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ORIGINAL ARTICLE

Initial *in vitro* evaluations of the antibacterial activities of glucosinolate enzymatic hydrolysis products against plant pathogenic bacteria

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Keywords
antibacterial activity, glucosinolates, isothiocyanates, phytochemicals, phytopathogenic bacteria.

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Abstract

Aims: The aim of the study was to evaluate the *in vitro* antibacterial effects of glucosinolate hydrolysis products (GHP) against plant pathogenic micro-organisms namely *Agrobacterium tumefaciens*, *Erwinia chrysanthemi*, *Pseudomonas cichorii*, *Pseudomonas tomato*, *Xanthomonas campestris* and *Xanthomonas juglandis*.

Methods and Results: Using a disc diffusion assay, seven different doses of 10 GHP were tested against each bacteria. The results showed that the isothiocyanates were potent antibacterials, whilst the other GHP were much less efficient. Moreover, the antibacterial effects were dose-dependent, increasing with the dose applied; 2-phenylethylisothiocyanate and sulforaphane showed the strongest inhibitory effects. The overall results show a great potential for using the isothiocyanates as an alternative tool to control undesired bacterial growth in plants.

Conclusions: Glucosinolate hydrolysis products and more specifically the isothiocyanates: benzylisothiocyanate, 2-phenylethylisothiocyanate, the isothiocyanate Mix and sulforaphane, were effective phytochemicals against the *in vitro* growth of the phytopathogenic bacteria. The antibacterial activity exhibited by these phytochemicals reinforces their potential as alternatives to the traditional chemical control of phytopathogenic bacteria.

Significance and Impact of the Study: This current *in vitro* study is the first providing comparative data on GHP as potential control agents for plant pathogenic bacteria. However, more studies are needed to determine their possible allelopathic impacts e.g. inhibition of plant growth and negative effects on beneficial soil bacteria and fungi (mycorrhizae).

Introduction

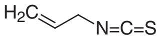
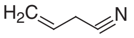
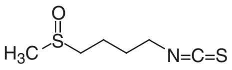
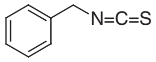
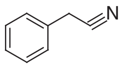
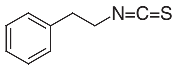
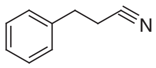
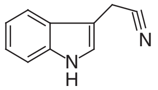
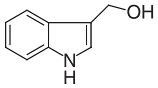
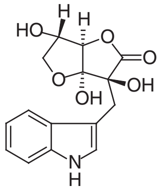
Glucosinolates are a group of organic anions containing β-D-thioglucose and sulfonated oxime moieties. These phytochemicals (plant secondary metabolites) are commonly present in Brassicaceae (Syn. Cruciferae) plants, comprising at least 120 compounds with well-defined structures (Grubb and Abel 2006; Halkier and Gershenzon 2006). They are biosynthesized from amino acids and the biosynthesis pathways have been elucidated by biochemical and genetics approaches using *Arabidopsis* plants (Grubb and Abel 2006; Halkier and Gershenzon

2006). It comprises amino acid side-chain elongation, oxidative decarboxylation, conversion into basic structure and secondary modifications (Kroymann *et al.* 2001, 2003; Textor *et al.* 2007). They are one of the main classes of secondary metabolites found in cruciferous crops, based on a core structure with side chain modifications (radical group – R) fitting into three basic groups (aliphatic, aromatic or indolyl), strictly related to the amino acid from which the glucosinolate is derived (Fenwick *et al.* 1983; Mithen 2001). The type and concentration of glucosinolates have been found to vary between *Brassica* species as well as between cultivars of the same species (Kushad *et al.* 1999; Rangkadilok *et al.* 2002).

Present in many members of the order *Capparales*, including important crops (e.g. oil seed rape, broccoli and cabbage), glucosinolates co-exist with myrosinase enzyme (thioglucoside glucohydrolase, EC 3.2.3.1), which is responsible for glucosinolate hydrolysis, when direct contact occurs (Fenwick *et al.* 1983; Rosa 1999; Yan and Chen 2007).

