African Journal of Microbiology Research Vol. 7(8), pp. 669-677, 19 February, 2013
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.1906
ISSN 1996-0808 ©2013 Academic Journals

Full Length Research Paper

Antibacterial effect of hydrosoluble extracts of garlic (*Allium sativum*) against *Bifidobacterium* spp. and *Lactobacillus acidophilus*

Jemma Booyens and Mapitsi S. Thantsha*

Department of Microbiology and Plant Pathology, New Agricultural Sciences building, University of Pretoria, Pretoria, 0002, South Africa.

Accepted 8 February, 2013

The antimicrobial effects of garlic (*Allium sativum*) against pathogenic microorganisms have been well documented. It is generally stated that garlic exhibits differential inhibition between pathogenic and beneficial bacteria. Though there is substantial evidence to support the claim for pathogens, there is limited literature on its effects on beneficial bacteria, specifically probiotic bifidobacteria. This study aimed to investigate the antimicrobial effects of different garlic preparations on five strains of bifidobacteria. The disk diffusion assay revealed antibacterial activity of different garlic preparations characterised by zones of inhibition ranging from 13.0 ± 1.7 to 36.7 ± 1.2 mm. Minimum inhibitory concentration (MIC) values for garlic clove extract ranged from 75.9 to 303.5 mg/ml (estimated 24.84 to 99.37 µg/ml allicin). *Bifidobacterium lactis* Bi-07 300B was on average the most resistant to garlic, followed by *B. lactis* Bb12, *B. longum* LMG 13197, *B. longum* Bb356 and *B. bifidum* 11041, being most sensitive. This study reveals for the first time, susceptibility of bifidobacteria to antibacterial activity of garlic. Caution is therefore advised when using probiotic bifidobacteria and garlic simultaneously.

Key words: Allicin, *Allium sativum*, *Bifidobacterium,* garlic, probiotic.

INTRODUCTION

Garlic [*Allium sativum* L. (Liliaceae)] has been used worldwide for centuries as a spice, food and folklore medicine to cure and prevent various illnesses (Haciseferoğullari et al., 2005). It has numerous health benefits confirmed by numerous studies, which include its antiarthritic, antithrombotic, anticancer and antimicrobial activities (Amagase et al., 2001; Corzo-Martínez et al., 2007). Garlic has also been used to treat acne, ringworm, high blood pressure, gastrointestinal problems as well as asthma (Deresse and Mohammed, 2009; Kumar et al., 2010).

*Corresponding author. E-mail:mapitsi.thantsha@up.ac.za. Tel: +2712 420 4562. Fax: +2712 420 3266.

Allicin is the main active compound in crushed garlic cloves responsible for its antibacterial activity (Ankri and Mirelman, 1999; Groppo et al., 2007). This compound is produced after intact garlic tissues are damaged by crushing or cutting, when alliin is converted into allicin by the enzyme allinase (Ruddock et al., 2005). It has been found that garlic exhibits antibacterial activities against a wide range of Gram-positive and Gram-negative bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Bacillus, Clostridium*, *Neisseria*, *Proteus*, *Pseudomonas*, *Shigella* and *Mycobacterium* (Ankri and Mirelman, 1999; Belguith et al., 2010; Deresse, 2010; Gupta and Ravishanka, 2005; Harris et al., 2001; Rees et al., 1993; Ruddock et al., 2005; Uchida et al., 1975). *Helicobacter pylori*, the causative agent of

stomach ulcers, is also susceptible to garlic (Cellini et al., 1996; Sivam, 2001). Garlic also has antifungal, antiprotozoal and antiviral properties (Ankri and Mirelman, 1999; Harris et al., 2001). It has been documented that garlic inactivates and harms virulent microorganisms but not helpful ones in the body (Hayes, 1996). Rees et al. (1993) stated that garlic exerts differential inhibition between beneficial and potentially harmful enterobacteria.

Probiotics are live microorganisms which when administered in sufficient amounts exhibit various health benefits for the host (Moubareck et al., 2005; Wohlgemuth et al., 2010). The most commonly used probiotic strains which are commercially available are *Bifidobacterium* and *Lactobacillus* strains, which belong to the lactic acid bacteria (LAB) and which are normal inhabitants of the human gut (Rolfe, 2000; Kolida et al., 2006; Wohlgemuth et al., 2010). Bifidobacteria are probiotics that are beneficial microorganisms with acclaimed health beneficial effects on the host if ingested in sufficient amounts. At the same time that probiotics are recommended to consumers for health benefits, there are herbs such as garlic, which are also recommended for the same reason.

Although there have been numerous studies on the effects of garlic on pathogens, there are few studies on the susceptibility of beneficial bacteria, specifically probiotic strains of *Lactobacillus* (Rees et al., 1993; Naganawa et al., 1996; Ross et al., 2001). Susceptibility of lactic acid bacteria such as *Enterobacter* spp. and *Lactobacillus acidophilus* has been tested (Banerjee and Sarkar, 2003; O'Gara et al., 2000; Ross et al., 2001; Ruddock et al., 2005). The use of garlic alone or together with other herbs and spices in foods has the potential to extent to its use as an alternative food preservative in foods in which garlic flavour is desirable, or for extending the shelf life of raw meat products.

