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Full Length Research Paper

Inhibitory effect of aqueous garlic extract *(Allium sativum)* on some isolated *Salmonella* serovars

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There has been a consistent increase in the search for alternative and efficient compound for food conservation, aiming a partial or total replacement of antimicrobial chemical additives. Garlic offers a promising alternative for food safety and bioconservation. Filter sterilized, aqueous garlic extract was tested for ability to inhibit the growth of some isolated *Salmonella* serovars. The aqueous garlic extract (A. G. E., 57.1% (w/v), containing 324 µg/ml allicin) inhibited the growth and killed most of the tested *Salmonella* serovars. The effect of bacteriostatic concentration of A. G. E. on the growth of the different tested serovars, revealed a pattern of inhibition characterized by: (i) a transitory inhibition phase whose duration was proportional to A. G. E concentration (ii) a resumed growth phase which showed a lower rate of growth than in uninhibited controls and (iii) an entry into stationary phase at a lower culture density. The minimal inhibitory concentration and minimum bactericidal concentrations were very close; garlic MIC range 10 - 12.5 mg/ml; MBC range 13 - 15 mg/ml. Garlic extract could be stored at 4°C because no detectable loss of antibacterial activity at this temperature over several days was observed. However, excessive warming, or longer periods at higher temperatures should be avoided. Among enzymatic activities followed with the API-ZYM system, significant changes during the inhibition phase were detected. These biochemical changes represent an adaptative response towards the garlic stress.

Key words: *Allium sativum*, allicin, garlic, antimicrobial, *Salmonella*.

INTRODUCTION

The increasingly high numbers of bacteria that are developing resistance to classical antibiotics (Levy, 1997; Cohen, 1992; Walsh, 2000; Kiessling et al., 2002; Cole et al., 2002) drive much of the current interest on plant antimicrobial molecules in hope that they may provide useful leads into anti-infective drug candidates. Several antimicrobial agents were isolated from plant including secondary metabolites as essential oil and terenoides, amongst which can be cited xanthones, benzophenones, coumarins and flavonoids (Nkengfack et al., 2002; Ouahouo et al., 2004; Komgeum et al., 2005).

Garlic (*Allium sativum*) has traditional dietary and medicinal applications as an anti-infective agent (Ross et al., 2001). Garlic is a common food spice widely distributed and used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases (Rivlin, 2001). Garlic is thought to have various pharmacologic properties and medical applications. It is mainly consumed as a condiment in various prepared food (Amagase et al., 2001).

Garlic is a strong antibacterial agent and acts as an inhibitor on both Gram-positive and Gram-negative bacteria including such species as *Escherichia*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Proteus* and *Helicobacter pylori* (Ankri and Mirelman, 1999; Reuter et al., 1996).

The main antimicrobial constituent of garlic has been

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Abbreviations: AGE; Aqueous garlic extract, **MBC;** minimal bactericidal concentration, **MIC;** minimal inhibitory concentration, **EDTA;** ethylene diamine tetraacetic acid, **CFU;** colony-forming units, **ZYM A;** enzyme-linked reagent A, **ZYM B;** enzyme-linked reagent B.

Table 1. *Salmonella* serovars characteristics.

Serovars	Isolat	Antigenic formula	Source	Origin
S. hadar	287	(6.8 : Z10/ENX)	Turkish meat	Ariana
S. enteritidis	49	(9 : gm :-)	One-day-old chicken	Tunis
S. lindenburg	320	(6.8 : i :1,2)	Fresh milk	Tunis
S. nikolaifleet	63	(1.6: gms : -)	Coproculture	Nabeul
S. cerro	291	(1.8 : Z4Z23 : 1,5)	Retail ground beef meat	Ariana
S. montevideo	297	(6.7: gms : -)	Waste water	Ariana

identified as the oxygenated sulphur compound, thio- 2 -propene- 1 -sulfinic acid S-allyl ester, which is usually referred to as allicin. Allicin is produced catalytically when garlic cloves are crushed and the enzyme allinase (alliin lyase E.C. 4.4.1.4) of the bundle sheath cells mixes with its substrate, alliin, which is released from mesophyll cells (Miron et al., 2000; Curtis et al., 2004).

Allicin undergoes thiol-disulphide exchange reactions and can react with free thiol groups in proteins (Ankri and Mirelman, 1999; Miron et al., 2002). Thiol (-SH) containing enzymes so far have been shown to be inhibited by allicin; examples of which include: succinic acid dehydrogenase, urease, papain, xanthine oxidase, choline oxidase, hexokinase, cholinesterase, glyoxylase, triose phosphate dehydrogenase, alcohol dehydrogenase and cysteine proteases (Ankri et al., 1997; Wills, 1956). Additionally, Focke et al. (1990) provided evidence for specific inhibition of acetyl-CoA synthetase (E.C.6.2.1.1) by allicin which occurred by a non-covalent, reversible binding of allicin to the enzyme. In contrast to others reports of enzymes inhibition by allicin this effect could not be competed out by thiol reagents such as dithioerythritol (Wills, 1956). Allicin's reactivity with enzymes and its radical-trapping properties and ready membrane permeability, are regarded as the basis of its biological activity (Miron et al., 2000; Focke et al., 1990).

The anti-microbial effects is due to the chemical reaction of the allicine with the thiol groups of several enzymes such as ARN Polymerase, by delaying and inhibiting DNA, RNA and protein synthesis (Ankri and Mirelman, 1999; Rabinkov, 1998).

Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including such species as *Esherichia*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Proteus* and *H. pylori* (Ankri and Mirelman, 1999; Reuter et al., 1996; Feldberg et al., 1988). Even acid-fast bacteria such us *Mycobacterium tuberculosis* are sensitive to garlic (Reuter, 1995).

The aim of this study was first to investigate the antimicrobial effect of aqueous garlic extract against six serovars of *Salmonella*, isolated from Tunisian foods, coproculture and wastewater. Second, to study the garlic cell growth effects and third to evaluate the bioresponses of treated bacteria, particularly biochemical changes induced by bacteriostatic aqueous garlic extract concentrations.

Salmonella serovars was chosen as the Gram-negative model organism, as it is one of the major human pathogens and food poisoning cases (Reed, 1993).

MATERIALS AND METHODS

Bacteria and growth conditions

Salmonella serovars included in this study were recovered from different samples, purchased from different region in Tunisia (Table 1).

A total of six *Salmonella* serovars, isolated from Tunisian fast foods, coproculture and wastewater were used in this study. These were *Salmonella cerro*, *Salmonella enteritidis*, *Salmonella lindenburg*, *Salmonella montevideo*, *S. hadar* and *Salmonella nikolaifleet* which are also listed in Table 1. Long-term storage of *Salmonella* samples was at - 20°C in sterile glycerol (15%). A preculture was prepared by transfer from this culture to fresh sterile liquid medium (Pronadisa, Hispanlab) and cultivated for 18 h at 37°C with shaking.

Inhibition studies: overnight cultures of the six serovars of *Salmonella* were grown in liquid medium and diluted into 50 ml of fresh sterile broth medium. Aqueous garlic extract was added at various concentrations directly to the flask and turbidity was monitored by measuring the optical density at 600 nm of the medium.

Preparation of aqueous garlic extracts (A. G. E.)

Fresh garlic cloves (70 g) were blended in 35 ml sterile distilled water, centrifuged at 5000 rpm and sterilised by filtration (0.45 µm). Aliquots were stored at - 20°C until required. The concentration of allicin in each preparation was determined spectrophotometrically by reaction with the thiol, 4 -mercaptopyridine (Miron et al., 2002). Briefly, varying quantities of garlic extract were incubated with 4 -mercaptopyridine (10^{-4} mM) in 50 mM phosphate buffer, 2 mM EDTA pH 7.2 which results in the formation of a mixed disulphide, 4 -allylmercaptothiopyridine and the consequent shift in absorbance at 324 nm was monitored.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determination by broth dilution

For MIC and MBC determinations, serial two-fold dilutions of aqueous garlic extract in 5 ml of broth inoculated with 50 µl of fresh precultures (inoculum, ~10^8 CFU/ml). The tubes were incubated at

37°C overnight with shaking and the highest dilution in which there were no growth was recorded as the MIC. For MBC testing, aliquots (20 µl) of broth from tubes containing no growth were plated onto solid medium and again incubated overnight at 37°C. The highest dilution in which there were no survivors was recorded as the MBC. In the above method, controls for each organism were performed using the sterile liquid medium without aqueous garlic extract. All MICs and MBCs were confirmed by triplicate assays.

Stability and heat inactivation of garlic extract

To assess the effects of storage on the antimicrobial activity in garlic extract an aliquot was kept at room temperature while another sample was stored at 4°C. Afterwards, the residual antibacterial activity was measured.

To study heat-inactivation, 1 ml aliquots of extract in 1.5 ml Eppendorf tubes were exposed to various temperatures for 10 min in a heating block, cooled to room temperature and 20 µl plated in the standard assay to assess the effect on antimicrobial activity.

Effect of garlic extract on some enzyme activity

The changes of different cellular enzymatic activities of treated or untreated bacteria, collected from the inhibition and the exponential growth phase culture, was evaluated using the API-ZYM system (BioMérieux). The cells were harvested, washed twice with cold 5 x 10^{-2} mol l^{-1} phosphate buffer (pH 7) and resuspended in 0.85% (w/v) sterile NaCl solution.

Each API-ZYM gallery was inoculated with two drops of adequate suspension of cells and incubated for 4 h at 28°C. The galleries were then activated by adding one drop of ZYM A and ZYM B reagents and after 5 min, values ranging from 0 to 5 in relation to the color developed in each enzymatic reaction, were visually assigned by means of the color chart supplied with the system.

RESULTS

Effect of aqueous garlic extract on *Salmonella* growth

The aqueous garlic extract (57.1% w/v), containing 324 µg/ml allicin) inhibited the growth and killed most of the tested *Salmonella* serovars. In order to study the effect of the A. G. E., different concentrations were tested. No effect on the different cell growth curves was observed when we added an A. G. E. to a final concentration less than 11 mg/ml (mean 89.1 µg/ml of allicin). So, we had studied the effect of garlic extract added to a final concentration, which range from 11 to 13 mg/ml.

Compared with the control cell suspensions without garlic extract, we observed a modification of the classical cell growth curves of the different *Salmonella* serovars. Figure 1 shows the basic phenomena observed in the inhibition of cell growth by aqueous garlic extract.