The hydrolysis products of glucosinolates include isothiocyanates, nitriles, epithionitriles and thiocyanates. The type of compounds produced are specific to the

Table 1 Chemical structures of the glucosinolate hydrolysis products (GHP) used in *in vitro* assays

Class	Precursor glucosinolate	GHP structure	Abbreviation
Aliphatic	Sinigrin	Allylisothiocyanate 	AITC
		Allyl cyanide 	ACN
	Glucoraphanin	Sulforaphane 	SFN
Aromatic	Glucotropaeolin	Benzylisothiocyanate 	BITC
		Benzyl cyanide 	BCN
	Gluconasturtiin	2-Phenylethylisothiocyanate 	PEITC
		2-Phenylethyl cyanide 	PCN
Indolyl	Glucobrassicin	Indole-3-acetonitrile 	IAN
		Indole-3-carbinol 	I3C
		Ascorbigen 	ASC
Mixture		Mixture of ITCs (AITC + BITC + PEITC)	ITC Mix

respective glucosinolates present in the tissue and conditions under which hydrolysis occurs (Underhill 1980; Larsen 1981; Fahey *et al.* 2001; Bones and Rossiter 2006). The difference in terms of chemical properties and biological activity between glucosinolates and their hydrolysed products is largely determined by the side-chain structure. Among the most common and predominant glucosinolate hydrolysis products (GHP) are isothiocyanates (ITCs), also recognized as major inhibitors of microbial activity (Wallsgrove *et al.* 1998; O′Callaghan *et al.* 2000). The *in vitro* experiments (Potter *et al.* 1998) have also shown that high levels of ITCs can be effective in the suppression of soil-borne plant pathogens (Brown and Morra 1997). Moreover, because of their general toxicity and volatility, GHP can play an important role in plant – pathogen interactions (Giamoustaris and Mithen 1995; Rask *et al.* 2000; Barth and Jander 2006).

In recent years, several studies have been conducted with a large number of compounds from different plants in order to investigate their antimicrobial properties against plant pathogenic micro-organisms (Daferera *et al.* 2003; Curtis *et al.* 2004; Vasinauskiene *et al.* 2006; Tabanca *et al.* 2007; Ozturk and Ercisli 2007). However, few studies have been conducted with GHP, particularly those with antibacterial potential (Ludwig-Müller 2008; Kowalska and Urszula Smolińska 2008). Thus in the present study the *in vitro* antibacterial activity of GHP at seven different doses (0·0, 0·015, 0·15, 0·75, 1·5, 3·0 and 15·0 μmoles) was tested against six relevant plant pathogenic Gram-negative bacteria: *Agrobacterium tumefaciens*, *Erwinia chrysanthemi*, *Pseudomonas cichorii*, *Pseudomonas tomato*, *Xanthomonas campestris* and *Xanthomonas juglandis*.

Materials and methods

Glucosinolate hydrolysis products

The GHP used in the *in vitro* assays are presented in Table 1. The ITCs (allyl-, benzyl-, 2-phenylthyl-), the nitriles/cyanides (allyl-, benzyl-, 2-phenylethyl-, indole-3-acetonitrile), and the amines (allyl-, benzyl-, 2-phenylethyl-) and indole-3-carbinol were obtained from Sigma-Aldrich. DL-sulforaphane was obtained from LKT Labs (St Paul, MN). Ascorbigen (ASC) was synthesized from indole-3-carbinol and indole-3-carbinol/ascorbic acid according to previously published methods (Agerbirk *et al.* 1996, 1998), and the purity was confirmed by HPLC as >98 %. The ITC Mix was produced by dissolving AITC (allylisothiocyanate), BITC (benzylisothiocyanate) and PEITC (2-phenylethylisothiocyanate) at the same concentrations directly in dimethyl sulfoxide (DMSO) (Sigma-Adrich). Each compound was tested at 0·0, 0·015, 0·15, 0·75, 1·50 and 3·0 and 15·0 μmoles with the exception of sulforaphane (SFN) which was only tested up to 3·0 μmoles.

