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IMPACT OF BACTERIAL *QUORUM SENSING* SYSTEM ON CHANGES OF ORGANOLEPTIC MARKERS OF STORAGE CABBAGE

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ABSTRACT

The regulation of phenotypes of vegetable-associated bacteria can be mediated through the production of acylated homoserine lactones (AHLs). This knowledge is essential for successful control of bacterial diseases of vegetable. The purpose of these investigations was to define the AHL patterns of gram-negative bacteria presented in storage cabbage by LC/MS technique. The phenomenon of regulating the pectinolytic activity and the exopolysaccharide (EPS) production by AHLs that are associated with microbial spoilage of cabbage was also examined. Among 100 strains isolated from storage cabbage, 47 isolates produced AHLs. The results of the 16S rDNA sequence analysis indicated that selected microflora was highly closely related to *Pantoea agglomerans*, *Rahnella aquatilis*, *Pseudomonas fluorescens*, *Pseudomonas syringae*, and *Pseudomonas cedrina* (approximately 98%-99% confidence). The chemical nature of AHLs produced by selected microflora differ from species to species. The pattern of AHLs of *Ps. cedrina* consisting of C_8-HSL, 3-oxo-C_{10}-HSL, and 3-hydroxy-C_{14}-HSL, has not been previously reported. The present study demonstrates that bacterial spoilage of storage cabbage is influenced by *quorum sensing*. Application of furanone C-30 that acts as *quorum sensing* inhibitor, caused the significant reduction in the production of EPS and pectinolytic enzymes by examined bacteria.

Keywords: *Enterobacteriaceae*, *quorum sensing*, acylated homoserine lactones, storage cabbage, microbiological spoilage

INTRODUCTION

Microbial spoilage of vegetables during storage is an area of global concern. Despite modern storage facilities, approximate 25-30% of all vegetables are lost due to microbial activity **(Ragaert *et al.*, 2007)**. An improved science-based understanding of the activity of microorganisms present in storage vegetables is crucial for the increase reduction of the rate of microbial degradation of foods and development of novel prevention techniques.
The studies of **Rasch *et al.* (2005)** and **Medina-Martinez *et al.* (2006)** have demonstrated that metabolism of plant-associated bacteria is *quorum sensing*-regulated. In gram-negative bacteria this regulation is typically mediated by chemical signals such as *N*-acyl-L-homoserine lactones (AHLs). AHLs have a conserved homoserine lactone (HSL) ring which is acylated with a fatty acyl group at the α-position. AHLs may contain C4 to C18 acyl side chains and, at the third carbon, either an oxo, or a hydroxyl, or no substitution **(Whitehead *et al.*, 2001 and Myszka and Czaczyk, 2012)**. The key regulatory components of the *quorum sensing* mechanism are LuxI-type proteins acting as AHL synthases, serving as AHL molecules receptors, and AHL-dependent transcription factors **(Whitehead *et al.*, 2001)**. The studies of phytopathogens have revealed that degradative enzymes production can be AHL-regulated and such activities may play a role in food spoilage **(Burger *et al.*, 2000)**. In addition, a variety of phytopathogens can produce the same AHLs, but whether these molecules are involved in the regulation of different phenotypes in each strain, or not, needs further investigations **(Whitehead *et al.*, 2001)**.
As foods spoilage is the result of not only bacterial activity, but also chemical changes, the discovery of AHL-positive strains in products by **Rasch *et al.* (2005)** and **Medina-Martinez *et al.* (2006)** inspired us to investigate if and how *quorum sensing* influences on the metabolism of bacteria colonizing the vegetables during postharvest storage. Our interest was focused on chopping cabbage. Increasing the consumers' health awareness, together with their demand for convenience foods, have accelerated the global sale of the cabbage. Cabbage contains sulforaphane, which has anticancer properties. Consumption of salads composed of raw chopping cabbage has been shown to reduce the risk of various cancers in human studies **(Verkerk *et al.*, 2009)**. Our preliminary study of microbiological quality of cruciferous vegetables cultivating in Wielkopolska District revealed the domination of psychrotrophic gram-negative bacteria belonging the genera *Pseudomonas*. In all examined samples the amount of lactic acid bacteria, *Clostridium* spp., molds and yeasts did not exceed the value of 10^1CFU/g (unpublished data).
The aim of this study was to define the AHL patterns of gram-negative bacteria presented in chopping cabbage. The phenomena of regulating the phenotypes by *quorum sensing* that are associated with microbial food spoilage were also examined.