To our knowledge, there has been no study thus far on how garlic affects the probiotic strains of bifidobacteria. Since viability is crucial to probiotics' success, it is important to test their susceptibility to foods or food ingredients with antimicrobial effects. The objective of this study was therefore to investigate the antibacterial activity of different garlic preparations against selected strains of bifidobacteria.

MATERIALS AND METHODS

Microorganisms and growth conditions

Commercial probiotic cultures of *Bifidobacterium lactis* Bb12 (CHR-Hansen), *B. longum* Bb536 (Morinaga Milk company), *B. lactis* Bi-07 300B and *Lactobacillus acidophilus* La14 150B (Danisco) were used. *Bifidobacterium longum* LMG 13197 and *B. bifidum* LMG 11041 type strains were obtained from BCCM/LMG culture collection, and revived as specified. *L. acidophilus* was grown in MRS broth while bifidobacteria were grown in MRS supplemented with 0.05% cysteine hydrochloride (MRS-cys-HCl) broth. All cultures were incubated at 37°C for 48 h in anaerobic jars containing Anaerocult A gaspacks (Merck Ltd. Modderfontein, SA).

Preparation of garlic extracts

Garlic cloves, garlic paste, garlic powder and garlic spice were bought from a local supermarket in Pretoria. These were stored at 4°C for no longer than two weeks. To prepare garlic clove extract (GC), 10 g were crushed in 5 ml sterile distilled water (sdH_2O) using a mortar and pestle, centrifuged at 3500 rpm using Eppendorf miniSpin centrifuge and then filtered through a 0.22 µm filter membrane (Minisart). The weight of the insoluble material was subtracted from the weight of the original cloves and the final concentration of garlic extract in solution was determined (Bakri and Douglas, 2005). Extracts of garlic paste (GP), garlic powder (Gp) and garlic spice (GS) were prepared the same way except that 10 g Gp and GS were suspended in 10 ml autoclaved distilled water (dH_2O).

Allicin concentration

The concentration of allicin in each garlic preparation was determined spectrophotometrically by reaction with thiol, 4-mercaptopyridine (Miron et al., 2002). Briefly, a 1:1 dilution of each garlic extract was incubated at room temperature in 1 ml 4-mercaptopyridine (10^{-4} M) in 50 mM phosphate buffer, 2 mM EDTA, pH 7.2, which results in formation of 4-allylmercapthiopyridine, causing a shift in absorbance. The decrease in optical density at 324 nm after 1 h was used to calculate the allicin concentrations in each garlic preparation. ε_M 39, 600 $M^{-1}cm^{-1}$ at 324 nm was used for the calculation.

Disk diffusion assay

The antimicrobial activity of the garlic preparations were tested using the disk diffusion method according to Benkeblia (2004), with minor modifications. Bacterial cell suspensions were adjusted to a 0.5 McFarland's standard. A lawn of bifidobacteria was prepared by spreading 100 µl of each of the broth cultures (incubated at 37°C for 48 h) onto MRS-cys-HCl agar plates. Filter disks (1 cm) were impregnated with 30 µl of the GC, GP, Gp and GS extract. Sterile dH_20 and ampicillin (Amp) at a final concentration of 5 mg/ml) were used as negative and positive controls, respectively. Plates were then incubated anaerobically at 37°C for 48 to 72 h, after which the diameters of any resultant inhibition zones were measured (mm). This assay was repeated in triplicate.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the garlic extracts was determined according to a method described by Bakri and Douglas (2005) with minor modifications. Extracts were diluted in MRS-cys-HCl broth and inoculated with 100 µl of the bifidobacterial cultures which were all adjusted to a concentration equivalent to 0.5 McFarland's standard beforehand. Tubes were then incubated anaerobically for 24 h at 37°C and the highest dilution where there

Table 1. The antibacterial activity (inhibition zones) of different garlic extracts and ampicillin on the probiotic strains tested.

Probiotic strain	Antimicrobial compound				
	GC	Gp	GP	GS	Amp
	Zone of inhibition (mean diameter (mm) ± SD)				
B. bifidum LMG 11041	36.7 ± 1.2	28.0 ± 2.0	21.3 ± 2.5	22.3 ± 0.6	52.0 ± 2.0
B. lactis Bb12	22.3 ± 1.5	17.3 ± 2.1	[a]	19.3 ± 2.1	34.7 ± 2.1
B. lactis Bi-07 300B	13.0 ± 1.7	[a]	[a]	[a]	31.0 ± 1.7
B. longum Bb536	31.3 ± 2.3	24.0 ± 3.0	20.7 ± 2.1	19.7 ± 1.5	44.7 ± 1.5
B. longum LMG 13197	28.0 ± 1.0	19.7 ± 2.1	[a]	24.0 ± 2.0	43.3 ± 2.5
Lactobacillus acidophilus La14 150B	[a]	[a]	[a]	[a]	44.0 ± 2.6

Each value is mean of 3 replicates ± standard deviation (SD); GC, garlic clove extract; GP, garlic paste extract; Gp, garlic powder extract; GS, garlic spice extract; [a]no inhibition zone.

was no bacterial growth was recorded as the MIC. Dilutions showing no visible growth were plated out (100 µl) onto MRS-cys-HCl agar plates and incubated anaerobically for a further 24 h at 37°C. The highest dilution where there was no growth at all was recorded as the MBC. Sterile MRS-cys-HCl broth and Amp were used as a negative and positive control, respectively. MIC and MBC determinations were performed in triplicate.

