Effect of Varied Culture Conditions on Bacteriocin Production of Four Lactobacillus Species Isolated From Locally Fermented Maize (Ogi)

Onwuakor[1], Onwuakor Chijioke Ethel[2] and Onwuakor Chijioke Ethel[3]

[1] Michael Okpara University of Agriculture Umudike

Received: 6 December 2013 Accepted: 5 January 2014 Published: 15 January 2014

Abstract

Background: Lactic acid bacteria (LAB) predominates the micro flora of fermented products. They produce metabolites that inhibit the growth of food borne pathogens and spoilage microorganisms.Materials and methods: Four (4) isolates of bacteriocin producing lactobacillus species (L. lactis, L. fermentum, L. casei and L. plantarum) with antibacterial activity against Salmonella typhimurium (ATCC 14028) and Shigella dysenteriae (ATCC 23351) were subjected to varied growth medium conditions. Bacteriocin production was tested at different physical and cultural conditions such as temperature (25, 30, 35 and 400C), pH (5, 6, 7 and 8), sodium chloride (NaCl) concentration (2, 4, 6 and 8

Index terms— bacteriocin, optimum, varied, culture, condition, fermented, maize, ogi.

Effect of Varied Culture Conditions on Bacteriocin Production of Four Lactobacillus Species Isolated From Locally Fermented Maize (Ogi)

Onwuakor, C.E ? , Nwaugo, V.O ? , Nnadi, C.J ? & Emetole, J.M. ? Abstract-Background: Lactic acid bacteria (LAB) predominates the micro flora of fermented products. They produce metabolites that inhibit the growth of food borne pathogens and spoilage microorganisms.

Aim:

To isolate and identify LAB species from fermented maize (Ogi) and to determine the effect of varied culture conditions on bacteriocin production and antibacterial activity against indicator organisms.

1 Materials and methods:

Four (4) isolates of bacteriocin producing lactobacillus species (L. lactis, L. fermentum, L. casei and L. plantarum) with antibacterial activity against Salmonella typhimurium (ATCC 14028) and Shigella dysenteriae (ATCC 23351) were subjected to varied growth medium conditions. Bacteriocin production was tested at different physical and cultural conditions such as temperature (25,30, **??**5 and 40 0 C), pH **??**5, 6, 7 and 8), sodium chloride (NaCl) concentration (2, 4, 6 and 8%) and incubation duration (12,24, **??**8 and 72 hours).

Results: The optimum bacteriocin production judged by their different zones of inhibition was recorded at temperature, 30 0 C and then 35 0 C. There were significant differences between all the incubation temperatures at $P<0.05$. Duration of incubation showed highest bacteriocin activity after 72 hours. Furthermore, optimal conditions for bacteriocin production were observed to be highest at pH 6.0 followed by 5.0 and then in 2% NaCl concentration. There were significant differences between the zones of inhibition of bacteriocins produced against the indicator organisms at various media pH and salt concentrations at $P<0.05$.

2 Conclusion:

These bacteriocins may have a potential use as food preservative and may help in improving the gastrointestinal tract by fighting off pathogenic bacteria.

3 Introduction

ood is any substance or mixture of substances both solid and liquid, which are intended for human consumption or ingestion for their nutritional support for the body or pleasurable benefits. It usually consists of plant or animal origin, which contains essential nutrients such as carbohydrates, fats, proteins, vitamins or minerals and is ingested and assimilated by an organism to produce energy, stimulate growth and maintain life [1] [2].

The lactic acid bacteria (LAB) are a group of Gram positive bacteria, non-respiring, non-spore forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates. They are the most important bacteria in desirable food fermentations, being responsible for the fermentation of sour dough bread, sorghum beer, all fermented milk, cassava (to produce garri and fufu) and most "pickled" (fermented) vegetables **??**3] [4]. Lactic acid bacteria occur naturally in several raw materials like milk, meat and flour used to produce foods. LAB is used as natural or selected starter cultures in food fermentations in which they perform acidification due to production of lactic acids. Protection of food from spoilage and pathogenic microorganisms by LAB is through producing organic acids [5]. The LAB produces an array of antimicrobial substances (such as organic acids, diacetyl, acetone, hydrogen peroxide, reuterin, antifungal peptides and bacteriocins [6] [7] [8]. Bacteriocins are ribosomally synthesized antimicrobial peptides or proteins [9].