MATERIALS AND METHODS

Plant material and preparation

White cabbage (*Brassica oleracea* L. spp. Olearacea convar capitata) came from a farm in western Poland. The cabbage was grown in the open air on loam soil. After harvesting vegetables were storied at 4°C until further experiments. Samples were washed upon running water. The outer leaves of the cabbage were removed and dried on blotting paper. After drying, the samples of cabbage were mechanically comminuted with a Nagema HU-1 cabbage slicer (Dresden, Germany). Comminuted product was weighed in the amount of 200g to polypropylene trays of 205 x 160 x 60mm, with oxygen permeability of 7–8 cm^3/m^2/24 h. Tested vegetable product was packaged in air atmosphere and storied at 4°C for 12 days.

Enumeration and isolation of psychrotrophic gram-negative bacteria

The samples of cabbage were diluted 1:9 with physiological saline (0.9% NaCl), homogenized in a stomacher for 2 min, and serially 10-fold diluted. Aerobic counts of bacteria of the family *Enterobacteriaceae* were made on MacConkey agar (Oxoid, UK) incubated at 15°C for 3 days. After incubation period, colonies from MacConkey agar were isolated and inoculated in LB according to Bertani (1951) (1% peptone, 0.5% yeast extract, 0.5% NaCl). Then cultures were incubated at 15°C for 3 days.

Screening for AHL-producing of gram-negative bacteria by replica planting

The screening of obtained isolates for AHL-production was performed according to the methods of **Ravn *et al*. (2001)**. According to the method *Agrobacterium tumefaciens* ATCC 51350 and *Chromobacterium violaceum* ATCC 31532 were used as AHL monitor strains to screen for AHL-producing microflora. The tested strains were streaked in parallel to the monitor strains on LB agar plates. *A. tumefaciens* carries a *lac*Z fusion to a *tra*I and produces a blue color in a presence of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) in response to AHL **(Ravn *et al*., 2001)**. *A. tumefaciens* have a high affinity for long chained AHL (≥ C8). The CviR of *C. violaceum* regulates the production of a purple pigment when induced by AHL. *C. violaceum* is induced by C4-C8 unsubstituted AHL **(McClean *et al*., 1997)**. All plates were incubated for 3 days at 15ºC.

Sequencing and phylogenetic analysis

After initial bacterial biomass incubation in 10 mM Tris-Cl pH 7.5 with 50 mg/mL lysozyme (Sigma, USA) for 0.5 h at 37ºC, total DNA from cells was extracted using Genomie Mini AX Bacteria Kit (A&A Biotechnology, Gdańsk, Poland). Sequences encoding small subunit of rRNA were amplified in PCR reaction using primers SDBact0008aS20 and SUniv1492bA21 **(Suau *et al*., 1999)**. PCR was performed in total volume of 50 uL containing 1xPCR buffer (20mM Tris-HCl, pH8.8, 2.0mM $MgSO_4$, 10mMKCl and 0.1% Triton X-100) additional $MgCl_2$ to concentration of 2.5 mM $MgCl_2$, 100 ng DNA template, 0.44 mM concentration of each primer, 200uM dNTP, 1.25 U RUN DNA polymerase (A&A Biotechnology, Poland). PCR was carried out in Biometra T Gradient thermocycler (Biometra, Germany). The amplification of 16S rDNA consisted 30 cycles of 1 min denaturation step at 94ºC, 1 min annealing step at 48ºC, and 1.5 min extension at 72ºC. Negative controls containing no DNA template were included in parallel. Four microliter samples of PCR product were analyzed by electrophoresis in 1.0% (w/v) agarose in 1x TBE buffer (89mM Tris-borate, 2mM EDTA pH 8.3) and then they were subsequently visualized by illumination after ethidium bromide staining. PCR products were purified using Clean-up Kit according to user manual (A&A Biotechnology, Gdańsk, Poland) and sequenced at Genomed (Warszawa, Poland) with primers used for PCR and additionally for inner sequence with GTGCCAGCMGCCGCCCTAA primer. Obtained sequences were arranged into contigs and identified in BLAST service of the GenBank database **(Altschul *et al*., 1990)**.

LC-MS analysis

Extracts for LC-MS analysis were prepared from 100ml cultures after growth in LB medium for 3 days at 15ºC. Bacteria were removed by centrifugation; the supernatants were extracted twice with equal volumes of ethyl acetate acidified with 0.5% of formic acid. Extracts were evaporated to dryness, redissolved in methanol (gradient grade), and analyzed on an Agilent 1200 series liquid chromatograph equipped with a DAD detector coupled to a high resolution, time-of-flight (TOF) mass spectrometer (Agilent 6224) with a ESI source. Extracts at volume of 20µl were injected on the Nucleosil C_{18} column (particle size 5µm, 125 x 4mm). The analyzes were done at a flow rate of 1ml/min with a water-acetonitrile gradient, starting at acetonitrile-water (5:95), going to 100% acetonitrile in 30 min, and maintaining 100% acetonitrile for 5min, before returning to the start conditions in 15 min, and equilibrating for 10 min. The UV spectra were collected by DAD at 195 nm. MS spectra were obtained in positive ion electrospray mode. The mass spectrometer parameters were as follows: capillary voltage - 3500V, scan acquisition – from 100 to 500 *m/z*, desolvation gas flow and temperature: 11 L/min and 325ºC respectively, nebulizer gas – 20 psi, fragmentor voltage – 150V.