Time kill curves

MRS-cys-HCl broths were inoculated with each of the *Bifidobacterium* spp. respectively and incubated overnight anaerobically at 37°C. Broth cultures were adjusted to a 0.5 McFarland's standard and viable plate counts were performed in order to confirm the initial amount of bacteria before exposure to the preparations. The cultures were exposed to MBC concentrations of garlic extracts or ampicillin (positive control) in triplicate. Tubes were then incubated at 37°C for 6 h. A 1 ml sample was taken from each of the tubes immediately after addition of garlic, after 0.5, 1, 2, 3, 4, 5 and 6 h of incubation. The samples were serially diluted in sterile ¼ strength Ringer's solution up to 10^{-6} and 100 µl of each dilution was pour-plated onto MRS-cys-HCl agar plates and then incubated anaerobically at 37°C for 72 h. An uninoculated culture was used as a negative control.

Statistical analysis

Statistical analysis of the data (standard deviations of means and Student's t-tests at the 5 % significance level) was performed using StatSoft STATISTICA 10. $p < 0.05$ showed marked significant difference, and $p > 0.05$ was non-significant.

RESULTS AND DISCUSSION

Disk diffusion assay

The final concentration of garlic extract in solution was determined to be 60.7% (w/v) for GC, 10.7 % (w/v) for GP, 9% for Gp and 8.9% (w/v) for GS. The estimated allicin concentrations in these extracts were 198.74 µg/ml, 124.98 µg/ml, 26.63 µg/ml and 10.24 µg/ml for GC, GP, Gp and GS, respectively. Table 1 shows diameters of zones of inhibition for the tested *Bifidobacterium* strains. The reported values are means of triplicate measurements. All bifidobacterial strains were inhibited by GC extract and inhibition zones ranged from 13.0 ± 1.7 to 36.7 ± 1.2 mm. Gp and GS inhibited all the strains, except *B. lactis* Bi-07 300B. No inhibition was obtained for GP for most bifidobacterial strains except for *B. bifidum* LMG 11041 and *B. longum* Bb536. Therefore GC had the highest antimicrobial activity compared to other tested preparations. Ampicillin was used as a positive control and all strains were susceptible with inhibition zones ranging from 31.0 ± 1.7 to 52.0 ± 2.0 mm. The susceptibility of the tested strains to the different garlic preparations differed. The resistance pattern (from the most to the least resistant) of the strains to GC extract was *B. lactis* Bi-07 300B > *B. lactis* Bb12 > *B. longum* LMG 13197 > *B. longum* Bb536 > *B. bifidum* LMG 11041. There was no correlation between the sensitivities of the strains to GC extract compared to GP, Gp and GS. The resistance pattern of the strains to GP was *B. lactis* Bb12 ~ *B. lactis* Bi-07 300B ~ *B. longum* LMG 13197 > *B. longum* Bb536 > *B. bifidum* LMG 11041. For Gp, it was *B. lactis* Bi-07 300B > *B. lactis* Bb12 > *B. longum* LMG 13197 > *B. longum* Bb536 > *B. bifidum* LMG 11041. The pattern for GS resistance was *B. lactis* Bi-07 300B > *B. lactis* Bb12 > *B. longum* Bb536 > *B. bifidum* LMG 11041 > *B. longum* LMG 13197. Overall, B. *bifidum* LMG 11041 was the most sensitive ($p < 0.05$) to the antibacterial effects of the garlic preparations and *B. lactis* Bi-07 300B was most resistant. No inhibition zones for *L. acidophilus* due to any of the garlic extracts was observed but its susceptibility to ampicillin was observed as was expected. *Lactobacillus* is reported to be very sensitive to this antibiotic (D'Aimmo et al., 2007). This is the first time that sensitivity of bifidobacterial species to garlic is being reported.

Table 2. Inhibitory effect (minimum inhibitory concentration (MIC)) of different garlic extracts on the probiotic strains tested.

Probiotic strain	Antimicrobial compound				
	GC	Gp	GP	GS	Amp (µg/ml)
	Garlic concentration (mg/ml) (estimated allicin concentration µg/ml)				
B. bifidum LMG 11041	75.9 (24.84)	30 (8.88)	107 (124.98)	44.5 (5.12)	0.00000035
B. lactis Bb12	202.3 (66.25)	45 (13.32)	>107 (124.98)*	89 (10.24)	0.035
B. lactis Bi-07 300B	303.5 (99.37)	>90 (>26.63)*	>107 (>124.98)*	>89 (>10.24)	0.05
B. longum Bb536	86.7 (28.39)	45 (13.32)	107 (124.98)	44.5 (5.12)	0.00005
B. longum LMG 13197	151.75 (49.69)	90 (26.63)	>107 (>124.98)*	89 (10.24)	0.002
L. acidophilus La14 150B	303.5 (99.37)	>90 (>26.63)*	>107 (>124.98)*	>89 (10.24)*	0.5

GC, garlic clove extract; GP, Garlic paste extract; Gp, garlic powder extract; GS, garlic spice extract; *not inhibited by the highest concentration of the garlic extract tested.

