Effect of N-acyl-homoserine lactones (AHL) on biogenic amine formation by *Pantoea agglomerans*

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Abstract

The effect of N-acyl-homoserine lactone (AHL)-based quorum sensing (QS) on biogenic amine (BA) formation by *Pantoea agglomerans* was studied. *Agrobacterium tumefaciens* A136 and KYC55-based bioassays confirmed AHLs production by *Pantoea agglomerans*. The production ability of AHLs was quantified on the basis of β -galactosidase activity. The influence of temperature (10°C and 20°C) and pH (5.5 and 6.5) on β -galactosidase activity and BAs production by *Pantoea agglomerans* was determined. Acidification of the environment adversely affected the growth and β -galactosidase activity of *Pantoea agglomerans*, and AHLs production and BAs accumulation by *Pantoea agglomerans* was inhibited at low temperature. A significant correlation between β -galactosidase activity and BAs (putrescine, histamine, putrescine and tryptamine) was identified ($P < 0.01$). Based on the results of this study, the AHL-based QS system influences the concentrations and types of BAs produced by *Pantoea agglomerans*.

Introduction

Biogenic amines (BAs) are low-molecular-weight nitrogenous organic bases that are frequently formed by the metabolisms of living organisms from amino acid precursors (1). BAs occur in many foods, especially in high-protein fermented foods, with the most important BAs found in fermented sausage being putrescine (PUT), cadaverine (CAD), β -phenylethylamine (PHE), spermine (SPM), spermidine (SPD), histamine (HIS), tryptamine (TRY) and tyramine (TYR)(2). BAs have been used as a chemical quality index for fermented sausage, and as good indicators of spoilage(3).

The determination of BAs in food products has received considerable interest due to their detrimental effects on human health. Common levels of BAs in foodstuffs and beverages (< 100 mg/kg) are not a serious risk to health because they are metabolized by detoxification enzymes in the human intestines. However, excessive intake of BAs may cause headache, migraine, and intestinal problems(4). The toxic effects of BAs on the gastrointestinal system also provoke nausea vomiting, abdominal pain and indigestion(5).

Temperature, pH and microbiological characteristics have been reported to promote or inhibit BAs formation(6). Microbial growth, biochemical reactions and the production of metabolites increase with temperature, leading to increased BAs content. The formation of BAs in fermented sausage is also catalyzed by decarboxylases, which are formed and released from the bacteria in the early stages of sausage production or storage. *Escherichia coli*, *Klebsiella pneumoniae*, *staphylococci*, *lactic acid bacteria*, *Pseudomonas* spp., *Serratia* spp. and *Enterobacteriaceae* are known to produce Bas(7), with *Enterobacteriaceae* exhibiting the highest decarboxylase activity and PUT and CAD accumulation.

Bacteria were initially thought to function as individual cells in obtaining nutrients and multiplying. However, bacteria are now known to communicate intercellularly by producing and detecting diffusible signaling molecules, which facilitate coordinated activities within a bacterial community in response to their environment; this is known as quorum sensing (QS)(8). The QS-signaling molecules in Gram-

negative bacteria are N-Acyl homoserine lactones (AHLs). These molecules have been found either to be present or to increase in concentration in different food systems⁹. AHLs are produced by certain members of a microbial community in various food products, such as fish, poultry, meats, dairy products or vegetables. For instance, AHL-based communication systems could enable *Enterobacteriaceae* to upregulate the production of enzyme systems at certain cell densities; thus, contributing to quality changes in vacuum-packed spoiled meat at relatively low cell numbers¹⁰. Fuqua et al¹¹ reported that environmental conditions may affect AHLs production and that pH and temperature are important factors that affects the stability of AHLs.

Within microbial groups, the capacity to produce BAs is a strain-specific characteristic, more widely distributed among certain genera and species. However, little is known about the effect of AHLs on BAs formation. The purpose of the present study was to identify AHLs using the bioreporter strains A136 and KYC55 and to determine the relative amounts of AHLs by *Pantoea agglomerans* in vitro. Furthermore, the effects of AHLs on the formation of BAs were then analyzed.