Investigation of AHL-regulated phenotypes

An involvement of AHL in regulation of EPS production and pectinolytic activity of bacterial isolates was examined by adding 0.9µg/ml of furanone C-30 (Sigma, USA) to freshly inoculated cultures in LB medium **(Wu *et al*., 2004)**. Furanone C-30 acts as bacterial *quorum sensing* inhibitor. The cultures that grew on LB medium without furanone C-30 component were used as reference. The incubation was conducted at 15ºC for 3 days. The amount of viable cells in the samples was examined by pour plate method.

The presence of pectinolytic activity was evaluated by spotting 10 µl of examined cultures on agar plates containing 1% (w/v) of polygalacturonic acid (Sigma, USA). The plates were incubated for 3 days at 15ºC. After the colonies reached around 3 mm, iodine-potassium iodide solution (1.0g iodine, 5.0g potassium iodide and 330ml H_2O) was added to detect clearance zones **(Bowen and Kominos, 1979)**.

The ability to synthesize EPS was determined using the procedure employed by **Forde and Fitzgerald (1999)**. The bacteria were harvested by centrifugation at 3000g for 20 min at the room temperature. The cells were resuspended in 1.5 ml of 30% (w/v) NaOH. Samples were boiled for 15 min, centrifuged at 15000g for 15 min, and the supernatant fluids were added dropwise to 60% (v/v) ethanol. The total EPS (expressed as µg/CFU) was determined using the acid hydrolysis method of **Parkar *et al*. (2001)**. The precipitated EPS was collected by centrifugation (15000g, 20 min), and was resuspended in 1ml of sterile water. The samples were mixed with 7 ml of 77% (v/v) H_2SO_4 and cooled for 10 min in an ice-bath. 1 ml of 1% (w/v) of cold tryptophan was added and the samples were heated in a boiling bath for 20 min to affect hydrolysis. The acid hydrolysis of EPS produced a furan which condenses with the tryptophan and forms a colored product. The calibration curves were prepared against standard dextran (Mp. 40000) solutions (Sigma, USA).

RESULTS AND DISCUSSION

Isolation and identification of AHL-producing psychrotrophic strains

Psychrotrophic strains of the family *Enterobacteriaceae* are frequent members of the spoilage microflora of storage vegetables **(Ragaert *et al*., 2007)**. The total counts of gram-negative rods in refrigerated cabbage can range from 10^3 to 10^5 CFU/g (**Ragaert *et al*., 2007)**. In this work the initial population of psychrotrophic members of *Enterobacteriaceae* in storage cabbage reached the average level of 5.0 x 10^2 CFU/g (Tab 1). The population increased to 2.0 x 10^4 CFU/g after 6 days and then gradually reached the value of 3.5 x 10^7 CFU/g during the remainder 6-days period of storage (Tab 1).

In this study for further investigations one hundred randomly picked colonies were isolated from MacConkey agar plates. All isolates produced pink to red mucoid colonies with a reddish bile precipitate surrounding. The ability of selected bacteria to synthesize AHL molecules was evaluated using *A. tumefaciens* and *C. violaceum* as AHL-monitoring strains. Of 100 strains isolated from storage cabbage, 47 elicited a response *A. tumefaciens*, which detects mainly 3-oxo-AHLs with acyl chain lengths from 6 to 14 C atoms. In the work, 45 isolates were positive in the *C. violaceum* assay, which detects nonsubstitued AHL molecules.

The results of the 16S rDNA sequence analysis indicated that AHL-positive isolates were closely related to *Pseudomonas* spp. and *Pantoea* spp. and *Rahnella* spp. (approximately 98%-99% confidence). *Pantoea agglomerans*, *Pseudomonas fluorescens*, and *Pseudomonas orientalis* were the dominant AHL-positive microflora in examined samples, except for an occasional isolation of *Rahnella aquatilis* and *Pseudomonas cedrina* (Tab 1). These observations were similar to the findings of **Simon-Sarkadi *et al*. (1993)** who reported that *Enterobacteriaceae* population represents up to 90% of the total microbial number of cut cabbage *Brassica pekinensis* Rupr. stored at 5ºC. Microbiological populations were mostly situated on cut surface of the cabbage **(Simon-Sarkadi *et al*., 1993)**.