Ogi (Akamu) is a product of fermented maize (Zea mays) widely eaten in Africa [10] [11]. Similar maize preparations in Ghana are referred to as "Akana" or "Kenkey". Ogi is often marketed as a wet cake formerly wrapped in leaves but presently in transparent polythene bags. Gelatinized Ogi (a porridge) called "pap" is mainly used as a breakfast meal for adults and weaning food by low income earners who cannot afford the more expensive imported weaning foods [12]. In most parts of Africa especially in Nigeria, children are fed with mashed adult foods. These foods are bulky and(D D D D) Year 2014 C

this therefore reduces food intake by a child, often resulting in malnutrition. The development of nutritionally balanced calorie less dense, low bulk and easily digestible weaning food becomes necessary. This involves the use of simple but time consuming traditional technology called fermentation [13]. The traditional fermentation method employed in Ogi production is a wild process and microorganisms are not controlled [14]. Microbiological analyses have shown the presence of several genera of bacteria, moulds and yeasts in the fermented maize product-Ogi [15] [16].

In the present study, different culture conditions were adjusted for bacteriocin production using Lactobacillus isolates from locally fermented maize (Ogi) to determine optimal fermentation conditions for bacteriocin production.

4 II.

5 Material and Methods

6 c) Isolation of lactic acid bacteria from Ogi

One (1) gram of a 72hrs fermented Ogi was transferred into 5ml peptone water (Merck, Germany) and serially diluted (10 fold dilutions). Then 1ml of each of the dilution was aseptically transferred into sterile Petri dishes (Pyrex and Anumbra) and 15ml of de Man Rogosa Sharpe (MRS) medium (Merck, Germany) was added using pour plate technique then incubated at 370C for 48hrs in an anaerobic flask (Oxoid). After incubation, colonies with different morphologies were randomly selected using a flamed platinum wire loop, streak plated and sub -cultured on MRS agar plates to obtain pure colonies. All isolates were examined for Gram reaction, production of catalase and oxidase activity.

7 d) Identification of LAB Isolates

Isolates were identified using the following tests: ammonia production from arginine, CO2 production from glucose and growth at different pH values, growth at different NaCl concentrations and carbohydrate fermentation. LAB isolates were tested for characteristics of Gram staining, cell morphology, colony morphology, motility, carbon dioxide production from glucose, growth at 100C and 450C, growth at pH of 4.4 and 9.6, growth in 6.5% and 18% NaCl, catalase reaction by 3% hydrogen peroxide and carbohydrate fermentation [17]. e) Detection of inhibitory activity of crude bacteriocin from selected isolates Selected LAB isolates were grown in MRS broth at 37°C for 24 hrs. Cell free supernatant of each isolate was obtained by centrifugation at 3,000xg at 4°C for 20 min. The supernatant was adjusted to pH 6.5 with 1M NaOH and subsequently filter sterilized through a 0.2µm membrane filter (Whatman, Germany). Inhibitory activity was determined using agar well diffusion assay [18]. Inhibitory effect of the hydrogen peroxide in the supernatant was eliminated by reacting with 5mg/ml catalase added. Suitable agar medium containing 1% agar (45°C) was inoculated with each of the two indicator strains. Agar wells of 5 mm diameter were cut and the filter-sterilized supernatant (20µl) was added into each well. The plates were incubated at 37°C for 24hrs. The inhibition zones around the wells were measured.

8 f) Optimization of Culture Conditions

The selected lactic acid strains were subjected to different culture conditions to derive optimum conditions for bacteriocin production g) Effect of varying culture conditions on bacteriocin activity

To study the effect of varying culture conditions, growth and bacteriocin production was estimated at varied temperatures (25,30, **??**5, and 400C) pH (5.0, 6.0, 7.0 and 8.0), sodium chloride (NaCl) concentrations (2.0, 4.0, 6.0 and 8.0% w/v) and duration of incubation (12, 24, 48, and 72hrs) in MRS broth. All samples were collected after 48hrs, except for those measuring incubation time effects before inhibitory activity was determined by agar well diffusion assay as described above.

h) Data presentation and statistical analysis Data were represented as means ± standard error of mean as well as bar charts. Two -way analysis of variance and Bonferroni's multiple comparison tests using GraphPad Prism (version 6.0) software were used to analyze data. Values were considered significant when $P<0.05$.