Table 3. Inhibitory effect (minimum bactericidal concentration (MBC)) of different garlic extracts on the probiotic strains tested.

Probiotic strain	Antimicrobial compound				
	GC	Gp	GP	GS	Amp MBC (µg/ml)
	Garlic extract dilution (estimated allicin concentration µg/ml)				
B. bifidum LMG 11041	1:5 (39.75)	1:2 (13.32)	>107 (>124.98)*	1:1 (10.24)	1:7 (0.000005)
B. lactis Bb12	1:1 (198.74)	1:1 (26.63)	>107 (>124.98)*	>89 (>10.24)*	1:2 (0.5)
B. lactis Bi-07 300B	1:1 (198.74)	>90 (>26.63)*	>107 (>124.98)*	>89 (>10.24)*	1:2 (0.5)
B. longum Bb536	1:4 (49.69)	1:1 (26.63)	>107 (>124.98)*	1:1 (10.24)	1:4 (0.005)
B. longum LMG 13197	1:2 (99.37)	>90 (>26.63)*	>107 (>124.98)*	>89 (>10.24)*	1:3 (0.05)
L. acidophilus La14 150B	>607 (>198.74)*	>90 (>26.63)*	>107 (>124.98)*	>89 (>10.24)*	1:1 (5)

GC, garlic clove extract; GP, Garlic paste extract; Gp, garlic powder extract; GS, garlic spice extract; *not inhibited by the highest concentration of the garlic extract tested.

It was expected that if there was any antimicrobial effects, it would be observed in fresh GC extract as it possess the active ingredient allicin, which is released upon crushing of the cloves. This compound is responsible for the antimicrobial activity of garlic and is active against a wide variety of Gram-positive and Gram-negative microorganisms (Benkeblia, 2004; Durairaj et al., 2009). Only two strains were susceptible to GP (Table 1), while all strains besides one were susceptible to Gp and GS. GS, Gp and GP had in general lower inhibition zones for the bifidobacterial strains. This is probably due to the harsh preparation processes of these preparations. Gp and garlic granules, used as GS, are dehydrated, dried and stored for long periods of time before they are utilized. Therefore active ingredients found in fresh garlic extract, such as allinase, are often eliminated or become inactive thereby producing insignificant amounts of allicin and thus negligible antimicrobial properties (Yu and Shi-ying, 2007).

MIC and MBC determination

Tables 2 and 3 show MIC and MBC values obtained. The garlic extract concentrations as well as the deduced allicin concentrations were used to calculate MIC and MBC values. MIC values for the garlic preparations, except GP, were lower for most bifidobacterial strains compared to the control *Lactobacillus* strain. This indicated that the bifidobacterial strains were more

sensitive to the garlic preparations than *Lactobacillus acidophilus*. For GC, Gp, GP and GS extracts the MIC values ranged from 75.9 to 303.5 mg/ml garlic (estimated 24.84 to 99.37 µg/ml allicin), 30 to > 90 mg/ml garlic (estimated 8.88 to > 26.63 µg/ml allicin), 107 to > 107 mg/ml garlic (estimated 124.98 to > 124.98 µg/ml allicin) and 44.5 to > 89 mg/ml (estimated 5.12 - > 10.24 µg/ml allicin), respectively. *B. bifidum* LMG 11041 had significantly lower MIC and MBC values overall for all the garlic extracts, indicating that it was by far the most susceptible of the bifidobacterial strains tested. On the contrary, *B. lactis* Bi-07 300B had significantly higher ($p < 0.05$) MIC and MBC values than all the other test strains and was therefore the most resistant. MBC values obtained for GC and Gp were double or more than the MIC values.

A further interesting observation made from the MIC values obtained is that garlic preparations containing minimal levels of allicin still exhibited antibacterial effects on bifidobacteria. It would be expected that allicin concentrations lower than those recorded as MIC for GC would not inhibit bacterial growth. However, as indicated by results obtained specifically for Gp and GS, allicin levels five times lower than MIC for GC were inhibitory to bifidobacteria growth (Table 2). This observation suggests that these garlic preparations have other antimicrobial compounds, though less potent than allicin, that act synergistically with low levels of allicin in these garlic preparations to inhibit bacterial growth. Compounds such as ajoene and vinyl dithiins (Harris et al., 2001) and other thiosulfanates (Hovana et al., 2011) with antimicrobial effects were isolated as products of garlic degradation. It has been shown that addition of ajoene to fungal cultures resulted in inhibition at concentrations lower than experienced with allicin (Harris et al., 2001). Inhibitions observed with low levels of allicin in this study could be attributed to this factor.