Plant pathogenic bacteria strains

The plant pathogenic bacteria isolates used in this study were the Gram-negative bacteria *A. tumefaciens*, *E. chrysanthemi*, *P. cichorii*, *P. tomato*, *X. juglandis*, and *X. campestris* provided by Dr António Monteiro, from Instituto Superior de Agronomia (ISA), Universidade Técnica de Lisboa, Portugal.

Antibacterial activity assessment

Colonies of bacteria were picked from overnight cultures in PCA solid medium, inoculated into 4·0 ml of 0·9% NaCl solution. The suspensions were prepared by adjusting the turbidity to match 0·5 McFarland standards. Antibacterial activity was tested using a modification of the disc diffusion method originally described by Bauer *et al.* (1966). A loop of bacteria from the agar-slant stock was cultured in nutrient broth overnight and spread with a sterile cotton swab into Petri dishes (90 mm of diameter) containing 20 ml of Mueller–Hinton Agar (Oxoid). Sterile filtre paper discs (6 mm in diameter) (Oxoid) impregnated with 15 μl of the GHP were placed on the agar plate seeded with respective micro-organism, and the plates were incubated in an inverted position overnight at 37°C. The equivalent volume of solvent without extracts served as negative control. Gentamicin (10 μg disc^{-1}) (Oxoid) was used as positive control. After overnight incubation, the diameter in mm of the inhibitory or clear zones around the disc was recorded.

All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced.

Antibacterial activity classification

The antibacterial effects of the tested GHP were classified according to the following scheme: noneffective (−) – inhibition halo = 0; moderate efficacy (+) – $0 <$ inhibition halo $<$ antibiotic inhibition halo; good efficacy (++) – antibiotic inhibition halo $<$ inhibition halo $< 2 \times$ inhibition halo; strong efficacy (+++) – inhibition halo $> 2 \times$ antibiotic inhibition halo.

Statistical analysis

All experiments were performed in triplicate. The data were analysed using one-way ANOVA. The differences

Table 2 Antimicrobial activity of GHP against phythopatogenic bacteria observed by the disc diffusion assay. The mean (mm) ± SD for at least three replicates is illustrated