Table 1 Detection and identification of AHL-synthesizing bacteria in chopped cabbage

Number of sample	Storage time (days)	Total number of psychrotrophic Enterobacteriaceae [CFU/g]	number of isolates	Number of AHL-positive isolates	Identification of AHL-positive isolates				
					P. agglomerans	*R. aquatilis*	*Ps. fluorescnes*	*Ps. orientalis*	*Ps. cedrina*
1	0	9.0 x 10^2	6	2	1			1	
	6	2.1 x 10^4	12	5	4			1	
	12	3.6 x 10^7	15	8	5	1		2	
2	0	3.0 x 10^2	3	1			1		
	6	1.9 x 10^4	14	6	3		3		
	12	3.3 x 10^7	28	10	5		3		2
3	0	5.0 x 10^2	2	1			1		
	6	1.9 x 10^4	8	4			3	1	
	12	3.5 x 10^7	12	10	5		2	3	

Profiles of AHL molecules of selected bacteria

In the work, for screening the capability of isolated microflora for the production of AHLs, the liquid chromatography (LC) coupled to MS was applied. The attribution of chromatographic peaks to AHLs was confirmed by the MS at the range of *m/z* 100 to 500. Selected results of LC-MS analyses for cell-free supernatant of *Ps. fluorescens* PF7 are shown in Figure 1, 2 and 3.

The examples of AHL profiles of isolates obtained in this work were presented in Tab 2. In the study, in extracts of cell-free supernatants of gram-negative isolates, several AHLs were identified. Interestingly, all examined samples showed the presence of C_8-HSL. Significant differences between the AHLs profiles of tested microflora were noted at the level of late-eluting signal molecules (AHLs with C9 and C14 acyl chains) (Tab 2). Results obtained in this work also indicate that the chemical nature of *quorum sensing* signal molecules differs from species to species. All of isolates of the genus of *Pseudomonas* produced different AHLs, which vary in the length and substitution of the acyl chain. The pattern of AHLs of *Ps. cedrina* consisting of C_8-HSL, 3-oxo-C_{10}-HSL, and 3-hydroxy-C_{14}-HSL, has not been previously reported.

Our results show essential differences in the spectra of AHLs in comparison to the published data for the same bacterial cultures. According to **Whitehead *et al.* (2001)** the production of AHLs by gram-negative bacteria is dominated by synergistic effect of the surrounding environment. Through signaling mechanisms bacteria also respond to metabolic activity of other species colonizing the same niche **(Whitehead *et al*., 2001)**. Bacterial cultures isolated from: potato (*P. agglomerans*), drinking water (*R. aquatilis*), and root hairs of a sugar beet plants (*Ps. fluorescens*) were capable to synthesize mostly the short-chained signal molecules **(Lane *et al*., 2000; Dong *et al*., 2001; Steidle *et al*., 2001; Liu and Griffiths, 2003)**. In our study LC-MS analysis of the AHLs produced by *P. agglomerans*, *R. aquatilis*, *Ps. fluorescens*, and *Ps. syringae* revealed the long-chained signal molecules (AHLs with C8 and C14 acyl chains) (Tab 2).