The addition of A. G. E. to these final concentrations at cell density of 0.05 induces the apparition of an inhibition phase in all the *Salmonella* growth curves. After this inhibition phase, cell growth resumed but at rate inferior to the control cell suspensions. In addition, cultures exposed to A. G.E. entered stationary phase at a cell density substantially lower than that of the control culture. This phenomenon was observed in all cases of A. G. E. treated *Salmonella*.

The effect of increasing the A. G. E. concentration on bacterial growth is indicated in Figure 1. As the aqueous garlic extract concentration was increased from 11 to 13 mg/ml (89.1 to 97.2 µg/ml of allicin), the duration of the inhibition phase increased, the rate of the growth after inhibition decreased and the cell density at which stationary phase was entered also decreased. The duration of the inhibition phase was proportional to the aqueous garlic extract concentration and variable according to the different *Salmonella* serovars tested.

The garlic extract added at these final concentrations had a bacteriostatic effect on *S. cerro*, *S. lindenburg*, *S. montevideo* and *S. hadar*, but the final concentration 13 mg/ml had a bactericide effect on *S. enteritidis* and *S. nikolaifleet*.

Examination of the effect of A. G. E. concentration on the different *Salmonella* serovars cell growth revealed that (i) the duration of inhibition varied from the different *Salmonella* serovars and it was clearly proportional to the amount of A. G. E. applied. A longer growth inhabitation phase was observed using increased A. G. E. concentration (Table 2). (ii) The resumed growth rate, expressed as a percentage of uninhibited growth rates, was proportional to A. G. E. concentration and it was different on the *Salmonella* serovars tested. The lowest resumed growth rate was observed in the case of *S. enteritidis* using 13 mg/ml of A. G. E. Our results demonstrate that the different *Salmonella* serovars did not present the same duration of inhibition and resumed growth rate. It seems that the sensibility to A. G. E. depend on *Salmonella* serovars.

In order to study the proportionality between the amount of active substance and diameter of inhibition zone, garlic extract (20 µl) or dilutions were applied directly to the surface of the plate or pipetted onto a stack of six, 5 mm diameter filter-paper discs cut with a hole-punch. The size of the inhibition halo was clearly proportional to the amount of A. G. E. applied and it showed a linear relationship when plotted against the log of the diameter of the inhibition zone (Figure 2b). This positive correlation was observed using the different *Salmonella* serovars.

Determination of MIC and MBC

Table 3 shows the MIC and MBC values obtained, expressed in terms of the garlic extract concentration and the deduced allicin concentration. The MIC values shown were determined by broth dilution. MBC values were mostly higher than MIC values, although they were occasionally identical. MICs range from 10 mg/ml garlic (estimated 8.1 µg/ml allicin) to 12.5 mg/ml garlic (estimated 10.1 µg/ml allicin). *S. lindenburg* present the lowest MIC value overall, whereas, *S. nikolaifleet* had the