		Dose applied (μmoles)				
Bacteria strain	Phytochemicals	0·15	0·75	1·5	3·0	15·0
Agrobacterium tumefaciens	AITC	n.d.	n.d.	n.d.	n.d.	9·7 ± 1·2
	ACN	n.d.	n.d.	n.d.	n.d.	n.d.
	SFN	9·7 ± 0·3	14·7 ± 0·3	16·3 ± 0·3	19·3 ± 0·3	n.t.
	BITC	n.d.	7·3 ± 0·3	8·7 ± 0·3	10·0 ± 0·0	12·3 ± 0·3
	BCN	n.d.	n.d.	n.d.	n.d.	n.d.
	PEITC	n.d.	n.d.	n.d.	n.d.	n.d.
	PCN	n.d.	n.d.	n.d.	n.d.	10·7 ± 0·9
	IAN	n.d.	n.d.	n.d.	9·0 ± 0·6	14·7 ± 0·7
	I3C	n.d.	n.d.	n.d.	n.d.	15·7 ± 0·3
	ASC	n.d.	n.d.	n.d.	n.d.	n.d.
	ITC Mix	n.d.	n.d.	8·0 ± 0·0	9·0 ± 0·0	23·0 ± 6·0
Erwinia chrysantemi	AITC	n.d.	n.d.	n.d.	8·3 ± 4·2	t.i
	ACN	n.d.	n.d.	n.d.	n.d.	n.d.
	SFN	9·3 ± 0·3	14·3 ± 0·3	18·3 ± 0·9	22·7 ± 0·9	n.t.
	BITC	28·7 ± 0·7	54·0 ± 4·0	51·3 ± 0·9	56·7 ± 2·0	t.i.
	BCN	n.d.	n.d.	n.d.	n.d.	8·3 ± 0·7
	PEITC	19·3 ± 0·7	28·7 ± 1·3	34·0 ± 1·2	35·7 ± 0·3	36·7 ± 0·7
	PCN	n.d.	n.d.	n.d.	7·7 ± 0·3	20·0 ± 1·2
	IAN	n.d.	14·3 ± 1·2	22·0 ± 1·2	30·7 ± 0·7	34·0 ± 0·0
	I3C	n.d.	n.d.	n.d.	8·7 ± 0·3	16·3 ± 0·3
	ASC	n.d.	n.d.	n.d.	8·0 ± 0·0	12·3 ± 0·7
	ITC Mix	22·7 ± 0·3	42·0 ± 1·7	46·0 ± 1·2	t.i.	t.i.
Pseudomonas cichorii	AITC	n.d.	n.d.	n.d.	7·0 ± 0·0	t.i.
	ACN	n.d.	n.d.	n.d.	n.d.	n.d.
	SFN	19·3 ± 0·7	31·3 ± 0·7	37·3 ± 0·7	44·0 ± 0·0	n.t.
	BITC	7·0 ± 0·0	t.i.	t.i.	t.i.	t.i.
	BCN	n.d.	n.d.	n.d.	7·0 ± 0·0	18·7 ± 0·7
	PEITC	n.d.	19·3 ± 0·7	23·7 ± 0·3	26·3 ± 0·9	31·3 ± 0·7
	PCN	n.d.	n.d.	7·0 ± 0·0	14·0 ± 0·0	28·7 ± 0·7
	IAN	n.d.	13·0 ± 0·6	24·0 ± 1·2	30·7 ± 1·8	34·7 ± 0·7
	I3C	n.d.	n.d.	n.d.	12·7 ± 0·7	27·3 ± 0·7
	ASC	n.d.	n.d.	n.d.	10·3 ± 0·9	18·7 ± 0·7
	ITC Mix	11·0 ± 0·6	53·0 ± 0·6	t.i.	t.i.	t.i.
Pseudomonas tomato	AITC	n.d.	n.d.	7·0 ± 0·0	8·3 ± 0·7	t.i.
	ACN	n.d.	n.d.	n.d.	n.d.	n.d.
	SFN	7·7 ± 0·3	11·3 ± 0·3	26·7 ± 0·7	34·7 ± 1·3	n.t.
	BITC	10·3 ± 0·3	30·0 ± 1·2	33·3 ± 0·3	39·7 ± 1·2	t.i.
	BCN	n.d.	n.d.	n.d.	7·0 ± 0·0	9·3 ± 0·3
	PEITC	7·3 ± 0·3	9·7 ± 0·3	11·3 ± 0·3	13·0 ± 0·6	13·7 ± 0·3
	PCN	n.d.	n.d.	n.d.	n.d.	16·3 ± 0·3
	IAN	n.d.	n.d.	10·0 ± 0·0	18·7 ± 1·3	24·7 ± 0·7
	I3C	n.d.	n.d.	n.d.	9·7 ± 0·3	22·7 ± 0·9
	ASC	n.d.	n.d.	n.d.	7·0 ± 0·0	10·0 ± 0·0
	ITC Mix	10·0 ± 0·6	25·0 ± 0·6	t.i.	t.i.	t.i.
Xanthomonas campestris	AITC	n.d.	n.d.	7·0 ± 0·0	t.i	t.i.
	ACN	n.d.	n.d.	n.d.	n.d.	n.d.
	SFN	8·0 ± 0·0	13·7 ± 0·3	26·7 ± 0·7	33·3 ± 0·7	n.t.
	BITC	n.d.	30·3 ± 1·5	38·7 ± 1·7	t.i.	t.i
	BCN	n.d.	n.d.	n.d.	n.d.	21·0 ± 0·6
	PEITC	9·0 ± 0·6	13·7 ± 0·7	16·0 ± 1·2	17·7 ± 0·9	21·7 ± 0·3
	PCN	n.d.	n.d.	n.d.	7·3 ± 0·3	19·3 ± 0·3
	IAN	n.d.	8·3 ± 0·3	13·7 ± 0·3	23·3 ± 0·7	31·3 ± 0·7
	I3C	n.d.	8·7 ± 0·3	15·3 ± 0·3	28·0 ± 0·0	35·3 ± 0·7

Table 2 (Continued)