Material And Methods

Strains and culture conditions

Pantoea agglomerans was originally isolated from smoked-horsemeat fermented sausage and obtained from the culture collection of the Animal Product Processing Laboratory (Shi he zi, China). A loop-full of slant cultures of *Pantoea agglomerans* was inoculated into 10 mL of nutrient-rich broth (NB, AoBoXing, Beijing, China) and incubated at 37°C for 24 h. A sample of this culture (1 mL) was then transferred to 100 mL of NB and incubated at 37°C for a further 24 h. *Pantoea agglomerans* was incubated in NB according to the methods described by Lorenzo .

A. tumefaciens KYC55, *A. tumefaciens*A136 and *A. tumefaciens* R10 for use in AHLs bioassays (a kind gift from Dr. Zeng, Ocean University of China, Qingdao, China) were cultured in Luria-Bertani broth (LB, AoBoXing, Beijing, China) at 28°C for 24 h. Antibiotics were added to the media at the following concentrations: spectinomycin (Sp; 50 µg/mL), and tetracycline (Tc; 4.5 µg/mL) for A136, spectinomycin (Sp; 50 µg/mL), tetracycline (Tc; 4.5 µg/mL), and gentamicin (Gm; 50 µg/mL) for KYC55, tetracycline (Tc; 4.5 µg/mL) for R10.

Preparation of samples by *Pantoea agglomerans* in broth at different pH and temperature

Samples of *Pantoea agglomerans* (5 mL) was inoculated into 45 mL NB (supplemented with 0.05% L-lysine, L-tyrosine, L-phenylalanine, D-tryptophan, L-histidine, L-methionine and 0.005% pyridoxal phosphate⁽¹²⁾ adjusted to pH 5.5, and pH 6.5). Culture was grown at 37°C for 48 h being counted. Cells was then harvested every 4 h by centrifugation at 12,000 ×g (AllegraX-22, USA) for 10 min at 4°C.

Samples of *Pantoea agglomerans* (5 mL) was inoculated into 45 mL NB (supplemented with 0.05% L-lysine, L-tyrosine, L-phenylalanine, D-tryptophan, L-histidine, L-methionine and 0.005% pyridoxal phosphate(12) was incubated at 10°C and 20°C for 48 h being counted. Cells were then harvested every 4 h by centrifugation at 12,000 ×g for 10 min at 4°C.

For each set of conditions, duplicate microbial counts were obtained and the mean values were expressed as OD₆₀₀. The supernatants were frozen at -20°C prior to the analysis of β-galactosidase activity and BAs.

AHLs determination

The plate assay for detection of AHLs was performed as described previously (13) with some modifications. The strains to be tested for the induction of KYC55 and A136 were streaked onto LB agar plates in parallel to the monitor strains. KYC55 and A136 were streaked onto plates supplemented with the 20 µL of the β-galactosidase substrate 5-bromo-4-chloro-3-indolylβ-d-galactopyranoside (X-gal, 20 mg/mL). *A. tumefaciens* R10 was used as a positive control for the induction of KYC55 and A136 in the assays. The monitor strains themselves served as negative controls. Plates were incubated for 24 h at 28°C.

Bioassay of β-galactosidase activity

Most AHL-lactonases exhibit significant sequence and structural homology with the metallo-β-lactamase superfamily(14). Therefore, β-galactosidase activity assays represent a relatively unbiased method for determining the types and relative amounts of AHLs present. The activity of β-galactosidase was measured using o-nitrophenyl β-D-galactopyranoside (ONPG) as a substrate¹⁵. One unit of β-galactosidase activity (U) was defined as the amount of enzyme that liberates of 1 nmol ONPG per min under the assay conditions¹⁵.