9 III.

10 Results

11 a) Isolation of lactic acid bacteria

In this study, Lactobacillus strains producing antimicrobial compounds were isolated from fermented maize (Ogi). Out of a total of seven (7) isolates that met basic characteristics of Lactobacilli, only four (4) showed antibacterial activity against both indicator organisms. They include L. lactis, L. fermentum, L. casei and L. plantarum. The effect of varied incubating duration (Hrs) on bacteriocin production was noted as different zones of inhibition were observed among the various isolates against the indicator organisms (Figures **??** and 4).

12 Volume XIV Issue IV Version I

The effect of varied medium pH and Sodium chloride concentration on bacteriocin production was affected as different zones of inhibition were observed among the various isolates against the indicator organisms (Figures **??** and 6; Figures 7 and 8) respectively

13 Discussion

These identified Lactobacillus species were in agreement with those earlier identified from similar fermented food products by [19]. The isolates were then tested for antibacterial activity against the indicator organisms (Salmonella typhimurium and Shigella dysenteriae). The bacteriocin activity of the isolates that showed antibacterial activities were further tested after the culture conditions were varied to determine the effect of different cultural conditions on the antibacterial action of bacteriocins produced. Parameters such as incubation duration and temperature, media pH and salt concentrations were varied.

The effect of varied incubating temperatures on bacteriocin production by the isolates in de Man Rogosa Sharpe broth was determined by its antibacterial activity against the indicator strains. The result showed that antibacterial activity was highest at 30.0 0 C followed by 35.0 0 C as seen in figures 1 and 2. There were significant differences between the different zones of inhibition produced for all the incubating temperatures (at $P<0.05$). This was in agreement with the work of [20] and [21], which showed that bacteriocin production was affected by different incubating temperatures. The maximum bacteriocin activity recorded at 30 0 C suggests that ambient growth temperature is most ideal for bacteriocin production by Lactobacillus species.

The effect of varied incubating duration (Hrs) on bacteriocin production was affected as different zones of inhibition were observed among the various isolates against the indicator organisms (Tables **??** and 4). Optimum bacteriocin production was observed after 72 hours judged by the zones of inhibition against the indicators. There were observable reduction bacteriocin activities as incubation time dropped. There were significant differences between the various incubation times (at $P<0.05$). This result was in complete agreement with [22], which showed that incubation time affects bacteriocin production.

The results obtained in this study regarding bacteriocin activity from media incubated at varied pH values (Figures **??** and 6) showed optimum activity at pH 6 followed closely by pH 5 for both indicator organisms. There were clear significant differences between pH 7 and 8 but not in pH 5 and 6 as both showed similar zones of inhibition for all the isolates against indicator organisms. This result was consistent with the reports of [23], which showed influence of pH on growth of vaginal Lactobacilli. This pH tolerance is an extremely important feature since the isolates have the ability to survive, grow and produce bacteriocins under acidic and alkaline conditions.

Every microorganism has a minimal, a maximal and an optimal pH for growth and metabolism. Microbial Furthermore, the effect of varied medium percentage sodium chloride (NaCl) concentration on bacteriocin production and activity was also evaluated. Highest zones of inhibition and consequent optimum bacteriocin production was observed at NaCl concentration of 4%, but started reducing as salt concentration increased further (Figures 7 and 8). There was significant difference between zones of inhibition obtained at all the salt concentrations. This was in agreement with [22] who studied cultural parameter for bacteriocin production.

Besides the strong acid medium in the stomach, the probiotic microorganisms taken orally have to defend against the bile salt in the gastrointestinal tract [24]. Hence, bile tolerance is considered to be one of the important properties required for high survival and as a consequence of probiotic activity. The decrease in bacteriocin production as salt concentration increased could be attributed to stress on the isolates.

Microbial food safety is an increasing public health concern worldwide [25] [26] and many gram negative bacteria like Escherichia coli, Salmonella serovars, Campylobacter species, Shigella species etc, have been implicated in food borne diseases [19]. Alternate methods for controlling pathogenic bacteria by the production of antimicrobial peptides called bacteriocins are now highly considered. Bacteriocins from lactic acid bacteria have attracted much attention and have been the subject of intensive investigation due to their ability to act as a bio-preservative agent, which led to their incorporation into foods, particularly in the dairy foods and also in human therapeutics [27] [28] [29] [30].