Bacteria strain	Phytochemicals	Dose applied (μmoles)				
		0·15	0·75	1·5	3·0	15·0
Xanthomonas campestris	ASC	n.d.	n.d.	n.d.	7·3 ± 0·3	14·7 ± 0·7
	ITC Mix	16·7 ± 0·3	31·7 ± 0·3	46·7 ± 0·3	t.i	t.i.
Xanthomonas juglandis	AITC	n.d.	n.d.	11·7 ± 0·3	t.i.	t.i.
	ACN	n.d.	n.d.	n.d.	n.d.	n.d.
	SFN	n.d.	8·7 ± 0·3	9·7 ± 0·7	14·7 ± 0·3	n. t.
	BITC	n.d.	n.d.	10·0 ± 0·0	11·7 ± 0·3	43·7 ± 1·5
	BCN	n.d.	n.d.	n.d.	7·3 ± 0·6	11·3 ± 0·7
	PEITC	n.d.	n.d.	n.d.	n.d.	7·3 ± 0·3
	PCN	n.d.	n.d.	n.d.	n.d.	7·7 ± 4·6
	IAN	n.d.	n.d.	7·0 ± 0·0	8·3 ± 0·3	13·7 ± 0·3
	I3C	n.d.	n.d.	7·3 ± 0·3	8·3 ± 0·3	13·0 ± 0·0
	ASC	n.d.	n.d.	n.d.	n.d.	n.d.
	ITC Mix	7·0 ± 0·0	11·0 ± 0·6	12·3 ± 6·2	t.i.	t.i.

GHP, glucosinolate hydrolysis products; n.d., antibacterial activity not detected; t.i., total inhibition; n.t., not tested.

Table 3 Antibacterial activity of gentamicin

Bacteria	Diameter of inhibition zone (mm)*
Agrobacterium tumefaciens	18·0 ± 0·0
Erwinia chrysantemi	26·7 ± 0·9
Pseudomonas cichorii	18·3 ± 0·9
Pseudomonas tomato	29·7 ± 1·2
Xanthomonas campestris	20·3 ± 0·3
Xanthomonas juglandis	23·7 ± 0·9

*Average levels ± SEM of three replicates.

between the mean values were separated at Duncan's Comparison test. The results were presented as the Mean ± SEM. Significance level for the separation was set at $P < 0·05$. Statistical analyses were performed using the statistical program of SUPER ANOVA ver. 1.11 software (Abacus Concepts, Berkeley, CA, USA).

Results

The tested GHP have differential effects on *in vitro* bacterial growth, although the effects were predominantly positive, e.g. they inhibited bacterial growth. Only ACN had no antibacterial effect (Table 2). The GHP antibacterial effect was dependent on the chemical structure and, generally, proportional to the dose applied (Table 2). The negative control (only DMSO) had no effect on *in vitro* bacterial growth. Moreover, the GHP at 0·015 μmoles dose were only inhibitory against *E. chrysanthemi* for the compounds BITC and PEITC with a moderate antibacterial activity for both cases (inhibition halo of 8·7 ± 0·3 mm and 8·0 ± 0·0 mm for BITC and PEITC respectively).

The GHP antibacterial effect was strongly dependent on the dose applied ($P < 0·05$). BITC, PEITC, ITC Mix and SFN were the compounds with the strongest dose-dependent effect ($P < 0·05$). Although a dose of 15·0 μmoles for SFN was not tested, the results obtained for other compounds gave us an idea that for this dose and this compound, the antibacterial effect could be stronger. The application of AITC showed a discrete antibacterial effect, only effective at higher doses (3·0 and 15·0 μmoles) and not effective against all the bacteria. However, for the highest dose, when showing antibacterial action, it was very evident, and in most of the cases the bacteria were unable to grow (Table 2).