The measurement of relative AHLs production was carried out based on the method described by (16) with some modifications. Prior to experiment, the culture of bioreporter strain KYC55 was activated in LB broth at 28°C for overnight, then 1 mL of the KYC55 suspension and 4 mL of the preparation of supernatant by *Pantoea agglomerans* (from experiment 2.2) were incubated into 45 mL LB broth at 28°C with shaking 220 rpm. The β-galactosidase activity was initiated by adding 800 µL Z-buffer, 100 µL 0.5% sodium dodecyl sulfate (SDS), 150 µL chloroform and 400 µL bacterial suspensions (which was grown at 28°C with shaking 220 rpm) in a 2.0 mL sterile centrifuge tube. The mixture was shaken adequately before adding 100 µL ONPG with further shaking. The reaction time of starting was recorded of bacterial suspension which starts changing yellow coloration. The reaction was stopped by the addition of 600 µL 1 M Na₂CO₃; this time was recorded as the end of the reaction. Each sample was centrifuged at 12,000 ×g for 10 min at 4°C to clarify the supernatant and the absorbance was measured at 420 nm (OD₄₂₀). Meanwhile, the absorbance of 400 µL aliquots of bacterial suspension (which was grown at 28°C with shaking 220 rpm) was measured at 600 nm. The activity of β-galactosidase was estimated according to the following equation:

$$\text{Miller Units} = (1000 \times \text{OD}_{420}) / (\text{OD}_{600} \times T \times V);$$

where T and V are the reaction time of bacterial suspension and bacterial suspension volume, respectively. All measurements were carried out in triplicate.

Biogenic amine determination

BAs standards (PUT, TRY, PHE, HIS, and TYR [Sigma-USA]; CAD, SPM, and SPD [Sigma-Switzerland]) solutions were prepared in 0.4 M perchloric acid to a final concentration of 1 mg/mL for each amine. Solutions of each amine were prepared in 0.4 M perchloric acid to final concentrations of 0.0, 2.0, 5.0, 10, and 20 µg/mL.

BAs derivatization was performed with dansyl chloride using the methods reported by (2) with slight modifications. In brief, one mL of culture supernatant for each strain or each BAs standard solution was placed in a 5-mL volumetric flask. Sodium hydroxide (2 N, 200 µL), saturated sodium bicarbonate (300 µL), and dansyl chloride (10 mg/mL; Sigma, USA) were added to the sample extract, which was then incubated in darkness at 40°C for 45 min. The reaction was stopped by the addition of 100 µL ammonium hydroxide to the mixture. The final reaction volume was adjusted to 5 mL with acetonitrile. The reaction mixture was centrifuged at 3,000 × g for 5 min. The resulting supernatant was filtered (0.22 µm syringe filter with a PVDF membrane) for HPLC (Agilent 1100 system) analysis.

HPLC analysis was performed in a C₁₈ column (Spherisorb, 2.5 µm ODS, 250 cm × 4.6 mm internal diameter) equipped with a diode array detector set at 254 nm. The elution was performed with acetonitrile (solvent A) and water (solvent B). The gradient elution program was as follows: 0 min = 65% B, 5 min = 75% B, 20 min = 100% B, 24 min = 100% B, 25 min = 75% B, 30 min = 65% B.

Statistical analysis

Three replicates of each experiment were performed. The data were subjected to an analysis of variance (ANOVA) and are reported as the mean values ± the standard deviation (mean ± SD). $P < 0.01$ was considered to indicate statistical significance. All statistical analyses were performed using the Statistical Package for Social Sciences, SPSS Version 17.0 for windows (SPSS Inc., Chicago, IL, USA).

Result And Discussion

AHLs production by *Pantoea agglomerans*

A representative illustration of the cross-feeding plate bioassay for the detection of BA-producing strains using *A. tumefaciens* is shown in Fig. 1. Following 24 h of incubation at 28°C, a positive blue coloration was observed in the cross-feeding plate bioassay. The development of blue colour in plates confirmed the presence of AHLs compounds, thus, *Pantoea agglomerans* was identified as AHLs producers. Being *traI* and *luxI* positive, *Pantoea agglomerans* elicited the same response profile.