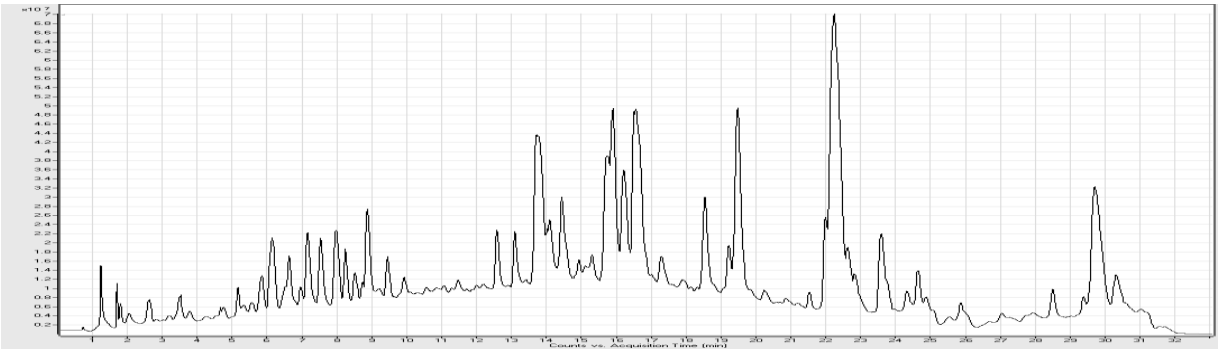

Figure 1 HPLC chromatogram of extracted AHL from *Ps. fluorescens* PF7 culture

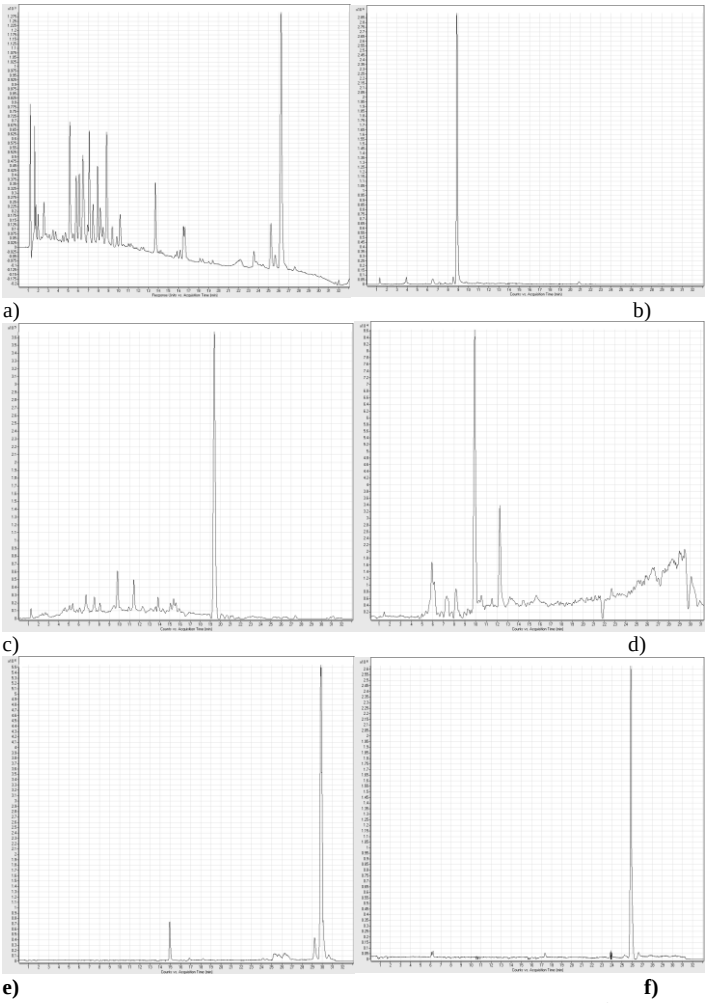

Figure 2 Positive electrospray ionization MS chromatograms (*a*-*f*), showing appropriately: the total ion current (TIC) (*a*) and extracted ions for $[M+H^+]$ adducts of five detected homoserine lactones: N-3-oxo-C_{10}-HSL (*b*), N-C_6-HSL (*c*), N-3-oxo-C_{14}-HSL (*d*), N-C_8-HSL (*e*), N-C_{10}-HSL (*f*)

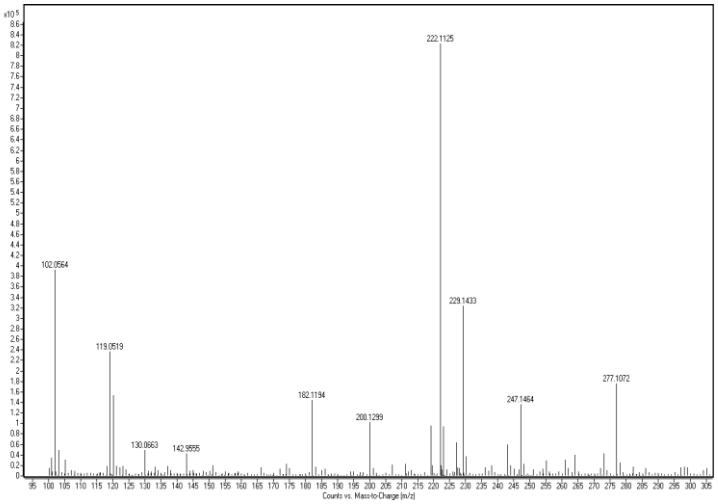

Figure 3 The mass spectrum for selected homoserine lactone (identified above) – N-C_6-HSL with the proper values of adducts:

1) m/z = 200.1299 - $[M+H^+]$,
2) m/z = 222.1125 – $[M+Na^+]$,
3) m/z = 182.1194 – $[M-H_20+H^+]$

Table 2 Comparison of the profiles of AHLs of obtained isolates with literature data

Bacteria	*AHLs identified by LC-MS in this work*	*literature data*	*Reference*
P. agglomerans PA 4	C_8-HSL; 3-oxo-C_{10}-HSL; 3-oxo-C_{11}-HSL	3-oxo-C_6-HSL	Dong et al. 2001
R. aqatilis RA 1	C_8-HSL; C_{10}-HSL; 3-oxo-C_{14}-HSL	3-oxo-C_6-HSL; 3-oxo-C_8-HSL	Steidle et a., 2001
Ps. fluorescens PF 7	C_6-HSL; C_8-HSL; C_{10}-HSL; 3-oxo-C_{10}-HSL; 3-oxo-C_{14}-HSL	C_4-HSL; C_6-HSL; C3-oxo-C_6-HSL; C_{14}-HSL	Lane et al., 2000; Liu and Griffiths, 2003
Ps. syringae PS 2	C_8-HSL; 3-oxo-C_8-HSL; C_9-HSL,	3-oxo-C_6-HSL	Cha et al., 1998
***Ps. cedrina* PC 2**	C_8-HSL; 3-oxo-C_{10}-HSL; 3-hydroxy-C_{14}-HSL	no data	no data