Comparing the antibacterial effect of GHP with those obtained with gentamicin application (Table 3), it was found that some GHP were more efficient than the antibiotic. This was true for the compounds BITC, ITC Mix, PEITC and SFN, particularly for *P. cichorii*, *X. campestris* and *E. chrysanthemi*. In fact, some of the GHP had a higher antibacterial (strong efficacy) than that of gentamicin, as seen by the two times larger inhibition zone diameters. Strong antibacterial effects were seen with: AITC at doses ≥3·0 μmoles (*X. juglandis* and *X. campestris*) and 15·0 μmoles (*P. cichorii*, *P. tomato* and *E. chrysantemi*); BITC at doses ≥1·5 (*P. cichorii*), 3·0 μmoles (*E. chrysantemi*, *X. juglandis* and *X. campestris*) and 15·0 μmoles (*P. tomato*); ITC Mix at doses ≥0·75 μmoles (*P. cichorii*), 1·5 (*P. tomato*) and 3·0 μmoles (*X. juglandis*, *X. campestris* and *E. chrysantemi*); SFN at doses ≥1·5 μmoles (*P. cichorii*) (Table 4). The most tolerant bacteria to both antibiotic (Table 3) and GHP (Table 4) was *A. tumefaciens*. For this bacterium, only the ITC Mix at 15·0 μmoles and SFN at 3·0 μmoles were more efficient than Gentamicin, and only with moderate effects (Table 4).

If we assemble the GHP into different chemical classes (aliphatic, aromatic and indole) (Table 1) we noted that

Table 4 Classification of GHP antibacterial activity

		Dose applied (μmoles)				
Bacteria	Phytochemicals	0·15	0·75	1·5	3·0	15·0
Pseudomonas cichorii	AITC	–	–	–	+	+++
	ACN	–	–	–	–	–
	SFN	+	++	+++	+++	n.t.
	BITC	+	+++	+++	+++	+++
	BCN	–	–	–	+	++
	PEITC	–	++	++	++	++
	PCN	–	–	+	+	++
	IAN	–	+	++	++	+++
	I3C	–	–	–	+	++
	ASC	–	–	–	+	++
	ITC Mix	+	+++	+++	+++	+++
Pseudomonas tomato	AITC	–	–	+	+	+++
	ACN	–	–	–	–	–
	SFN	+	+	+	++	n.t.
	BITC	+	++	++	++	+++
	BCN	–	–	–	+	+
	PEITC	+	+	+	+	+
	PCN	–	–	–	–	+
	IAN	–	–	+	+	+
	I3C	–	–	–	+	+
	ASC	–	–	–	+	+
	ITC Mix	+	+	+++	+++	+++
Xanthomonas juglandis	AITC	–	–	+	+++	+++
	ACN	–	–	–	–	–
	SFN	–	+	+	+	n.t.
	BITC	–	–	+	+	+++
	BCN	–	–	–	+	+
	PEITC	–	–	–	–	+
	PCN	–	–	–	–	+
	IAN	–	–	+	+	+
	I3C	–	–	+	+	+
	ASC	–	–	–	–	–
	ITC Mix	+	+	+	+++	+++
Xanthomonas campestris	AITC	–	–	+	+++	+++
	ACN	–	–	–	–	–
	SFN	+	+	++	++	n.t.
	BITC	–	++	++	+++	+++
	BCN	–	–	–	–	+
	PEITC	+	+	+	+	+
	PCN	–	–	–	+	+
	IAN	–	+	+	+	++
	I3C	–	+	+	++	++
	ASC	–	–	–	+	+
	ITC Mix	+	++	++	+++	+++
Agrobacterium tumefaciens	AITC	–	–	–	–	+
	ACN	–	–	–	–	–
	SFN	+	+	+	++	n.t.
	BITC	–	+	+	+	+
	BCN	–	–	–	–	–
	PEITC	–	–	–	–	–
	PCN	–	–	–	–	+
	IAN	–	–	–	+	+
	I3C	–	–	–	–	+
	ASC	–	–	–	–	–
	ITC Mix	–	–	+	+	++
Erwinia chrysantemi	AITC	–	–	–	+	+++
	ACN	–	–	–	–	–
	SFN	+	–	+	+	n.t.
	BITC	++	++	++	+++	+++
	BCN	–	–	–	–	+
	PEITC	+	++	++	++	++
	PCN	–	–	–	+	+
	IAN	–	+	+	++	++
	I3C	–	–	–	+	+
	ASC	–	–	–	+	+
	ITC Mix	+	++	++	+++	+++

GHP, glucosinolate hydrolysis products; –, noneffective; +, moderate efficacy; ++, good efficacy; +++, strong efficacy; n.t., not tested.

aliphatic group (AITC, ACN, SFN) had a lower effect, when compared with the aromatic group (aryl and indole GHP; BITC, BCN, PEITC, PCN, IAN, I3C, ASC and ITC mix), despite the strong effect of SFN, which shows that the GHP chemical structure is clearly related to the antibacterial effectiveness.