Effect of pH on β -galactosidase activity

AHLs production is clearly dependent on environmental growth parameters, such as temperature and pH. The detection of AHLs has been greatly facilitated by the development of sensitive bioassays that allow rapid screening of microorganisms for diffusible signal molecules. *A. tumefaciens* as a bioassay organism for QS has a transcriptional response regulator coupled to a reporter gene allowing a readily observable phenotype for the bioassay of QS molecules. Medina-Martínez established that food composition and pH affect the QS system, with most AHLs being unstable in alkaline conditions.

The results of β -galactosidase assays of *Pantoea agglomerans* incubated in NB adjusted to different initial pH values are shown in Fig. 2. The expression of β -galactosidase induced in *Pantoea agglomerans* by the *A. tumefaciens* KYC55 reporter showed extremely significant differences ($P < 0.01$) when the initial culture conditions were adjusted to pH 5.5 and pH 6.5. The β -galactosidase activity of *Pantoea agglomerans* correlated with the changes in cell numbers. This study also demonstrated that *Pantoea agglomerans* was able to synthesize AHLs when a sufficiently high population density was reached. The β -galactosidase activity increased progressively, reaching 193.05 Miller Units and 214.23 Miller Units after 32 h of incubation at initial pH 5.5 and pH 6.5, respectively. In accordance with this result, Rajesh and Ravi Shankar Rai(17) reported that the culture conditions play a major role in enhancing the production of enzymes, with variable conditions such as pH, incubation period, and agitation speed being important factors in AHLs production. Raol et al(18) noted that β -galactosidase activity was optimized by response surface methodology with a 4.69-fold increase in production achieved using low cost medium, and that the production of AHL-lactonase is highly influenced by pH. The relative activity of AHL-lactonase increased with the pH of the media to an optimum level at pH 7.0. Similarly, optimization of culture conditions to pH 7.5 for *Bacillus cereus* MTCC1305 yielded a 1.58-fold increase in β -galactosidase production (19).

Effect of temperature on AHLs activity

Temperature can affect the QS system because auto inducer production depends on environmental growth factors (temperature, pH and carbon source), which can have a strong influence on the expression levels of the signaling molecules. Similarly, the production of β -galactosidase is highly influenced by the temperature, with activity increasing with temperature to the optimum level at 20°C. β -galactosidase activity was measured in Miller Units (20) and the activity of *Pantoea agglomerans* at different temperatures is shown in Fig. 3.

Figure 3 shows the effect of temperature on the β -galactosidase activity of *Pantoea agglomerans*. At 10°C and 20°C, the β -galactosidase activity of *Pantoea agglomerans* increased progressively over the 32-h incubation period, reaching threshold concentrations of 150.68 Miller Units and 180.78 Miller Units, respectively. In accordance with these results, Medina-Martínez reported that AHLs production by *A. hydrophila* 519 was detected after 70 h of incubation at 12°C. The AHLs production was noted to reach levels of 940 nmol/10 mL after 43 h of incubation at 22°C. The β -galactosidase activity of *Pantoea*

agglomerans was detected during the growth phase. Similarly, Yates reported the production of AHLs by *Y. pseudotuberculosis* in the mid-logarithmic and early stationary phases when cultured in LB medium at 37°C.

Effect of pH on the formation of biogenic amines

The accumulation of BAs has been reported to require the presence of precursors, microorganisms expressing amino decarboxylases and conditions suitable for growth and decarboxylase activity(21). Amino acid decarboxylase activity is critically influenced by pH(22), with optimum activity at acid pH, which is compatible with bacterial growth and the neutralization mechanism. Therefore, low pH, in particular between pH 4 and pH 5.5, favors amine production(23).

