Végéphyl – 12e CONFÉRENCE INTERNATIONALE SUR LES MALADIES DES PLANTES
TOURS – 11 ET 12 DÉCEMBRE 2018

INHIBITORY EFFECT OF *OLEA EUROPAEA* L. AND *CERATONIA SILIQUA* L. LEAVES EXTRACTS AGAINST *PECTOBACTERIUM ATROSEPTICUM*

S. MEZIANI [(1)], F. ZAIDI [(1)], N. HUGOUVIEUX-COTTE-PATTAT [(2)], C. BERTRAND [(4)]

[(1)] Faculty of Nature and Life Sciences, University of Bejaia, 06000 Bejaia, Algeria.
[(2)] Université Lyon 1, INSA-Lyon, CNRS UMR 5240, Microbiologie Adaptation et Pathogénie, Villeurbanne, F-69622, France.
[(3)] Laboratory of Chemistry of Biomolecules and Environment (LCBE), University of Perpignan Via Domitia, Perpignan, France.

RÉSUMÉ

L'activité antimicrobienne des extraits de plantes a été déterminée par la méthode de microdilution contre la bactérie pectinolytique (*Pectobacterium atrosepticum*, CFBP-5384), l'agent causal de la pourriture molle sur pomme de terre. L'oleuropéine a montré une activité intéressante contre la croissance bactérienne (CI50 = 0,2 mg / mL) et pourrait être utilisé pour l'inhibition de la croissance de la bactérie. La meilleure activité antibactérienne (CI50 = 1,5 mg / mL) contre *P. atrosepticum* a ete enregistré avec l'extrait acétonique des feuilles de caroubier. L'extrait de feuilles de *Ceratonia siliqua* inhibe l'enzyme pectate lyase, réduit la virulence de *P. atrosepticum* et peut donc être utilisé dans la lutte contre la pourriture molle de la pomme de terre.

Mots-clés : olive, caroube, composés phénoliques, activité antibacterienne, *Pectobacterium atrosepticum*.

ABSTRACT

The antimicrobial activity of the plant extracts was determined by the microdilution method against pectinolytic bacteria (*Pectobacterium atrosepticum,* CFBP-5384), the causative agent of potato soft rot disease. Oleuropein showed interesting activity against bacterial growth (IC_{50} = 0.2 mg/mL) and could be a promising candidate for bacterial growth inhibition. the best antibacterial activity (IC50 = 1.5 mg/mL) against *P. atrosepticum* was recorded with the acetone extract of the locust leaf. Extract of *Ceratonia siliqua* leaf inhibited pectate lyase reducing the virulence of *P. atrosepticum* and can therefore be used in the management of potato soft rot disease.

Keywords: Olive, Carob, Phenolic compounds, Antibacterial activity, *Pectobacterium atrosepticum*.

INTRODUCTION

The potato (*Solanum tuberosum* L.), the fourth most important crop in the world, can be affected by many biotic and abiotic factors, including pathogens and environmental stresses (Rauscher *et al.*, 2006). However, soft rot caused by a bacteria belonging to *Enterobacteriaceae* is among the major factors contributing to yield loss in this area. Potato soft rot is primarily caused by *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya spp*. (Gardan *et al.*, 2003; Samson *et al.*, 2005). *P. atrosepticum* more specifically associated with potato causes soft rot and blackleg, affecting both yield and tuber quality (Ma *et al.*, 2007) under cold and temperate climates. *P. atrosepticum*, a pectinolytic gram negative bacteria, produces extracellular enzymes such as pectate lyases, pectinases, cellulases and proteases, resulting in the tissue maceration and rot symptom (Toth *et al.*, 2003) affecting postharvest storage contributing to significant economic losses (Mills *et al.*, 2004). Pectate-lyases have a predominant role in this process due to their capacity to depolymerize pectin, a polysaccharide that acts as cement in the plant cell wall (Hugouvieux-Cotte-Pattat *et al.*, 1996).
There are currently no efficient curative methods to protect potato against *Pectobacterium ssp* (Latour *et al.*, 2008); chemical bactericides are generally ineffective. Thus, control of these soft rot bacteria is based mainly on exclusion or reduction of potential inoculums (Gracia-Garza *et al.*, 2002). Methods reducing the development of diseases caused by *Pectobacterium spp*. are therefore essential. Secondary metabolites, natural plant products, could be used against the microbial pathogens, based on their toxic nature and repellence to herbivores and microorganisms (Mazid *et al.*, 2011). Polyphenols, synthesized through the phenylpropanoid pathway, are involved in many ecological and physiological functions in plants and could be an important part of their defence system against pathogens and diseases (Wuyts *et al.*, 2006).
Olea europaea L. and *Ceratonia siliqua* L., typical Mediterranean trees that are common in Algeria with many applications in food and traditional medicine, contain high quantities of polyphenol compounds. Phenolic compounds extracted from olive leaf possess antimicrobial activity against a wide variety of bacteria (Sudjana *et al.*, 2009). Carob leaves possess antioxidant and/or antimicrobial properties (Kivçak *et al.*, 2002; Makris & Kefalas, 2004, Meziani *et al.*, 2015).
This study aims to evaluate the effects of acetone and ethanol extracts of *O. europaea* and *C. siliqua* leaves against the growth and production of virulence factors of *P. atrosepticum in vitro*.