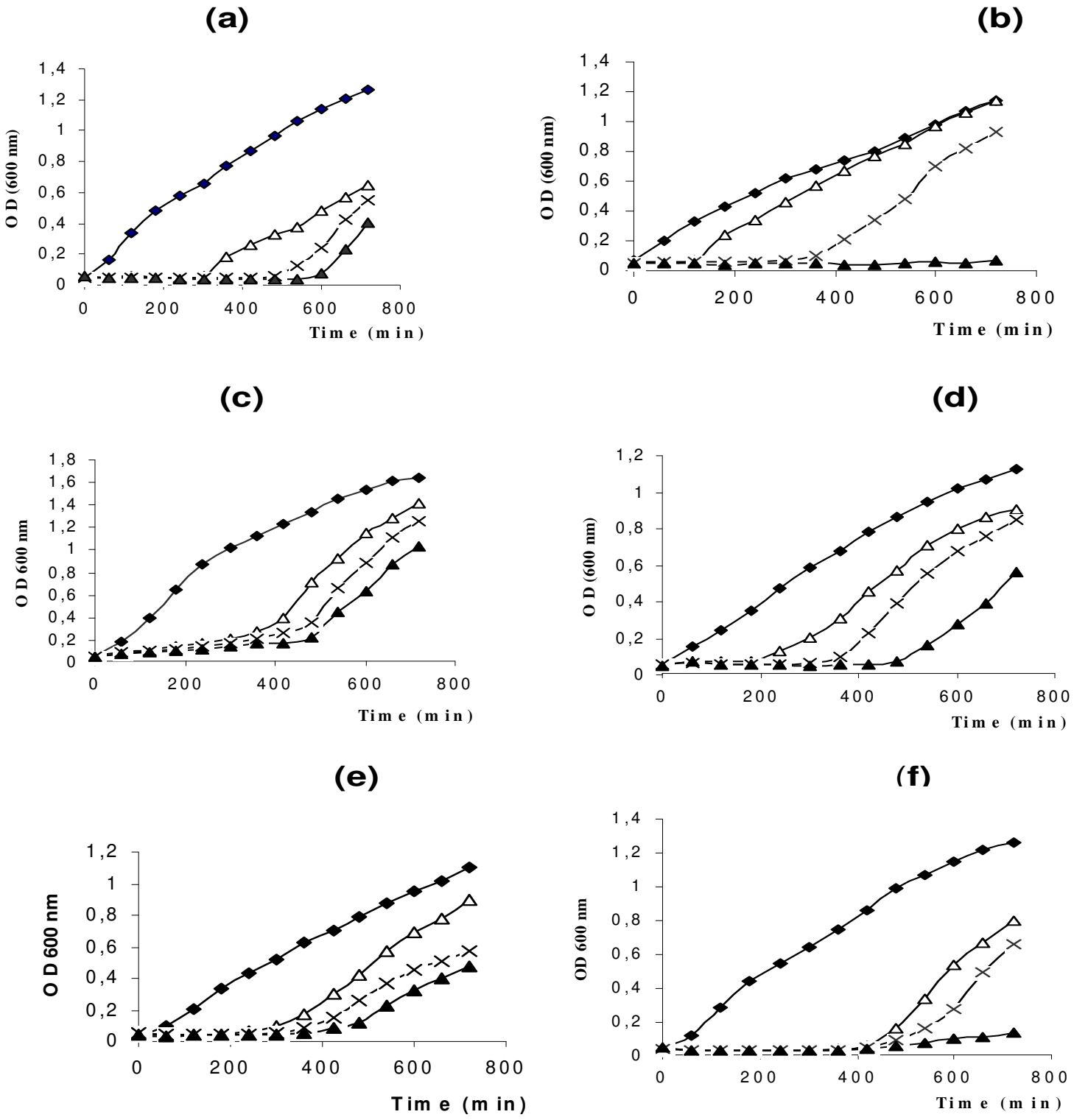

Figure 1. Growth curves of *Salmonella* serovars in liquid medium (Pronadisa, Hispanlab) at 37°C treated with different concentration of aqueous garlic extract. Aqueous garlic extract concentrations (mg/ml): (♦) 0; (▲) 11; (x) 12; (△) 13. **(a)** *Salmonella* cerro; **(b)** *Salmonella* enteritidis, **(c)** *Salmonella* lindenburg, **(d)** *Salmonella* montevideo, **(e)** *Salmonella* hadar and, **(f)** *Salmonella* nikolaifleet.

highest MIC of all *Salmonella* tested serovars.

The MBC values range from 13 mg/ml garlic (mean 10.5 µg/ml of allicin) to 15 mg/ml garlic (mean 12.1 µg/ml allicin). *S. nikolaifleet* present the lowest MBC value,

Table 2. Effect of A. G. E. concentration on the different *Salmonella* serovars cell growth.

Parameter	*Salmonella* serovars	11 mg/ml	12 mg/ml	13 mg/ml
Duration of inhibition (min)	*S. cerro*	360	540	600
	S. enteritidis	120	360	720
	S. lindenburg	240	364	480
	S. montevideo	480	480	600
	S. hadar	240	300	360
	S. nikolaifleet	480	480	600
Resumed growth rate (% of uninhibited rate)	*S. cerro*	51	44	32
	S. enteritidis	100	81	2.5
	S. lindenburg	74	69	46
	S. montevideo	87	77	63
	S. hadar	82	53	43
	S. nikolaifleet	63	53	11

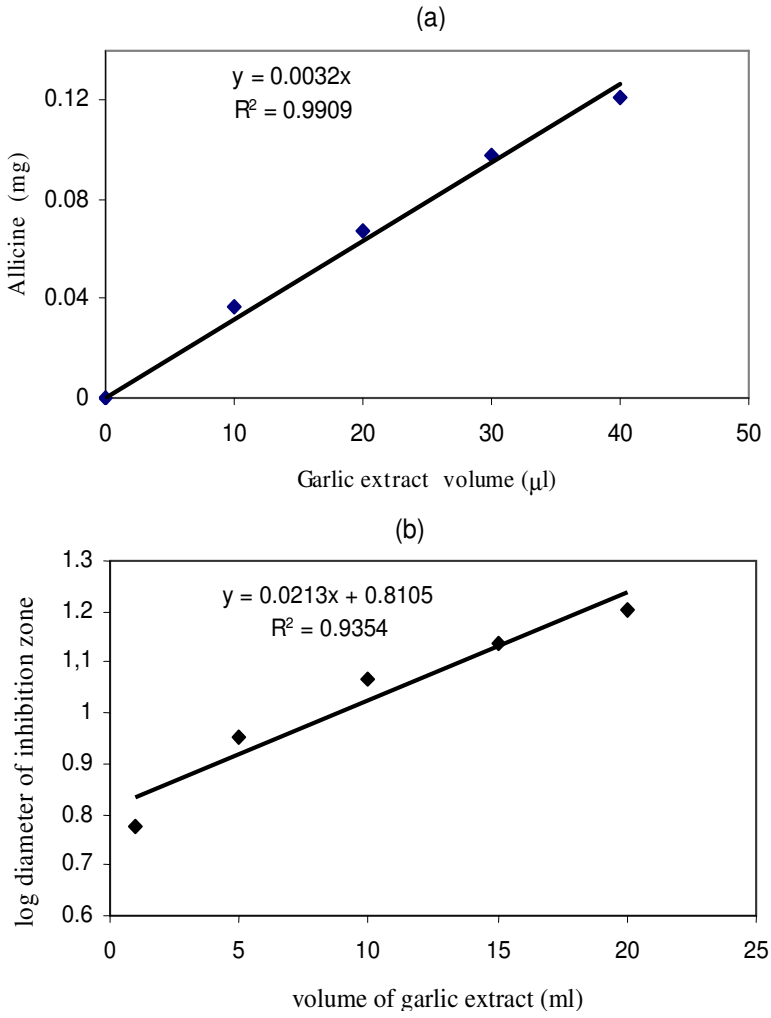

Figure 2. (a) Relationship curve between allicin amount aqueous and garlic extract volume. Reaction was done at room temperature in phosphate buffer, pH 7.2. **(b)** Regression plot of the log of the diameter of the inhibition zone against the volume of aqueous garlic extract. *S.* enteritidis-seeded Petri plate was used.