The concentrations of BAs produced by *Pantoea agglomerans* at initial pH 5.5 and pH 6.5 are shown in Fig. 4A and Fig. 4B, respectively. When cultured at initial pH 5.5, the concentrations of PUT and PHE produced by *Pantoea agglomerans* showed a general increase over the 48-h incubation period (PUT, 50.0–394.36 µg/mL (below 400 µg/mL); PHE, 14.0–322.79 µg/mL (below 400 µg/mL), respectively) (Fig. 4A). Based on these results, it can be concluded that the concentration of PUT and PHE produced by *Pantoea agglomerans* is affected by pH. An acid pH may favor decarboxylation reactions and amine formation as a protective mechanism adopted by microorganisms against unfavorable growth conditions (5). High concentrations of PUT and PHE were detected, which indicated that the low pH incubation conditions inhibited microbial growth and enzyme activity. Similar results have been reported by Roseiro et al.(23). Tosukhowong et al.(24) reported that the main reason for low levels of BAs is the low pH in the culture; however, in this study, PUT and HIS reached concentrations of 466.94 µg/mL and 218.21 µg/mL, respectively, when cultured for 48 h at initial pH 6.5 (Fig. 4B).

Effect of temperature on the formation of biogenic amines

It has been reported that temperature has a marked influence on the formation of BAs in fish and cheese products, with the amine content increasing with time and storage temperature(22). BAs are formed mainly by the action of bacteria; therefore, temperature and suppression of bacterial proliferation has a critical influence on BAs production. Temperature influences the relationships among the activities of the different microorganisms and can have opposing effects on BAs accumulation. It also can influence bacterial growth and enzyme activity. A significant and steady increase in the PHE and TRY concentrations produced by *Pantoea agglomerans* was observed at 10°C and 20°C (Fig. 5). Substantial differences were found in the extent of amine formation by *Pantoea agglomerans*.

The concentrations of PHE and TYR produced by *Pantoea agglomerans* cultured for 48 h at 10°C were consistently low (below 51.77 µg/mL and 14.38 µg/mL, respectively) (Fig. 5A). At 20°C, the concentrations of PHE ranged from 66.92 µg/mL to 489.28 µg/mL and the concentration of TYR was 325.22 µg/mL after incubation for 48 h (Fig. 5B). In accordance with these results, Koral et al. (25) reported that the formation of BAs depends on several factors, with low temperature slowing bacterial growth and enzyme activity. Similarly, Suzzi and Gardini(22) reported that PUT can be produced

at low temperatures (4°C) due to the action of *psychrotrophic pseudomonas*. It can be concluded that higher temperatures (20°C) favor proteolytic and decarboxylating reactions, resulting in increased amine concentrations. Similar results reported by Bover-Cid et al. [26] indicated that microbial decarboxylases remain active at 15°C, when most microbial populations have reached the stationary growth or death phases.

Correlation between biogenic amines and β-galactosidase activity

Table 1 shows that the Pearson correlation coefficients relating the concentrations of PUT, PHE, and HIS to β-galactosidase activity. At initial pH5.5, a significant correlation was identified between PUT, PHE and HIS and β-galactosidase activity by *Pantoea agglomerans* ($P<0.01$), indicating that the β-galactosidase activity of *Pantoea agglomerans* influences the formation of PUT, PHE and HIS. A significant positive correlation between β-galactosidase activity and PUT ($r = 0.900$, $P<0.01$), PHE ($r = 0.862$, $P<0.01$), and HIS ($r = 0.895$, $P<0.01$) levels in *Pantoea agglomerans* was determined; these correlations were also present at the initial pH of 6.5. Many important bacterial behaviors are regulated by AHL-dependent QS, including exoenzyme production and antibiotic production(27). Fuqua et al. [11] reported that pH is a major factor that affects the production and stability of AHLs. Suzzi & Gardini [22] also reported that amino acid decarboxylases are influenced by pH, with acidic pH levels being optimal for microbial mechanisms. Based on the results shown in Table 1, the accumulation of PUT, PHE, and HIS by *Pantoea agglomerans* was in accordance with the level of β-galactosidase activity at different initial pH levels. However, few studies have investigated whether the formation of BAs by *Pantoea agglomerans* is regulated AHLs.