Characterization of bacterial AHL-regulated phenotypes

Gram *et al.* (2002) demonstrated that nutrients composition of chill-stored vegetables allows the gram-negative bacteria to grow, but only the fraction of these microorganisms present in storage products (typically *Pseudomonas* spp. and *Pantoea* spp.) cause the spoilage characteristics. The metabolism of phytopathogenic bacteria can be oriented to secretion of pectinolytic enzymes and production of EPS **(Denny, 1995; Jayani *et al*., 2005)**. Pectinolytic enzymes cause the maceration of plant tissues **(Jayani *et al*., 2005)**. Bacterial EPS, due to their normally high viscosity, cause vascular plugging of plants leading to wilting. According to **Denny (1995)**, the production of EPS favors disease development by masking bacterial cell wall, thus protecting the cells from preformed antimicrobial compounds.

In the work we examined whether *quorum sensing* inhibition had any effect on pectinolytic activity and exopolysaccharides production by microflora of storage cabbage. The influence of the supplementation of the culture medium with furanone C-30 on tested bacterial growth is presented in Tab 3. The differences in metabolic activity of AHL-positives isolates and isolates with interrupting *quorum sensing* by furanone C-30, are shown in Tab4.

Table 3 The influence of the supplementation of the culture medium with furanone C-30 on tested bacterial growth

Isolate	Cell number in the medium (CFU/ml)	
	medium without furanone C-30	medium with furanone C-30
***P. agglomerans* PA4**	3.0×10^6	1.5×10^2
***R. aqatilis* RA1**	6.7×10^6	5.5×10^2
***Ps. fluorescens* PF7**	5.5×10^5	6.8×10^3
***Ps. syringae* PS2**	1.5×10^6	3.8×10^3
***Ps. cedrina* PC2**	3.5×10^5	2.8×10^2

In our study, furanone C-30 caused significant reduction of tested bacterial growth (Tab 3). In addition, in all of obtained isolates synthesis of EPS and pectinolytic enzymes were *quorum sensing*-regulated phenotypes (Tab 4). The EPS production capacity of examined bacteria varied between 4.80 to 6.69 [mgEPS/10^7CFU]. In this work, the supplementation of the culture medium with furanone C-30 caused significant reduction of EPS biosynthesis. Similarly, the production and secretion of pectinolytic enzymes by tested isolates was under the control of AHLs. Only in *Ps. cedrina* isolates we did not identify the ability to synthesize pectinolytic enzymes (Tab 4).

Our observations are consistent with the results obtained by **Rasch *et al.* (2005)**. The authors also noticed that food-associated *Enterobacteriaceae* were capable to synthesize AHLs. This ability allows bacteria, present at an average concentration of 10^6cfu/g, locally to induce expression of phenotypes that would otherwise require an average value of 10^8cfu/g **(Bruhn *et al*., 2004)**. **Bruhn *et al.* (2004)** also demonstrated the universal character of AHLs that facilitate inter-species communication between gram-negative strains. According to **Bruhn *et al.* (2004)** the ability of *Hafnia alvei* to produce AHLs facilitated the expression of *quorum sensing* system in other microorganisms present in stored meat products. If AHLs directly control of bacterial metabolism, such management could be the major mechanism determining the rate of microbiological spoilage of storage foods **(Hentzer *et al*., 2003)**. The application of *quorum sensing* inhibitors, which specifically block the AHL production, may enhance the shelf life of most fresh and lightly preserved foods **(Hentzer *et al*., 2003)**.

Table 4 Comparison of pectinolytic activity and synthesis of exopolysaccharides by AHL-positive isolates and isolates with inhibited production of AHLs by furanone C-30

Isolate	Production of exopolysaccharides [mgEPS/10^7CFU]		Pectinolytic activity[a]	
	medium without furanone C-30	medium with furanone C-30	medium without furanone C-30	medium with furanone C-30
***P. agglomerans* PA4**	6.14 (±0.003)	0.1 (±0.001)	+	-
***R. aqatilis* RA1**	4.80 (±0.1)	0.01 (±0.001)	+	-
***Ps. fluorescens* PF7**	5.13 (±0.5)	-	+	-
***Ps. syringae* PS2**	6.23 (±0.1)	-	+	-
***Ps. cedrina* PC2**	6.69 (±0.2)	0.01 (±0.001)	-	-

Legend: + positive reaction, - not detected,
average values ± standard deviations

CONCLUSION

Our work supports the notion that AHL-based *quorum sensing* is the common feature of gram-negative bacteria contaminated chill-stored products of vegetable origin. Despite the fact that chemical nature of AHLs differ from species to species, these molecules modulate the same metabolic activity of cells. Application of furanone C-30 that acts as *quorum sensing* inhibitor, caused the significant reduction in the production of EPS and pectinolytic enzymes by examined bacteria. Further studies on the impact of AHL regulation of spoilage of storage vegetables are currently in progress in our laboratory. Analyses of possible importance of AHL-based *quorum sensing* in spoilage reactions is essential for design novel preservation techniques eliminating the risk of resistance development of bacteria. This knowledge will permit for a more efficient food quality management.