Table 3. Inhibitory effect (MIC and MBC) of garlic extract on the tested *Salmonella* serovars.

Salmonella serovar	MIC		MBC	
	A. G. E. (mg/ml)	Allicin (µg/ml)	A. G. E. (mg/ml)	Allicin (µg/ml)
S. hadar (287)	12	9.7	14	11.3
S. enteritidis (49)	11.5	9.3	13.5	10.9
S. lindenburg (320)	10	8.1	15	12.1
S. nikolaifleet (63)	12.5	10.1	13	10.5
S. montevideo (291)	11	8.9	14	11.3
S. montevideo (297)	12	9.7	14.5	11.7

whereas, *S. montevideo* had the highest MBC.

Stability of fresh aqueous garlic extract

Figure 3 shows the stability of A. G. E. on storage at 4°C and room temperature (22°C) changes with the storage time.

While the extract stored at 4°C was found to be relatively stable and even after 10 days still retained over 90% of its original inhibitory potential, the solution stored at 22°C lost the antibacterial activity slowly over a period of 6 to 10 days.

In order to study the effect of heat treatment, several A. G. E. samples were incubated for 10 min at different temperature (20, 40, 60, 80 and 100°C). Afterwards, the residual antibacterial activity was measured.

We had found that a decrease in the A. G. E. inhibition strength is correlated with the decrease in the allicin content in the extract stored both at 4 and 22°C (data not shown).

Figure 4 shows that the antibacterial activity was relatively stable at higher temperatures (less than 80°C).

However, the antibacterial activity was however lost at 100°C. This result suggests that the antibacterial activity against the different *Salmonella* serovars is due to allicin and other compounds such as the antimicrobial peptides, which could be present in the A. G. E. and stable at high temperature (80°C).

Biochemical changes of *Salmonella* serovars

In order to assess biochemical changes of *Salmonella*, induced by A. G. E. during the cell growth, several bacterial enzymatic activities were analyzed during the inhibition (treated bacteria) and the exponential phase cells culture (treated and untreated bacteria). Using API ZYM plates, 19 enzyme activities were tested, the results are summarised in the Figure 5. Compared with the untreated cells, we observed that some enzyme activities were maintained at low level, others were already induced and others inhibited.

Our results showed that six enzyme activities were affected during the inhibition phase of the different treated *Salmonella* serovars, such as: Esterase (C 4), Esterase lipase (C 8), Leucine arylamidase, Naphtol-AS-BI-phosphohydrolase, α-glucosidase and α-mannosidase.

S. cerro, *S. enteritidis*, *S. montevideo* and *S. hadar* α-glucosidase activity was total inhibited but in the case of *S. lindenburg* and *S. nikolaifleet* we observed that it was maintained at a low level.

During the exponential phase cell culture, treated bacteria present different enzymatic activities compared to the untreated cells. We observed that the α-mannosidase activity was already induced in all stressed cells (Figure 5); whereas, the α-glucosidase activity was inhibited in *S. lindenburg* and *S. montevideo.* These results showed that the different *Salmonella* serovars did not also present the same biochemical changes during the inhibition and the exponential phase cell culture.

DISCUSSION

With the emergence of antibiotic-resistant bacteria, it is reasonable to explore new sources of natural compounds with antimicrobial activity. Edible plants have been proven to be harmless and are economical.

In this study, a marked inhibitory effect of aqueous garlic extract against *Salmonella* serovars, which has also been described against other bacteria, was observed and confirmed. The phenomenon of inhibition is characterized by: i) an inhibition phase observed during the lag phase which appears longer than that observed in the cell growth control, the same result was described by Feldberg et al. (1988) and O' Gara et al. (2000); (ii) a resumed secondary logarithmic growth rate less than the original rate of growth. These observations confirm those described by Feldberg et al. (1988).

The duration of inhibition was proportional to the final aqueous garlic extract concentration, there was a simple linear relationship between inhibition activity and A. G. E. amount (mean allicin). A differential effect of garlic extract on *Salmonella* serovars was also reflected in the cell growth curves performed in this study.

After this inhibition phase, cells were able to overcome the inhibition and resume growth, which suggests that they were able to metabolize the allicin to non-inhibitory