Table 1
Correlation between β-galactosidase activity and biogenic amines of *Pantoea agglomerans* cultured in sterilized medium at initial pH 5.5 and initial pH 6.5

	pH 5.5			pH 6.5		
Biogenic amines	PHE	PUT	HIS	PHE	PUT	HIS
β-galactosidase	0.711**	0.874**	0.723**	0.900**	0.862**	0.895**
	0.009	0.000	0.008	0.000	0.000	0.000
Note: **Correlation is significant at the $P<0.01$ level						
*Correlation is significant at the $P<0.05$ level						

Table 2 shows that the Pearson correlation coefficients relating the concentrations of PHE and TYR and β-galactosidase activity at 10°C and 20°C. At both 10°C and 20°C, the concentrations of TRY and PHE correlated significantly with β-galactosidase activity by *Pantoea agglomerans* ($P<0.05$), while the correlation was less significant of *Pantoea agglomerans* (PHE : $r = 0.584$, $P<0.05$; TRY: $r = 0.584$, $P<0.05$). These data indicate that the β-galactosidase activity of *Pantoea agglomerans* influences the formation

of TYR and PHE at both temperatures. Most of the growth-associated enzymes are known to exhibit optimal levels of activity at the same temperature at which optimal growth occurs. Microbial growth and enzyme activity were inhibited by temperature, as described previously by a number of authors(22)–28; It is well-known that microbial pathways represent the predominant mechanism by which BAs are produced. This is consistent with the findings of this study, in which BAs changes were reflected by alteration β -galactosidase activity at different temperature.

Table 2
Correlation of β -galactosidase activity and biogenic amines of *Pantoea agglomerans* cultured in sterilized medium at 10°C and 20°C.

	10°C		20°C	
Biogenic amines	PHE	TRY	PHE	TRY
β -galactosidase	0.641*	0.733**	0.823**	0.852**
	0.025	0.007	0.001	0.000
Note: **Correlation is significant at the $P < 0.01$ level				
*Correlation is significant at the $P < 0.05$ level				

Conclusions

In this study, the predominant BA-producing strain (*Pantoea agglomerans*) was shown to produce AHLs and exhibited the capacity to induce the β -galactosidase activity expressed by the biosensor strain KYC55 under different incubation conditions. The activity of β -galactosidase was produced by *Pantoea agglomerans* was higher at pH 6.5, compared with pH 5.5. AHLs production of *Pantoea agglomerans* was inhibited low temperature, the produnction ability of β -galactosidase at 10°C (150.68 Miller Units) was lower than that at 20°C (180.78 Miller Unit). Temperature and pH also can influence the accumulation of BAs by *Pantoea agglomerans*. The concentrations of HIS and PHE produced by *Pantoea agglomerans* at pH 6.5 showed higher than pH 5.5, and content of PUT at pH 6.5 was lower than that at pH 5.5. Furthermore, the accumulation of PHE and TRY by *Pantoea agglomerans* was inhibited by low temperature (10°C). Besides, A significant positive correlation obtained in the analysis of between BAs and β -galactosidase activity. Based on the results of above, it can be clearly indicated that the AHL-based QS system influences the concentrations and types of BAs by the BA-producing strain.