REFERENCES

ALTSCHUL, S.F., GISH, W., MILLER, W., MYERS, E.W., LIPMAN, D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403-410.
http://dx.doi.org/10.1002/047145916x.ch4

BERTANI, G. 1951. Studies on lysogenesis. 1. The mode of phage liberation by lysogenic *Escherichia coli*. *Journal of Bacteriology*, 62, 293-300

BOWEN, J.H., KOMINOS, S.D. 1979. Evaluation of a pectin agar medium for isolation of *Yersinia enterocolitica* within 48 hours. *American Journal of Clinical Pathology*, 72, 586-590.

BRUHN, J.B., CHRISTENSEN, A.B., FLODGAARD, L.R., NIELSEN, K.F., LARSEN, K.F., GIVSKOV, M., GRAM, L. 2004. Presence of acylated homoserine lactones (AHLs) and AHL-producing bacteria in meat and potential role of AHL in spoilage of meat. *Applied and Environmental Microbiology*, 70, 3149-3155.

http://dx.doi.org/10.1128/aem.70.7.4293-4302.2004
BURGER, M., WOODS, R.G., MCCARTHY, C., BEACHAM., I.R. 2000. Temperature regulation of protease in *Pseudomonas fluorescens* LS107d2 by an ECF sigma factor and a transmembrane activator. *Microbiology*, 146, 3149-3155.
DENNY, T.P. 1995. Involvement of bacterial polysaccharides in plant pathogens. *Annual Review of Phytopathology*, 33, 173-197.
http://dx.doi.org/10.1146/annurev.phyto.33.1.173
DONG, Y.H., WANG, L.H., XU, J.L., ZHANG, H.B., ZHANG, X.F., ZHANG, L.H. 2001. Quenching *quorum-sensing*-dependent bacterial infection by N-acylhomoserine lactone. *Nature*, 411, 813-817.
FORDE, A., FITZGERALD, G.F. 1999. Analysis of exopolysaccharide (EPS) production mediated by the bacteriphage adsorption blocking plasmid, pCI658, isolated from *Lactococcus lactis* ssp. *cremoris* HO2. *International Dairy Journal*, 9, 465-472.
http://dx.doi.org/10.1016/s0958-6946(99)00115-6
GRAM, L., RAVN, L., RASCH, M., BRUHM, J.B., CHRISTENSEN, A.B., GIVSKOV, M. 2002. Food spoilage-interactions between food spoilage bacteria. *International Journal of Food Microbiology*, 78, 79-97.
http://dx.doi.org/10.1016/s0168-1605(02)00233-7
HENTZER, M., WU, H., ANDERSEN, J.B., RIEDEL, K., RASMUSSEN, T.B., BAGGE, N., KUMAR, N., SCHEMBRI, M.A., SONG, Z., KRISTOFFERSEN, P., MANEFIELD, M., COSTERTON, J.W., MOLIN, S., EBERL, L., STENBERG, P., KJELLEBERG, S., HØIBY, N., GIVSKOV, M. 2003. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *The EMBO Journal*, 22, 2931-2944.
http://dx.doi.org/10.1093/emboj/cdg366
JAYANI, R.S., SAXENA, S., GUPTA, R. 2005. Microbial pectinolytic enzymes: a review. *Process Biochemistry*, 40, 2931-2944.
http://dx.doi.org/10.1016/j.procbio.2005.03.026
MCCLEAN, K.H., WINSON, M.K., FISH, L., TAYLOR, A., CHHABRA, S.R., CAMARA, M., DAYKIN, M., LAMB, J.H., SWIFT, S., BYCROFT, B.W., STEWART, G.S.A.B., WILLIAMS, P. 1997. *Quorum sensing* and *Chromobacterium violaceum*: explotation of violacein production and inhibition for the detection of *N*-acylhomoserine lactones. *Microbiology*, 143, 3703-3711.
http://dx.doi.org/10.1099/00221287-143-12-3703
MEDINA-MARTIZNEZ, M.S., UYTTENDAELE, M., MEIREMAN, S., DEBEVERE, J. 2006. Relevance of N-acyl-L-homoserine lactone production by *Yersinia enterocolitica* in fresh foods. *Journal of Applied Microbiology*, 100, 1-9.
http://dx.doi.org/10.1111/j.1365-2672.2006.03143.x
MYSZKA, K., CZACZYK, K. 2012. *N*-acylhomoserine lactones (AHLs) as phenotype control factors produced by gram-negative bacteria in natural ecosystems. *Polish Journal of Environmental Studies*, 21, 15-21.