Discussion

In recent years the research for new techniques and new strategies for plant disease management led to the development of studies with plant pathogen antagonists (Johnson and Dileone 1999; Bashan and de-Bashan 2002; Aysan *et al.* 2003). Increasing attention is given to glucosinolates and their enzymatic derivatives, because of their control activity against several plant pathogens, insects and nematodes (O'Callaghan *et al.* 2000; Buskov *et al.* 2002; Serra *et al.* 2002). However, the research on the potentials of these phytochemical antibacterials is scarce. Hogge *et al.* (1988) stated that the only known potential source of constitutive antimicrobial components from *Arabidopsis* is a group of sulfur-containing glucosides termed glucosinolates. Upon tissue damage, they are converted by an endogenous thioglucosidase into breakdown products, some of which are effective against some micro-organisms (Mithen *et al.* 1986; Manici *et al.* 1997). Li *et al.* (1999) founded a correlation between the increasing resistance of *Brassica napus* against *Sclerotinia sclerotiorum* and the levels of indole glucosinolates. More recently, Tierens *et al.* (2001) detected in a noninfected *Arabidopsis* species, one antimicrobial component, 4-methylsulinylbutyl isothiocyanate, which also has been described previously as an antimicrobial agent (Dornberger *et al.* 1975). However, several researchers stated that these correlations are uncertain and the mode of action of the GHP is still complex

(Giamoustaris and Mithen 1997; Sexton and Howlett 2000). In addition, other plant defence mechanisms should not be ignored such as the induction of phytoalexins, pathogenesis-related proteins and hypersensitive reactions for which there is good evidence for their role in plant defence against bacterial pathogens. Therefore, the function of GHP may be additive or synergistic in combination with other plant defences.

Despite the uncertainties of the role of GHP, some studies on the biological activity of the GHP against micro-organisms have been carried out (Giamoustaris and Mithen 1995; Rask *et al.* 2000; Barth and Jander 2006); these studies primarily focused on pathogenic fungi and a few studies on plant bacterial diseases. This study demonstrates that GHP can be effective, *in vitro*, against the plant pathogenic bacteria *A. tumefaciens*, *E. chrysanthemi*, *P. cichorii*, *P. tomato*, *X. campestris* and *X. juglandis*, very common in crops causing significant problems. Normally, these bacteria infect the inner parts of the plants and if the conditions are favourable for disease, they could be very aggressive and then symptoms develop quickly, which includes, yellowing and blackening of leaf and leaf veins, interruption of normal nutrient circulation, tumours, rot of fruits, leaves, stems and roots, and finally necrosis and death of plant, decreasing the production (DeCleene and DeLey 1976; Shaw and Kado 1988; Bashan and de-Bashan 2002; Aysan *et al.* 2003). Because these pathogens invade inner parts of the plant, the conventional chemical products such as copper may not provide adequate control for these diseases, and thus alternatives to their control are still needed.

In accordance with previous studies, ITC from glucosinolates have generally been shown to be more effective than other hydrolysis products and that ITC derived from aromatic glucosinolates were generally more effective than those derived from aliphatic glucosinolates (Manici *et al.* 1997; Sarwar and Kierkegaard 1998).