Declarations

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References

1. Gong, X., Qi, N., Wang, X., Lin, L., & Li, J. (2014). Ultra-performance convergence chromatography (UPC(2)) method for the analysis of biogenic amines in fermented foods. *Food Chem*, 162, 172-175.
2. Lu, S., Xu, X., Zhou, G., Zhu, Z., Meng, Y., & Sun, Y. (2010). Effect of starter cultures on microbial ecosystem and biogenic amines in fermented sausage. *Food Control*, 21(4), 444-449.
3. Onal, A., Tekkeli, S. E., & Onal, C. (2013). A review of the liquid chromatographic methods for the determination of biogenic amines in foods. *Food Chem*, 138(1), 509-515.
4. Bai, X., Byun, B. Y., & Mah, J. H. (2013). Formation and destruction of biogenic amines in Chunjang (a black soybean paste) and Jajang (a black soybean sauce). *Food Chem*, 141(2), 1026-1031.
5. Nie, X., Zhang, Q., & Lin, S. (2014). Biogenic amine accumulation in silver carp sausage inoculated with *Lactobacillus plantarum* plus *Saccharomyces cerevisiae*. *Food Chem*, 153, 432-436.
6. Loizzo, M. R., Menichini, F., Picci, N., Puoci, F., Spizzirri, U. G., & Restuccia, D. (2013). Technological aspects and analytical determination of biogenic amines in cheese. *Trends in Food Science & Technology*, 30(1), 38-55.
7. Landeta, G., Curiel, J. A., Carrascosa, A. V., Munoz, R., & de las Rivas, B. (2013). Technological and safety properties of lactic acid bacteria isolated from Spanish dry-cured sausages. *Meat Sci*, 95(2), 272-280.
8. Geske, G. D., Mattmann, M. E., & Blackwell, H. E. (2008). Evaluation of a focused library of N-aryl L-homoserine lactones reveals a new set of potent quorum sensing modulators. *Bioorg Med Chem Lett*, 18(22), 5978-5981.
9. Estephane, J., Dauvergne, J., Soulere, L., Reverchon, S., Queneau, Y., & Doutheau, A. (2008). N-Acyl-3-amino-5H-furanone derivatives as new inhibitors of LuxR-dependent quorum sensing: Synthesis, biological evaluation and binding mode study. *Bioorg Med Chem Lett*, 18(15), 4321-4324.
10. Gobbetti, M., De Angelis, M., Di Cagno, R., Minervini, F., & Limitone, A. (2007). Cell-cell communication in food related bacteria. *Int J Food Microbiol*, 120(1-2), 34-45.
11. Fuqua, C., Parsek, M.R., Greenberg, E.P., (2001): Regulation of gene expression by cell to cell communication: acyl-homoserine lactone quorum sensing. *Annual Review of Genetics*, (35), 439-468.
12. Munoz-Atienza, E., Landeta, G., de las Rivas, B., Gomez-Sala, B., Munoz, R., Hernandez, P. E., Cintas, L. M., & Herranz, C. (2011). Phenotypic and genetic evaluations of biogenic amine production by lactic acid bacteria isolated from fish and fish products. *Int J Food Microbiol*, 146(2), 212-216.

- 24.** Tosukhowong, A., Visessanguan, W., Pumpuang, L., Tepkasikul, P., Panya, A., & Valyasevi, R. (2011). Biogenic amine formation in Nham, a Thai fermented sausage, and the reduction by commercial starter culture, *Lactobacillus plantarum* BCC 9546. *Food Chem*, 129(3), 846-853.
- 25.** Serkan Koral, Bekir Tufan, Andrej Ščavničar, Drago Kočar, Matevž Pompe, Sevim Köse.(2013). Investigation of the contents of biogenic amines and some food safety parameters of various commercially salted fish products. *Food Control*,32, 597-606.
- 26.** Bover-Cid, S., Hugas, M., Izquierdo-Pulido, M., &Vidal-Carou, M.C.(2000). Amino acid-decarboxylase activity of bacteria isolated from fermented pork sausages. *International journal of Food Microbiology*, 66, 185-189.
- 27.** Kai, K., Kasamatsu, K., & Hayashi, H. (2012). (Z)-N-(4-Decenoyl) homoserine lactone, a new quorum-sensing molecule, produced by endobacteria associated with *Mortierella alpina* A-178. *Tetrahedron Letters*, 53(40), 5441-5444.
- 28.** Latorre-Moratalla, M. L., Bover-Cid, S., Talon, R., Garriga, M., Zanardi, E., Ianieri, A., Fraqueza, M. J., Elias, M., Drosinos, E. H., & Vidal-Carou, M. C. (2010). Strategies to reduce biogenic amine accumulation in traditional sausage manufacturing. *LWT - Food Science and Technology*, 43(1), 20-25.

Figures

Figure 1

Detection AHLs of *Pantoea agglomerans* by cross-feeding plate assay. AHLs production is indicated by blue coloration of the reported strain.