LANE, R.E., JIANG, Y., CHHABRA, S.R., JACOB, S., STEWART, G.S.A.B., HARDMAN, A., DOWNIE, J.A., O'GARA, F., WILLIAMS, P. 2000. The biocontrol strain *Pseudomonas fluorescens* F113 producers the *Rhizobium* small bacteriocin, N-(3-hydroxy-7-cis-tetradecenoyl) homoserine lactone, via HdtS, a putative novel N-acylhomoserine lactone synthase. *Microbiology*, 16, 2469-2480.
LIU, M., GROFFTHS, W. 2003. The role of *quorum sensing* in the regulation of protease by *Pseudomonas fluorescens* and its relation to food spoilage. *Annual Meeting Book Abstract*, IFT, 268-296.
PARKAR, S.G., FLINT, S.H., PALMER, J.S., BROOKS, J.D. 2001. Factors influencing attachment of thermophilic bacilli to stainless steel. *Journal of Applied Microbiology*, 11, 675-685.
http://dx.doi.org/10.1046/j.1365-2672.2001.01323.x
RAGAERT, P., DEVLIEGHERE, F., DEBEVERE, J. 2007. Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. *Postharvest Biology and Technology*, 44, 185-194.
http://dx.doi.org/10.1016/j.postharvbio.2007.01.001
RASCH, M., ANDERSEN, J.B., NIELSEN, K.F., RAVN FLOODGAARD, L., CHRISTENSEN, H., GIVSKOV, M., GRAM, L. 2005. Involvement of Bacterial quorum-sensing signals in spoilage of bean sprouts. *Applied and Environmental Microbiology*, 71, 3321-3330.
http://dx.doi.org/10.1128/aem.71.6.3321-3330.2005
RAVN, L., CHRISTENSEN, A.B., MOLIN, S., GIVSKOV, M., GRAM, L. 2001. Methods for detecting acylated homoserine lactones produced by gram-negative bacteria and their application in studies of AHL-production kinetics. *Journal of Microbiological Methods*, 44, 239-251.
http://dx.doi.org/10.1016/s0167-7012(01)00217-2
SIMON-SARKADI, L., HOLZAPFEL, W.H., HALASZ, A. 1993. Biogenic amine content and microbial contamination of leafy vegetables during storage at 5°C. *Journal of Food Biochemistry*, 17, 407-418.
http://dx.doi.org/10.1111/j.1745-4514.1993.tb00483.x
SIEIDLE, A., SIGL, K., SCHUHEGGER, R., IHRING, A., SCHMID, M., GANTNER, S., STOFFELS, M., RIEDEL, K., GIVSKOV, M., HARTMAN, A., LANGEBARTELS, C., EBERL, L. 2001. Visualisation of N-acylhomoserine lactone-mediated cell-cell communication between bacteria colonizing the tomato rhizosphere. *Applied and Environmental Microbiology*, 67, 5761-5770.
http://dx.doi.org/10.1128/aem.67.12.5761-5770.2001
SUAU, A., BONNET, R., SUTREN, M., GODON, J.J., GIBSON, G.R., COLLINS, M.D., DORE, J. 1999. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within human gut. *Applied and Environmental Microbiology*, 65, 4799-4807.
VERKERK, R., SCHREINER, M., KRUMBEIN, A., CISKA, E., HOLST, B., ROWLAND, I., DE SCHRIHVER, R., HANSEN, M., GERHÄUSER, C., MITHEN, R., DEKKER, M. 2009. Glucosinolares in *Brassica* vegetables: the influence of the food supply chain on intake, bioavailability and human health. *Molecular Nutrition and Food Research*, 53, S219-S265.
http://dx.doi.org/10.1002/mnfr.200800065
WHITEHEAD, N.A., BARNARD, A.M.L., SLATER, H., SIMPSON, N.J.L., SALMOND, G.P.C. 2001. *Quorum-sensing* in gram-negative bacteria. *FEMS Microbiology Reviews*, 25, 365-404.
http://dx.doi.org/10.1111/j.1574-6976.2001.tb00583.x
WU, H., SONG, Z., HENTZER, M., ANDERSEN, J.B., MOLIN, S., GIVSKOV, M., HØIBY, N. 2004. Synthetic furanones inhibit *quorum-sensing* and enhance bacterial clearance by *Pseudomonas aeruginosa* lung infections in mice. *Journal of Antimicrobial Chemotherapy*, 53, 1054-1061.
http://dx.doi.org/10.1093/jac/dkh223