V.

14 Conclusion

This study thus suggests the selection of bacteriocin -producing Lactobacillus strains as starter cultures for controlled fermentations at optimum cultural conditions. Bacteriocins from lactobacillus species harnessed under different culture conditions have shown different antimicrobial potencies. These findings could be applied in the food and pharmaceutical industries to further enhance maximum bacteriocin production at optimal levels to replace conventional antibiotics in combating pathogens that are vastly acquiring antimicrobial resistance.

Figure 1:

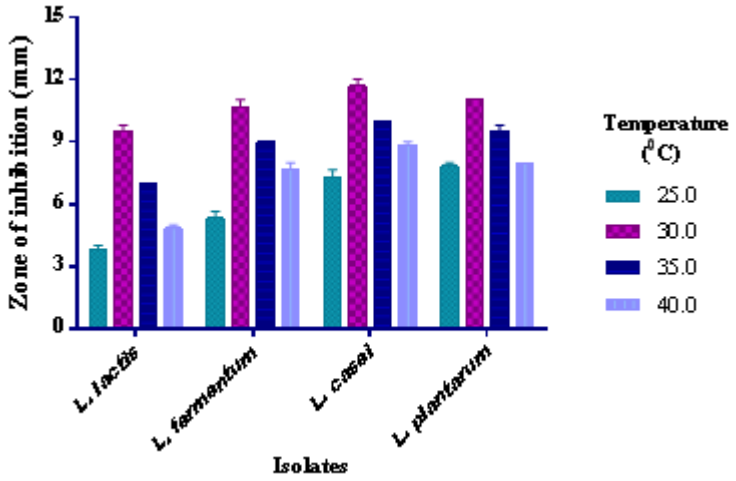
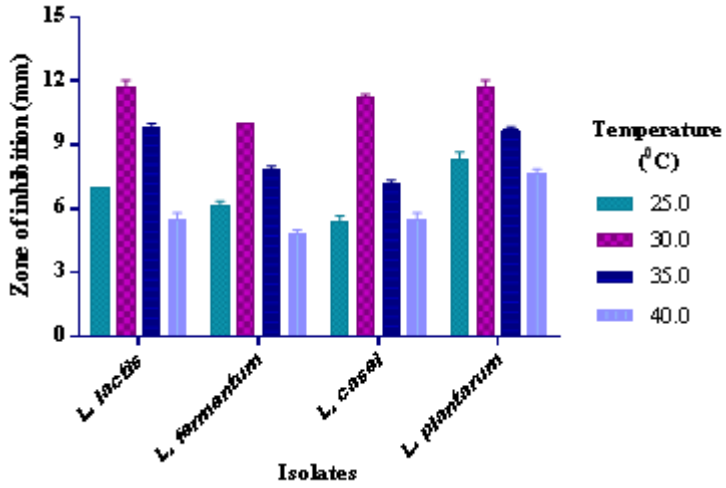
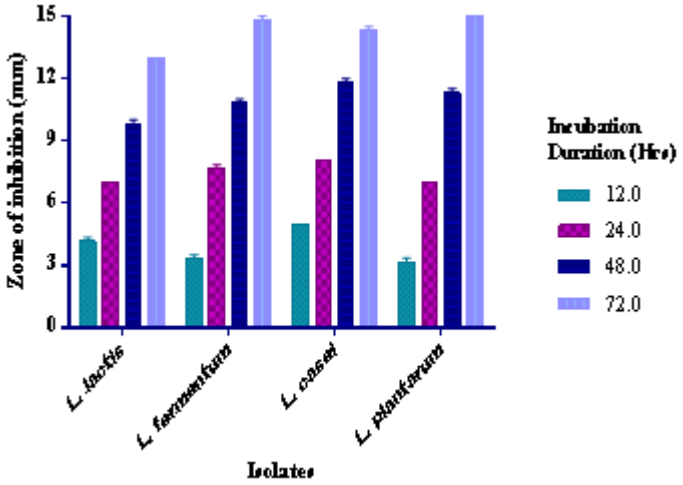


Figure 2:



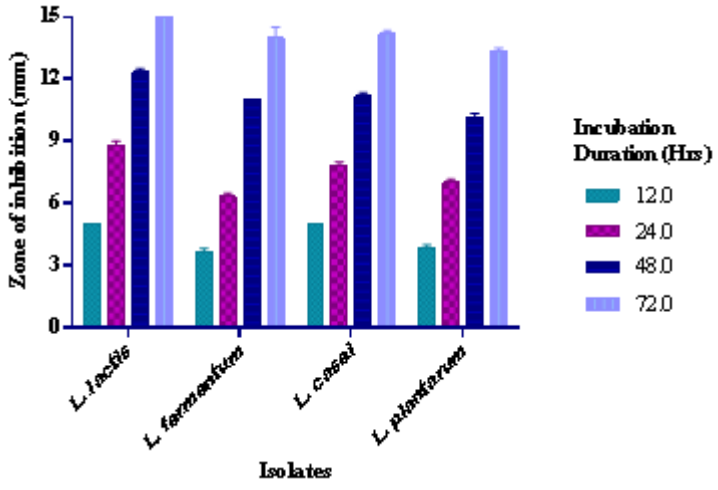
12

Figure 3: Fig. 1 :Fig. 2 :



346

Figure 4: Fig. 3 :Fig. 4 :Fig. 6 :



7

Figure 5: Fig. 7 :

[Turkish Journal of Medical Sciences ()] , *Turkish Journal of Medical Sciences* 2003. 33 p. .

[Pakistan Journal of Nutrition ()] , *Pakistan Journal of Nutrition* 2005. 5 (1) p. .

[African Journal of Biotechnology ()] , *African Journal of Biotechnology* 2006. 5 (9) p. .

[International Journal of Chemical and Biological Sciences ()] , *International Journal of Chemical and Biological Sciences* 2012. 1 p. .

[Odunfa ()] 'African fermented foods'. S A Odunfa . *Microbiology of Fermented Foods*, B J Wood (ed.) (New York) 1985. Elsevier Applied Science Publishers. p. .

[Schillinger and Lucke ()] 'Antibacterial activity of Lactobacillus sakei isolated from meat'. U Schillinger , F K Lucke . *Applied and Environmental Microbiology* 1989. 55 p. .

[Schnurer and Magnusson ()] *Antifungal lactic acid bacteria as bio-preservatives. Trends in Food Science and Technology*, J Schnurer , J Magnusson . 2005. 16 p. .

[Aly et al. ()] 'Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk'. S Aly , A T Cheik , I Ouattara , H N Bassole , S T Alfred . *Pakistan Journal of Nutrition* 2004. 3 (3) p. .

[Karaoglu et al.] *Antimicrobial activity and characteristics of bacteriocins produced by vaginal lactobacilli*, A S Karaoglu , A Faruk , S S Kilic , A O Kilic .

[Okereke et al. ()] 'Antimicrobial properties of probiotic bacteria from various sources'. H C Okereke , O K Achi , U N Ekwenye , F A Orji . *African Journal of Biotechnology* 2012. 11 (39) p. .

[Settanni and Corsetti ()] 'Application of bacteriocins in vegetable food bio-preservation'. L Settanni , A Corsetti . *International Journal of Food Microbiology* 2008. 121 p. .

[Galvez et al. ()] 'Bacteriocin -based strategies for biopreservation'. A Galvez , H Abriouel , L Lopez , N B Omar . *International Journal of Food Microbiology* 2007. 120 p. .

[Savadogo et al.] *Bacteriocin and lactic acid bacteria, a mini review*, A Savadogo , Cat Quattara , S A Bassole Ihn Traore .

[Sarika et al. ()] 'Bacteriocin production by new isolate of Lactobacillus rhamnosus GP1 under different culture conditions'. A R Sarika , A P Lipton , M S Aishwarya . *Advance Journal of food Science and Technology* 2010. 25 (5) p. .

[Vuyst ()] 'Bacteriocins from lactic acid bacteria: production, Purification and food Applications'. De Vuyst , LeroyF . *Journal of Molecular Microbiology and Biotechnology* 2007. 13 p. .

[El-Ziney et al. (ed.) ()] M G El-Ziney , J Debevere , M Jakobsen , Reuterin . *Natural Food Antimicrobial Systems*, A Naidu (ed.) (London.) 2000. CRC Press. p. .