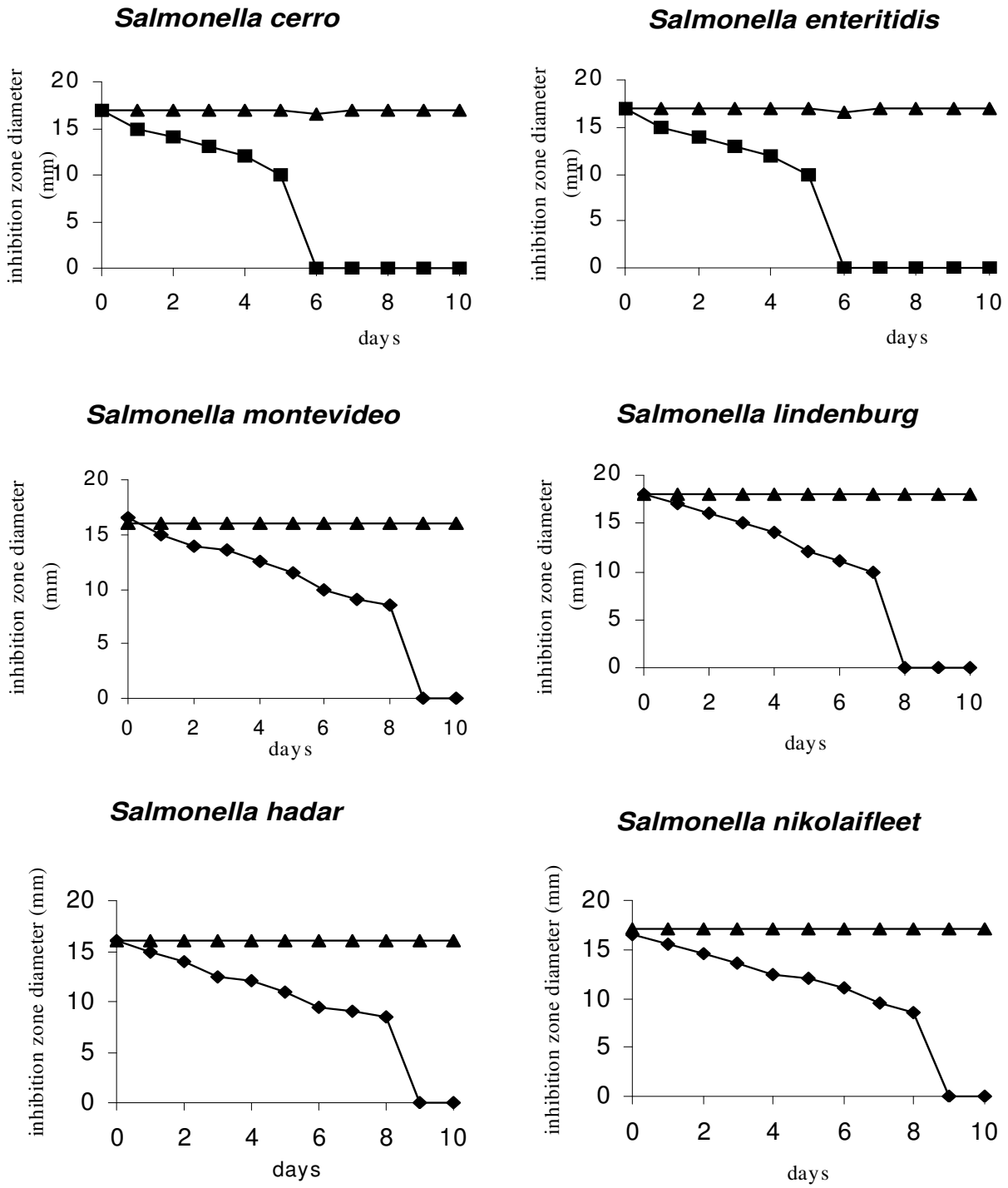

Figure 3. Antibacterial activity evolution of aqueous garlic extract during storage in the dark at room temperature (♦) or at 4°C (▲).

compound.

Although aqueous garlic extract at these concentrations is bacteriostatic rather than bacteriocidal, the observation that the resumed growth rate is substantially lower than the uninhibited-culture growth rate suggests that the inhibited cells are not totally able to recover from aqueous garlic extract inhibition. The lower secondary growth rate could indicate either the presence of some unrepaired lesion in the cells or the depletion of limiting nutrients from the medium during the inhibition phase.