Based on the differences detected between GHP, in which BITC, PEITC, ITC Mix and SFN were the most proficient, it is clear that the chemical structure of ITC affects the relative activities, and this has some parallels when comparing other biological activities, namely the anticancer activity, as referred to previously by Zhang and Talalay (1998). However, two ITC belonging to different structural groups, PEITC to the aromatic group and SFN to the aliphatic group, have similar inhibitory effects, which are in accordance with previous authors (Zhang and Talalay 1998) who stated that these two GHP chemical groups had the highest biological effects, and therefore could be very important in the mechanism of Capparales species plant resistance (Menard *et al.* 2001). Kirkegaard (1996), also suggested that the green material used for 'biofumigation' against soil-borne diseases must be carefully chosen because the roots of *Brassica* plants are generally more potent than the corresponding leaves as they are richer in aromatic GHP such as PEITC; SFN is regularly referred to as one of the most promising GHP, because of the anticarcinogenic activities (phase II xeonbiotic enzyme induction and signal transduction effects) in both animals and humans (Fahey *et al.* 1997; Talalay and Fahey 2001). Fewer studies (Yulianti *et al.* 2006) have been published on the potential role of GHP against plant pathogenic bacteria, and none in SFN. However, the results of the present study are corroborated by previous findings, due the inhibition effect of SFN seen with various pathogenic bacteria.

The minor effect detected for AITC at lower doses, in all bacteria analysed, could be explained either by its lipophilic nature and slower diffusion through the agar, or by its higher volatility when applied, as previously noted (Suhr and Nielsen 2003). Thus, to be effective, it must be applied at higher doses as illustrated by the results, because when applied at higher doses AITC had a strong inhibition effect against *E. chrysantemi*, *P. cichorii*, *P. thinsp;tomato*, *X. campestris* and *X. juglandi*s. Despite this compound being used in the food industry as a preservative (Delaquis and Mazza 1995), because of the higher antibacterial activity against *Escherichia coli*, *Listeria monocytogenes*, *Salmolella*, *Pseudomonas corrugate*, *Pseudomonas aeruginosa*, and *Vibrio parahaemolyticus* (Delaquis and Sholberg 1997; Isshiki *et al.* 1992), the positive effect detected in this study was overall less pronounced than found with other ITC.

The different *in vitro* inhibitions obtained for each bacteria tested with different GHP reflect either their nature (bacterial physiology and biochemistry) and/or the chemical nature of the GHP (volatility, diffusion properties, chemical reactivity etc), as referred above. *Agrobacterium tumefaciens* was less affected even though it is a Gram-negative bacterium like the other plant pathogens tested. Both Gram-positive and Gram-negative bacteria have a cell wall made of peptidoglycan and phospholipid bilayer with membrane of proteins. However, the Gram-negative bacteria have a unique outer membrane with lipopolysaccharides, a thinner layer of peptidoglycan and a periplasmic space between the cell wall and the membrane, which confers for this kind of bacteria higher resistance to lysozymes and antibiotic attacks (Salton and Kim 1996). However, these barriers are generally permeable to low molecular weight (phyto)chemicals with lipophilic properties as is the current case. Although the current study does not show the mechanism underlying the resistant behaviour, the higher resistance of *A. tumefaciens* to the tested GHP may be related to metabolic resistance (enzymatic inactivation of the ITC) or insensitivity to the GHP. ITCs are generally chemically very reactive. They can react with the -SH group in glutathione (thus affecting redox status of cells) and with -SH group in proteins (e.g. potential enzyme and signal transduction pathway interactions) forming dithiocarbamates. They can also react with $-NH_2$ groups of proteins forming thioureas, again potentially leading to the inhibition of enzymes or affecting signal transduction pathways (Holst and Williamson 2004; Juge *et al.* 2007). The rate of the ITC nonenzymatic reactions is related to their chemical structure and generally the aromatic ITCs are chemically more reactive than the aliphatic ITC.

In conclusion, the results obtained show that GHP could be an alternative tool in controlling plant pathogenic bacteria. The antibacterial effects exhibited against the different plant pathogenic bacteria used, reinforce the biological role of these compounds. However, more studies are needed to determine which concentration of these compounds is more suitable for application, taking into account their possible undesirable impact on healthy plants and on the soil e.g. inhibition of beneficial soil bacteria and other micro-organisms and fungi (mycorrhizae). Moreover, the economic costs of such measures must be considered and accordingly studied.

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