[Akinrele ()] 'Fermentation studies on maize during the preparation of a traditional African Starch-cake food'. I A Akinrele . *J. Sci. Food Agric* 1970. 21 p. .

[Mr and Mo ()] *Food Microbiology*, Adams Mr , Moss Mo . 1995. Tyne and Wear, London. p. . (3rd ed. Athenaeum Gateshead)

[Abdulmumeen et al.] *Food: Its preservatives, additives and applications*, H A Abdulmumeen , A N Risikat , A R Sururah .

[Ogunshe et al. ()] 'In vitro antimicrobial characteristics of bacteriocinproducing Lactobacillus strains from Nigerian indigenous fermented foods'. Aao Ogunshe , M A Omotoso , A Adeyeye . *African Journal of Biotechnology* 2007. 2 (8) p. .

[Amakoromo ()] *Indigenous Fermented Foods of Nigeria: Processing, composition and Improvement*, E R Amakoromo . 2011. PH, Nigeria: University of Port Harcourt Press. p. .

[Divakara and Manjunatha ()] 'Lactic acid bacteria as probiotics: Role in human health'. R Divakara , B K Manjunatha . *Research Reviews in Biomedicine and Biotechnology* 2010. 1 (1) p. .

[Axelsson ()] 'Lactic acid bacteria: classification and physiology'. L Axelsson . *Advance in Lactic Acid Bacteria: Microbiological and Functional Aspects*, S Salminen, A, Von Wright, Ouweland A Editors. Marcel Dekker (ed.) (New York, U.S.A.) 2004. p. .

[Magnusson and Schnurer ()] 'Lentibiotics: Structure, biosynthesis and mode of action'. J Magnusson , J Schnurer . *FEMS Microbiology Reviews* 2001. 25 p. .

[Pte and Kuyanbana ()] 'Microbial quality of pap prepared from cereals sold in Bauchi markets'. Ozoh Pte , Z U Kuyanbana . *Nigeria. Int. J. Environ. Health, Res* 1995. 5 p. .

[Ogunshe et al.] *Microbial studies on Aisa: A potential indigenous fermented food condiment from Albiziz saman*, Aao Ogunshe , A E Ayodele , O Okonko .

[Mbakwem-Aniebo and Udemgba ()] 'Microbiological quality of untreated and salt-treated Ogi (Akamu) kept at room temperature'. C Mbakwem-Aniebo , G Udemgba . *Nature and Science* 2012. 10 (8) p. .

[Marero et al. ()] 'Nutritional characteristics of weaning foods prepared from germinated cereals and legumes'. L M Marero , E M Pagumo , A R Aguinaldo , S Homma . *J. Food Sci* 1989. 53 (8) p. .

[Francis ()] *Pioneer in Food Sciences and Quality, In: A Century of Food Science. Institute of Food Technologists*, F J Francis . 2000.

[Ross et al. ()] 'Preservation and fermentation: Past, present and future'. R P Ross , S Morgan , C Hill . *International Journal of Food Microbiology* 2002. 79 p. .

[Zhao et al. ()] 'Prevalence of Campylobacter spp., Escherichia coli and Salmonella serovars in retail chicken, turkey, pork and beef from the greater Washington DC area'. C Zhao , G Beilei , J Vilena , R Sudler , E Yeh , S Zhao , D G White , D Wagner , A Meng . *Applied and Environmental Microbiology* 2000. 66 p. .

[Sourav and Arijit ()] 'Study of Physical and cultural Parameters on the bacteriocins produced by lactic acid bacteria Isolated from Traditional Indian fermented Foods'. B Sourav , D Arijit . *American Journal of Food Technology* 2010. 5 (2) p. .

[Onwuakor and Ukaegbu-Obi ()] 'Synergistic Bio-preservative Effects of Vernonia amygdalina Leaves and Sacoglottis gabonensis Stem Bark on Palm Wine from Elaeis guineensis and Raphia hookeri from Uturu, Nigeria'. C E Onwuakor , K M Ukaegbu-Obi . *American Journal of Microbiological Research* 2014. 2 (3) p. .

[Holtzel et al. ()] 'The first low molecular weight antibiotic from lactic acid bacteria: reuterincyclin, a new tetramic acid'. A Holtzel , M G Ganzle , G J Nicholson , W P Hammes , G Jung . *Angewandte Chemie. International Edition* 2000. 39 p. .