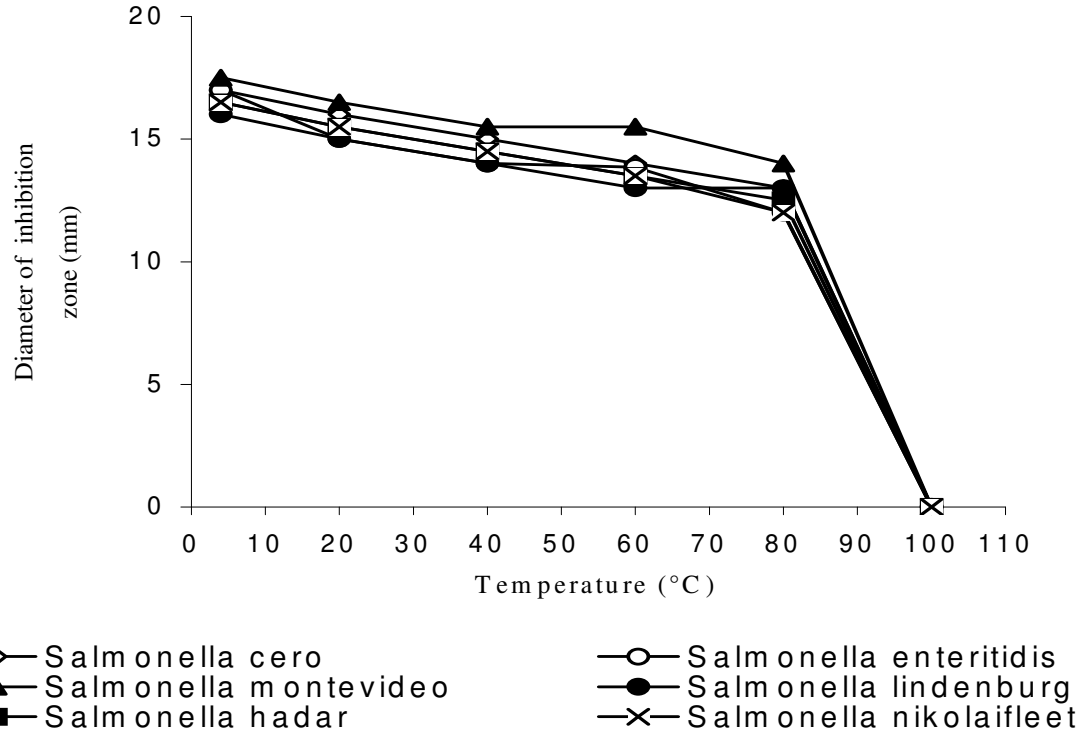

Figure 4. Effect on antibacterial activity in the *Salmonella* Petri plate test of heating garlic extract for 10 min at various temperatures.

Cellini et al. (1996) demonstrated that aqueous garlic extract effectively inhibited sixteen clinical isolates and three reference stains of *H. pylori* (Uchida et al., 1975, Chowdhury et al., 1991) also investigated the ability of garlic to inhibit antibiotic-resistant strains of bacteria. The *in vitro* MIC of garlic extract was 5 µl/ml and was affective against *Shigilla dysenteriae*, *Shigilla flexneri, Shigilla sonnei* and *Escherichia coli*. Multiple resistant strains of bacteria were used by Singh et al. (1984) to investigate garlic's antibiotic potential. They found that garlic was more effective than any of the test antibiotics (penicillin, ampicillin, doxycycline, streptomycin and cephalexin) against clinical strains of *Staphylococcus, Echerichia, proteus, Pseudomonas* and *Klebsiella* bacteria.

The mechanism of bacterial inhibition by allicin was investigated by Feldberg et al. (1988). They reported a typical cycle of inhibition: initially, there was a lag time of approximately 15 min between addition of allicin and onset of inhibition; then there was a "transitory inhibition phase" whose duration was directly proportional to the allicin concentration and inversely proportional to the culture density; this was followed by a resumed growth phase reached a stationary phase at a lower culture density than the uninhibited controls.

Feldberg et al. (1988) reported that the *in vitro* mechanism of *Salmonella typhimirium* inhibition growth by allicin at bacteriostatic concentrations (0.2 to 0.5 mM) was found to be due to a delayed and partial inhibition of DNA and protein synthesis and an immediate inhibition of RNA synthesis, suggesting that this is the primary target of allicin action.

The mechanism responsible for all these activities is believed to be allicin and its chemical reaction with thiol groups of various enzymes (Focke et al., 1990). It is of interest that cooked garlic and commercially available garlic tablets lost the antibacterial effect found in raw garlic.

The values of MIC and MBC were very close to those described in literature for other Gram-negative stains but they are lower than the Gram-positive stains (Small et al., 1947).

A good proportionality was observed firstly between allicin amounts and the A. G. E. volumes (Figure 2a) and secondly, between the diameter of inhibition zone and the A. G. E. volumes (Figure 2b).

This antibacterial activity diminution observed during room temperature of A. G. E. conservation seems to be due to the allicin instability and its transformation with time into more stable components: polysulfides and thiosulphonates (Cellini et al., 1996). Garlic extract could be stored at 4°C as our result showed that there was no detectable loss of activity at this temperature over several days (Figure 3). However, excessive warming or longer periods at higher temperatures should be avoided (Figure 4).

This heat stability would be a very useful characteristic if the antimicrobial peptides were to be used as a food preservative, because many food-processing procedures