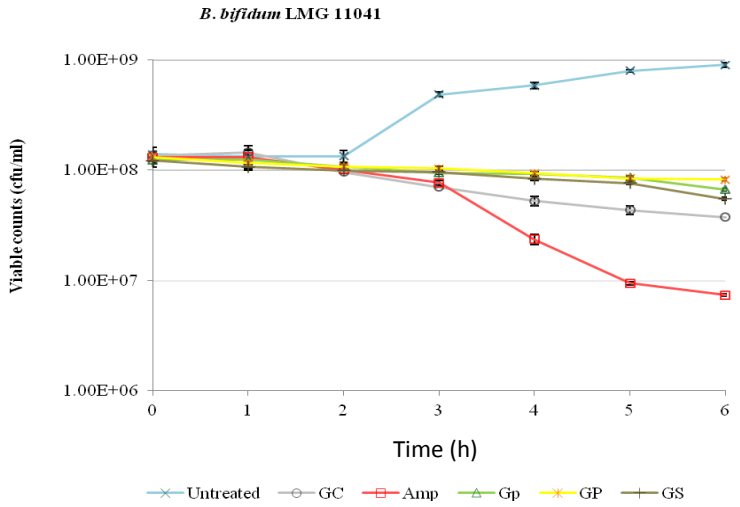
MIC values for GC and Gp extract against *L. acidophilus* were higher compared to those reported in literature (Owhe-Ureghe et al., 2010; Ross et al., 2001). Allicin concentration value for GC extract obtained in this study was also slightly lower than those reported by Bakri and Douglas (2005). These differences may be due to a number of reasons. According to Deresse (2010), garlic species tend to vary in different countries as well as the processing methods used for different garlic preparations. Lower allicin concentrations and higher MIC values may also be due to little precautions taken to prevent loss of garlic components, specifically allicin, by volatilization. Allicin is a very volatile and unstable compound which, depending on environmental conditions and processing actions, will undergo numerous reactions and form other derivatives (Hovana et al., 2011). Therefore this may result in lower or no antibacterial activity when garlic is exposed for long periods of time. The origin and type of strains used may also play a role in these differences.

GC extract was able to inhibit all bifidobacterial strains and had the strongest/highest ($p < 0.05$) antimicrobial activity of all the preparations tested. Gp and GS were found to have similar antimicrobial effects and only inhibited certain strains. GP had the lowest inhibitory effect with only *B. bifidum* LMG 11041 being slightly susceptible. There have been few studies on the antimicrobial activity of GP, Gp and GS, but it is known that Gp has a lower inhibitory effect than GC due to its high vegetable content (O'Gara et al., 2000). Different culture media may have an effect on antimicrobial activity of garlic components and it is known that cysteine has an effect on allicin (Ross et al., 2001). This may therefore have had an influence on the interpretation of our *in vitro* test results on the antimicrobial activity of GP preparation as cysteine was added to MRS broth used during the experiments. Therefore these results may be an underestimate of Gp, GP and GS's full antimicrobial potential.

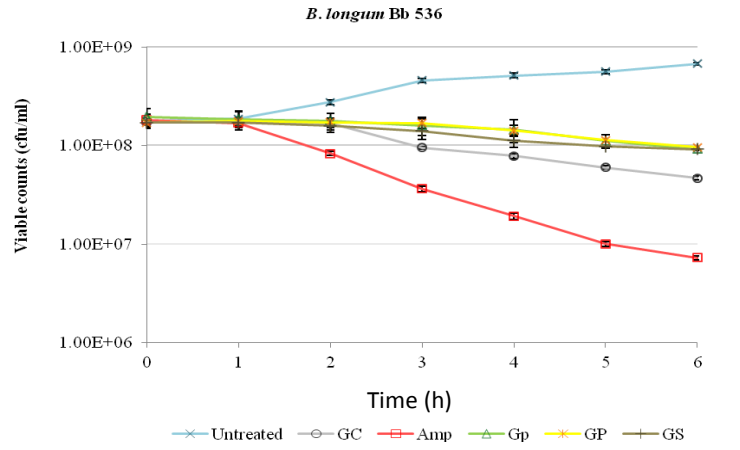
Time-kill curves

Figure 1 shows the time kill curves for the tested bifidobacteria strains. The initial average concentration of bacteria for all strains was approximately equal to 0.5 McFarland's standard. All the strains were sensitive to Amp, the positive control, with *B*. *longum* Bb 536 showing the highest reduction in viable numbers to that of the initial amount (Figure 1b). In general, all the bifidobacterial strains tested experienced a significant decrease ($p < 0.05$) in cell numbers when exposed to GC extract than other preparations. *B. bifidum* LMG 11041 (Figure 1a) and *B. longum* Bb536 were the only two strains that showed a reduction in viability for all the garlic preparations over the allocated time period. Overall, *B. bifidum* LMG 11041 showed the highest ($p < 0.05$) reduction in viability for all preparations (Figure 1a, b). Viability of *B. longum* LMG 13197 (Figure 1c) incubated in both Gp and GS decreased; while viability of *B. lactis* Bb12 (Figure 1d) incubated in the same preparations decreased slightly less. For both of these strains, there was no reduction in cell numbers when exposed to GP. *B. lactis* Bi-07 300B showed the closest trend to that of the comparison strain, *L. acidophilus* (Figure 1e, f). There was no significant reduction in viability for these strains upon exposure to GP, Gp and GS with a minor decline for GC. This could also possibly indicate that *B. lactis* Bi-07 300B requires a longer exposure period of more than 6 h to succumb to the antimicrobial effects of garlic compared to the other strains. The negative control, incubated with no garlic preparations, experienced no decline in cell numbers for all strains, as expected. On the contrary there was a gradual increase

(a)



(b)



(c)

