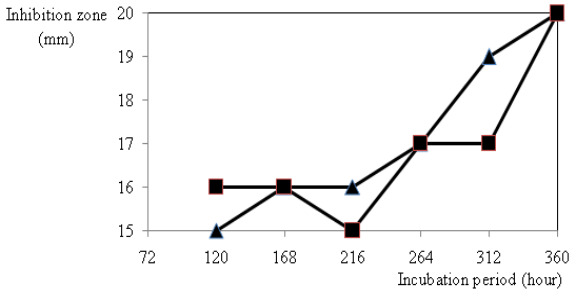

Fig. 2. Inhibition zones produced by *Lactococcus lactis* subsp. *lactis* against *Salmonella* with initial substrate pH 5.5 (▲) and pH 7.0 (■) incubated at 37°C.

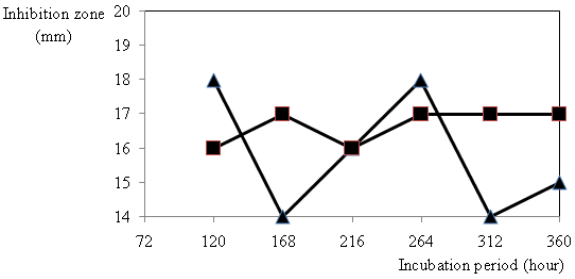

Fig. 3. Inhibition zones produced by *Lactococcus lactis* subsp. *lactis* against *Staphylococcus aureus* with initial substrate pH 5.5 (▲) and pH 7.0 (■) incubated at 35°C.

Fig. 3 illustrated inhibition zones produced by *Lactococcus lactis* subsp. *lactis* cultured in GYP medium against *Staphylococcus aureus* (incubated at room temperature). The effect of antimicrobial compound on GYP medium at 35°C with initial substrate pH 5.5 and pH 7.0 was elaborated in the graph. The highest inhibition zone for initial substrate pH 5.5 was 18mm for respective 120 and 264 hours. However, it was found that 17mm of inhibition zones were scored for respective times of incubation period

namely 168, 264, 312 and 360 hours for initial substrate pH 7.0.

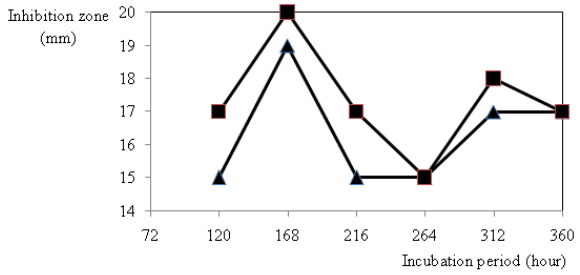

Fig. 4. Inhibition zones produced by *Lactococcus lactis* subsp. *lactis* against *Staphylococcus aureus* with initial substrate pH 5.5 (▲) and pH 7.0 (■) incubated at 37°C.

Shown in Figure 4 is the inhibition zones produced by *Lactococcus lactis* subsp. *lactis* against *Staphylococcus aureus* with initial substrate pH 5.5 and pH 7.0 incubated at 37°C. The overlapping occurred at 264 and 360 hours of incubation period and the trend of the inhibition zones for the incubation period is more less about the same. The highest inhibition zones for both initial substrate pH were 20 and 19 at 168hours of incubation period.

From the data shown above, the maximum incubation period performed was 360 hours. After 360 hours, it was found no inhibition zones were produced and no *Lactococcus lactis* subsp. *lactis* cultures were existed in the medium after re-streaked on the agar plates. The highest inhibition zones produced were subjective to the incubation period matters. The highest incubation zones were not necessarily produced at the longest incubation period. The suggestion of the phenomenon was supported by Aly et. al., (2004) which found the antimicrobial compounds was consumed by the probiotic as an energy sources.

The effect of antimicrobial compound on test organisms was only bacterial static and not bacterial cidal. After the one day of incubation, the agar which was streaked with a loop of sample taken from the clear zone showed the growth of the test organisms.

Conclusion

The optimum condition for maximum inhibition zone production for antimicrobial activity in which produced the largest inhibition zone was 312 hours (13 days) of anaerobic incubation, temperature at 37°C, pH 5.5 and GYP as fermentation medium. This study has shown the antimicrobial compound produced by *Lactococcus lactis* subsp. *lactis* was bacterial static and the maximum antimicrobial compound production was at 312 hour of incubation. GYP medium at an initial substrate pH of 5.5 and a temperature of 37°C ascertain to be the best conditions for maximum antimicrobial compound production for *Lactococcus lactis* subsp. *lactis*.

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