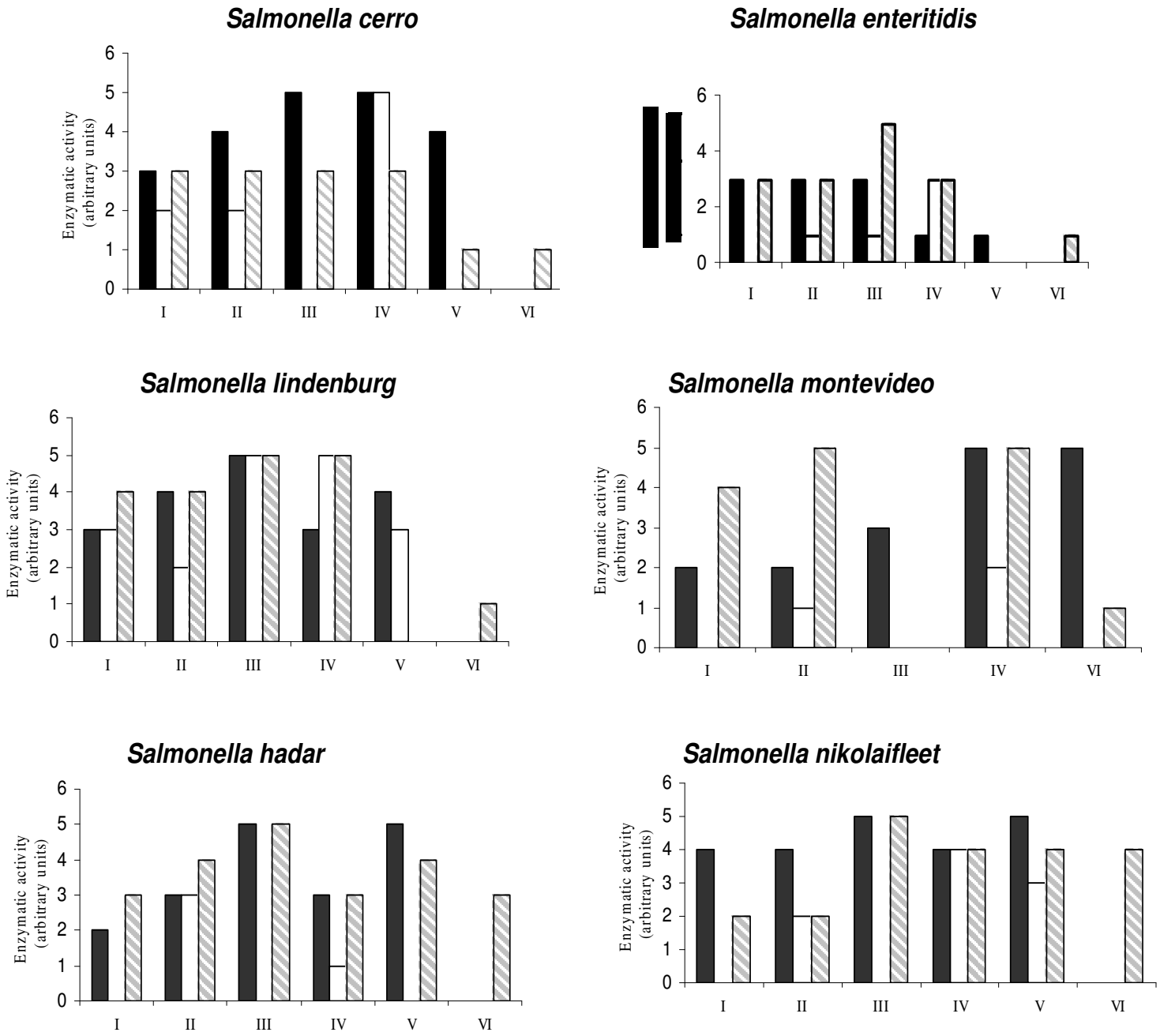

Figure 5. Changes in some enzymes activity levels of *Salmonella* cells during A. G. E. treatment (12 mg/ml). **I:** Esterase (C 4); **II:** Esterase lipase (C 8), **III:** Leucine arylamidase, **IV:** Naphtol-AS-BI-phosphohydrolase, **V:** α-glucosidase and, **VI:** α-mannosidase. (■) Control bacteria; (□) Treated bacteria (inhibition phase); (□) Treated bacteria (exponential phase).

involve a heating step. It seems that garlic extract contain antibacterial peptides or proteins, which exhibit an antibacterial activity stable at a high temperature less than 80°C. These antimicrobial peptides have a broad antimicrobial spectrum, including Gram-positive and Gram-negative bacteria and they are produced by plants to protect themselves from pathogen invasion (Singh and Shukla, 1984).

Several antimicrobial agents were isolated from plant including secondary metabolites as essential oil and terenoides, amongst which can be cited xanthones, benzophenones, coumarins and flavonoids (Nkengfack et al., 2002; Komgeum et al., 2005).

Recently, there has been increasing interest in discovering new natural antimicrobials. This has also been true in food microbiology. Plant products with antimicrobial properties have notably obtained emphasis for possible application in food production in order to

prevent bacterial and fungal growth (Bakri and Douglas, 2005; Block, 1992). Much focus on determining the antimicrobial activity of plant extracts is found in folk medicine (Zasloff, 2002).

Our results demonstrated that garlic has, by far, the highest antibacterial activity among the common vegetables and fruits. Furthermore, it is active against a large spectrum of bacteria. Garlic has long been known to have antibacterial activity from *in vitro* and *in vivo* (Reuter, 1995; Fattouch et al., 2007). It is effective against *H. pylori* (Lanciotti et al., 2004), a bacteria associated with peptic ulcer disease and gastric cancer. Garlic has synergic effect with omeprazole against *H. pylori* (Rios and Recio, 2005) and with streptomycin or chloramphenicol against *M. tuberculosis* (Sivam, 2001). It has been shown to prevent the formation of *Staphylococcus* enterotoxin (Jonkers et al., 1999) which causes food poisoning. Garlic also has antifungal (Gupta and Viswanathan, 1955; Gonzalez-Fandos et al., 1994), antiviral and antiparasitic properties (Yoshida et al., 1987, Yamada Y. and Azuma K., 1977), Tsai et al., 1985. It seems to be safe agents that have the potential for broader applications to take advantage of its antibacterial activities. It is suggested that garlic, as a natural herb, could be used to extend the shelf-life of meat products, providing the consumer with food containing natural additives, which might be seen more healthful than those of synthetic origin.

To sum up, the reported results shed new light on the behavior of pathogenic Gram-negative bacteria under A. G. E. stress. This antibacterial agent triggers several biological and biochemical changes enabling *Salmonella* to escape the deleterious effects of garlic.

Moreover, the natural resistance of such cells to garlic compounds is unknown. Work is in progress in our laboratory to determine the mechanisms of garlic toxicity and to identify molecules involved in these adaptive responses.

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