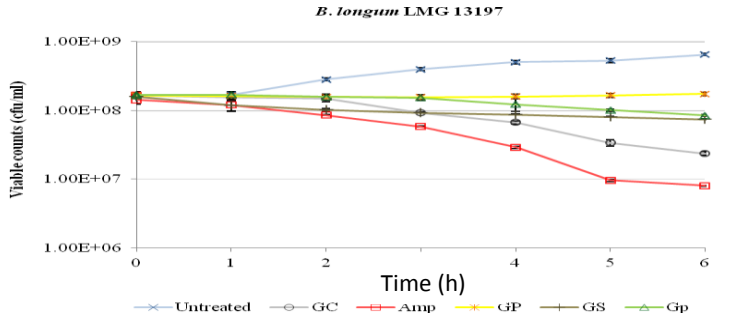
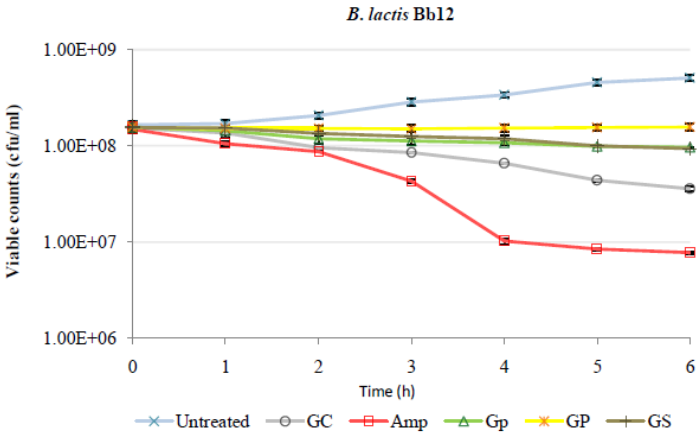
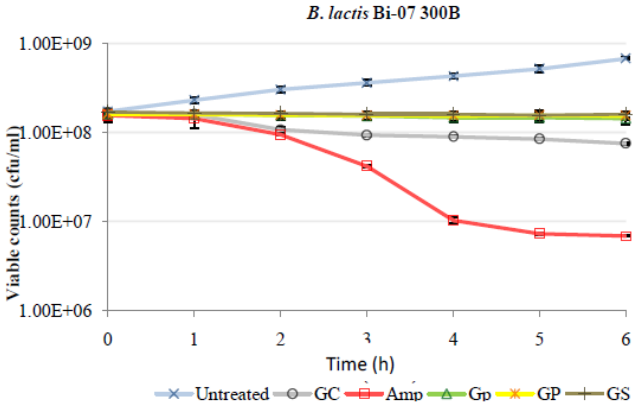


Figure 1. The effect of garlic preparations (GC, garlic clove extract; GP, Garlic paste extract; Gp, garlic powder extract; GS, garlic spice extract) at their respective MBC for each strain, on viability of *B. bifidum* LMG 11041 (a), *B. longum* Bb536 (b), *B. longum* LMG 13197 (c), *B. lactis* Bb12 (d), *B. lactis* Bi-07 300B (e) and *L. acidophilus* (f) over 6 h. Ampicillin (5 mg/ml) and broth cultures without garlic were used as positive and negative controls, respectively.



(e)



(f)

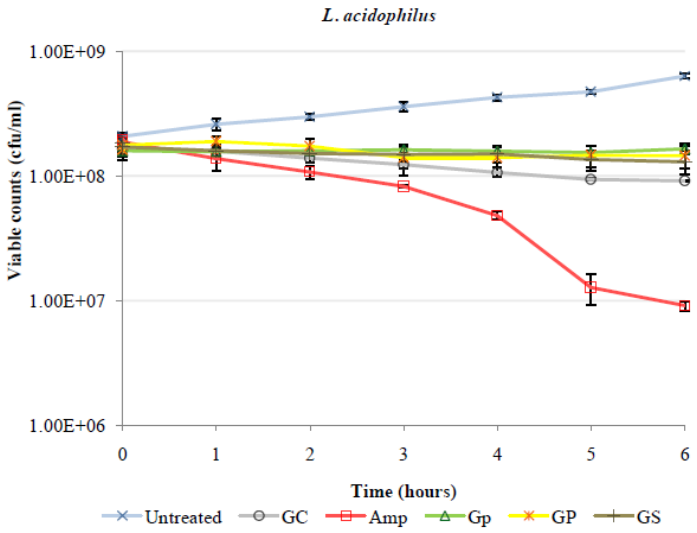


Figure 1. Contd.

in numbers over 6 h. There was a significant difference ($p < 0.05$) in viability for the negative control if compared with that of garlic treated cultures. The order of strains that demonstrated the highest drop in viability towards the garlic preparations was as follows: *B. bifidum* LMG 11041 > *B. longum* Bb 536 > *B. longum* LMG 13197 > *B. lactis* Bb12 > *B. lactis* Bi-07 300B.

All strains, including *L. acidophilus*, showed a decrease in viability from that of their initial bacterial concentration once exposed to GC for 6 h; therefore they were all affected by the GC, some not as much as others. Total reductions of 1.2 log, 0.8 log, 0.9 log, 0.8 log and 0.4 log were observed at the end of the 6 h exposure to GC for *B. bifidum* LMG 11041, *B. longum* Bb 536, *B. longum* LMG 1037, *B. lactis* Bb12 and *B. lactis* Bi-07 300B, respectively. For most strains, GC had the shortest lag phase when compared to the other garlic preparations and on average took 1 h to start inhibiting the bacterial cells. The most sensitive strain, *B. bifidum* LMG 11041 succumbed to the antibacterial activity of GC within 0.5 h. GP, Gp and GS had an average minimum exposure time of 2 h before they started having an inhibitory effect on the strains.

Upon exposure to GP, there was a slight increase in viability for *B. lactis* Bb12 (Figure 1d) and *B. longum* LMG 13197 (Figure 1c). This could be an indication that the active antimicrobial compounds of GP were diminished and the remaining population of cells started multiplying. The diminished antimicrobial activity could be due to instability of the active compound or its transformation into stable components such as polysulfides and thiosulphonates (Cellini et al., 1996; Belguith et al., 2010). Regrowth of bacteria exposed to garlic preparations was observed elsewhere. Margues et al. (2008) observed regrowth of *Salmonella enterica* exposed to garlic powder. Feldberg et al. (1988) suggested regrowth of *Salmonella typhimurium* could be due to its ability to titrate allicin or to metabolize it into noninhibitory compounds (Belguith et al., 2010). Exposure periods longer than 6 h to GP, Gp and GS may be required to observe a reduction in *B. lactis* Bi-07 300B viability as there was no decrease during the 6 h. These results may possibly indicate that this strain takes a longer time to succumb to the antimicrobial effect of GP than other strains do. However, comparing viable counts of all garlic exposed strains to the untreated cells, it is evident that in the presence of any garlic preparation, growth of bifidobacteria was inhibited. There was a significant increase in viable numbers of untreated bacteria overtime, whereas either a decline or no change in viable numbers was observed for garlic exposed cells (Figure 1). This highlights that the presence of garlic has negative effects on bifidobacteria.

A minimum exposure period (MEP) of 1 h to GC was required for all strains but *B. bifidum* LMG 11041, which required only 30 min. The MEP for all other garlic extracts was 2 h. *B. lactis* strains however, required longer than 6 h as no decrease in viability was observed until the end of the exposure period for all garlic extracts. There was a significant difference ($p < 0.05$) in viability for controls compared to garlic exposed cultures. The differences in minimum exposure period observed for the tested strains indicated that sensitivity of bifidobacteria to garlic preparations differed between strains. This highlights that no generalizations should be made for the different strains on their sensitivity to antimicrobial effects of garlic. Inter-strain variations in garlic extract sensitivity was also observed for strains of *Streptococcus mutans* by Chen et al. (2009) who attributed it to be partially due to different cell surface composition in different strains.

Conclusions

Results show that garlic does have antimicrobial effects against bifidobacteria, with fresh GC extract being the most potent. This is a significant information as many consumers may take probiotics and garlic simultaneously, which would therefore decrease the viability and hence the affectivity of the probiotics. This then becomes a huge disadvantage to the consumer. Therefore from this study we conclude that garlic exerts antibacterial activity against bifidobacteria. Caution is therefore needed when using probiotic bifidobacteria and garlic simultaneously. However, further research investigating the effect of food constituents on the antibacterial activity is recommended.

ACKNOWLEDGEMENTS

Thanks are due to the National Research Foundation (NRF) and the University of Pretoria for funding this project. Prof. E Buys of the Department of Food Science, University of Pretoria is thanked for providing some of the cultures.

REFERENCES

Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y (2001). Intake of garlic and its bioactive components. J. Nutr. 131:955-962.

Ankri S, Mirelman D (1999). Antimicrobial properties of allicin from garlic. Microbes Infect. 2:125-129.

Bakri IM, Douglas CWI (2005). Inhibitory effect of garlic extract on oral bacteria. Arch. Oral Biol. 50:645-651.

Banerjee M, Sarkar PK (2003). Inhibitory effect of garlic on bacterial pathogens from spices. World J. Microbiol. Biotechnol. 19:565-569.

Belguith HF, Kthiri A, Chati A, Abu Sofah J, Ben H, Ladoulsi A (2010). Inhibitory effect of aqueous garlic extract *(Allium sativum)* on some isolated *Salmonella* serovars. Afr. J. Microbiol. Res. 4:328-338.

Benkeblia N (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). Food Sci.

Technol. 37:263-268.
Cellini L, Campli D, Masulli E, Bartolomeo DS, Allocati N (1996). Inhibition of *Helicobacter pylori* by garlic extract (*Allium sativum*). FEMS Immunol. Med. Microbiol. 13:273-277.
Chen YY, Chiu HC, Wang YB (2009). Effects of garlic extract on acid production and growth of *Streptococcus mutans*. J. Food Drug Anal. 17:59-63.
Corzo-Martínez M, Corzo N, Villamiel M (2007). Biological properties of onions and garlic. Trends Food Sci. Tech. 18:609-625.
D'Aimmo MR, Modesto M, Biavati B (2007). Antibiotic resistance of lactic acid bacteria and *Bifidobacterium* spp. isolated from dairy and pharmaceutical products. Int. J. Food Microbiol. 115:35-42.
Deresse D (2010). Antibacterial effect of garlic (*Allium sativum*) on *Staphylococcu aureus*: An *in vitro* study. Asian J. Med. Sci. 2:62-65.
Deresse D, Mohammed A (2009). Assessment of antibacterial effect of crude preparation of garlic (*Allium sativum*) on diarrhea causing bacteria: An *in vitro* study. Asian J. Med. Sci. 1:12-14.
Durairaj S, Srinivasan S, Lakshmanaperumalsamy P (2009). *In vitro* antibacterial activity and stability of garlic extract at different pH and temperature. eJBio. 5:5-10.
Feldberg R, Chang S, Kotik A, Nadler M, Neuwirth Z, Sundstrom D, Thompson N (1988). *In vitro* mechanism of inhibition of bacteria cell growth by allicin. Antimicrob. Agents Chemother. 32:1763-1768.
Groppo FC, Ramacciato JC, Motta RHL, Ferraresi PM, Sartoratto A (2007). Antimicrobial activity of garlic against oral streptococci. Int. J. Dent. Hyg. 5:109-115.
Gupta S, Ravishankar S (2005). A comparison of the antimicrobial activity of garlic, ginger, carrot, and turmeric pastes against *Escherichia coli* O157:H7 in laboratory buffer and ground beef. Foodborne Pathog. Dis. 2:330-340.
Hacıseferoğullari H, Özcan M, Demir F, Çalışır S (2005). Some nutritional and technological properties of garlic (*Allium sativum* L.). J. Food Eng. 68:463-469.
Harris JC, Cottrell SL, Plummer S, Lloyd D (2001). Antimicrobial properties of *Allium sativum* (garlic). Appl. Microbiol. Biotechnol. 57:282-286.
Hayes A (1996). Fight flu with lots of garlic. Sydney morning herald. http://cabbagediet.com/au/cabbage-diet-articles/1996/5/29/fight-flu-with-lots-of-garlic/. Accessed 24 February 2009.
Hovana EIK, James US, James E, Egbobor EM, Egba AG, Eta ES, Nwakaku OA (2011). Antibacterial and phytochemical studies of *Allium sativum.* New York Sci. J. 4:123-128.
Kolida S, Saulnier DM, Gibson GR (2006). Gastrointestinal Microflora: Probiotics. Adv. Appl. Microbiol. 59:187-219.
Kumar KPS, Bhowmik D, Chiranjib, Tiwari P, Kharel R (2010). *Allium sativum* and its health benefits: An overview. J. Chem. Pharm. Res. 2:135-146.
Margues A, Encarnação S, Pedro S, Nunes ML (2008). In vitro antimicrobial activity of garlic, oregano and chitosan against *Salmonella enterica*. World J. Microbiol. Biotechnol. 24:2357-2360.
Miron T, Shin I, Feigenblat G, Weiner L, Mirelman D, Wilchek M, Rabinkov A (2002). A spectrophotometric assay for allicin, alliin, and allinase (alliin lyase) with a chromogenic thiol: reaction of 4-mercaptopyridine with thiosulfinates. Anal. Biochem. 307:76-83.
Moubareck C, Gavini F, Vaugien L, Butel MJ, Doucet-Popularie F (2005). Antimicrobial susceptibility of Bifidobacteria. J. Antimicrob. Chemoth. 55:38-44.
Naganawa R, Iwata N, Ishikawa K, Hiroyuki F, Tsuchiyoshi F, Suzuki A (1996). Inhibition of microbial growth by ajoene, a sulphur-containing compound derived from garlic. Appl. Environ. Microbiol. 62:4238-4242.
O'Gara EA, Hill DJ, Maslin DJ (2000). Activities of Garlic Oil, Garlic Powder, and their diallyl constituents against *Helicobacter pylori*. Appl. Environ. Microbiol. 66:2269-2273.
Owhe-Ureghe UB, Ehwarieme DA, Eboh DO (2010). Antibacterial activity of garlic and lime on isolates of extracted carious teeth. Afr. J. Biotechnol. 9:3163-3166.
Rees LP, Minney SF, Plummer NT, Slater JH, Skryme DA (1993). A quantitative assessment of the antimicrobial activity of garlic (*Allium sativum*). World J. Microbiol. Biotechnol. 9:303-307.
Rolfe RD (2000). Symposium: Probiotic Bacteria: Implications for Human Health. J. Nutr. 130:396S-402S.
Ross ZM, O'Gara EA, Hill DJ, Sleightholme HV, Maslin DJ (2001). Antimicrobial properties of garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl. Environ. Microbiol. 67:475-480.
Ruddock PS, Mingman L, Foster BC, Lawson L, Arnason JT, Dillon J-AR (2005). Garlic Natural Health Products Exhibit Variable Constituent Levels and Antimicrobial Activity against *Neisseria gonorrhoeae*, *Staphyloccocus aureus* and *Enterococcus faecalis.* Phytother. Res. 19:327-334.
Sivam GP (2001). Protection against *Helicobacter pylori* and other bacterial infections by garlic. J. Nutr. 131:1106S-1108S.
Uchida Y, Takahashi T, Sato N (1975). The characteristics of the antibacterial activity of garlic. Jpn. J. Antibiot. 28:638-646.
Wohlgemuth S, Gunnar L, Blaut M (2010). Recent developments and perspectives in the investigation of probiotic effects. Int. J. Med. Microbiol. 300:3-10.
Yu LI, Shi-ying XU (2007). Preparation of garlic powder with high allicin content. Agric. Sci. China 6:890-898.