MATERIAL AND METHODS

Plant material

Two typical Mediterranean trees common in Algeria were selected based on their traditional curative traits, natural abundance, and sustainable utilization. Leaves of olive (*Olea europaea* L. cultivar *aimel*) and carob (*Ceratonia siliqua* L.) were collected from Tazmalt (A/MIRA pilot farm) and Aftis, Bejaia. The leaves were washed with distilled water to remove all impurities, air dried at room temperature and ground to a fine powder in an electric blender (Super Blender National®, Japan). The samples were stored under vacuum in a desiccator prior to analysis.

Extraction

Samples (2 g) were extracted with 50 mL of acetone/water or ethanol/water (80/20, v/v) by magnetic stirring for 1 h at room temperature according to Ranalli *et al.* (2006). Extracts were filtered through filter paper (10 μm), centrifuged (5000 rpm, 10 min) and the extraction process repeated twice with the residue following the same protocol. The supernatants were pooled and the solvents evaporated under vacuum on a rotary evaporator (Büchi, R-114) at 40°C. The crude extracts were stored at -20°C until use.

Bacterial strain

The strain *Pectobacterium atrosepticum* (*Pca*, strain CFBP-5384) used in this study was provided by the Culture Stock Collection of Phytopathogenic Bacteria (CFBP, INRA Angers, France). It was maintained as deep-frozen cultures (-80°C) in Luria-Bertani (LB) medium supplemented with 30%

glycerol.
Before inoculation, a bacteria suspension was prepared in sterile distilled water (SDW) from cultures on King's B Agar medium, and incubated at 25°C.

Antibacterial activity

The National Committee for Clinical Laboratory Standard method (NCCLS, 2006) for the microplate bioassay (micro-dilution) was used with some modifications to evaluate the antimicrobial activities of *P. atrosepticum*. Extracts and oleuropein were dissolved in 1% dimethylsulphoxide (DMSO) (Merck, Darmstadt, HE, Germany); samples that were difficult to dissolve were sonicated, first diluted to highest concentration (10 mg/mL) to be tested and then serially diluted two-fold in a concentration range from 0.625 to 10 mg/mL. Bacterial suspension was prepared in liquid nutritive media; suspensions were diluted in distilled water to obtain final inoculums of 2.10^5 cfu/mL.
The 96-well microplates (250 µL volume, Fisher Scientific) were prepared by dispensing into each well 198 µL of microorganism suspension and 2 µL of the initially prepared diluted extracts. The last well containing 198 µL of liquid medium and 2 µL of diluted extract was used as negative control and the positive control well contained 200 µL of the bacterial inocula without the extract. The final volume in each well was 200 µL. The plates were covered with sterile plate sealer, agitated to mix the content of the wells using a plate shaker and incubated at 25°C for 24 h. Bacterial growth was estimated by measuring absorbance in microplate wells at 600 nm (Aviso Microplate Absorbance Reader, Sirius HT, Ebersberg, Germany). The MIC was defined as the lowest concentration preventing visible growth. The IC_{50}, lowest concentration of extracts inhibiting 50% of the bacterial growth was determined graphically using 100% of growth as the positive control (table I). All samples were tested in triplicate.

Pectate lyase activity

Pectate lyase activity was determined with a spectrophotometer by monitoring the formation of 4,5 unsaturated oligogalacturonides at 230 nm by the degradation of polygalacturonate (Tardy *et al.*, 1997). Three replicates were carried out for each extract. Specific activity was expressed as micromoles of unsaturated products liberated per minute per milligram of bacterial dry weight (µmol/min/mg).

Statistical analysis

Each assay was repeated in triplicate and the results were presented as mean±SD. Statistical difference between results was compared by un-paired t-test, p values less than 0.05 were considered as significant. Significant differences between means were determined by Fisher LSD test according to STATISTICA methods.

RESULTS

Antibacterial activity of plant extracts

Acetone and ethanol leaf extracts were tested for potential antibacterial activity against *P. atrosepticum* (CFBP-3584). The concentration of extracts giving 50% inhibition of the bacterial growth was determined (Table 1).
All extracts displayed antimicrobial activities against *P. atrosepticum* with IC_{50} values between 1.5 and 4.9 mg/mL. However, the highest antimicrobial activity was more observed with acetone extracts than ethanol extracts. Oleuropein, the major phenolic component of olive leaf extracts showed the most potent activity against the bacterial growth (IC_{50} = 0.2 mg/mL).

Table 1. Antimicrobial activity of plant extracts and oleuropein against *P. atrosepticum* expressed as MICs and IC_{50} (mg/mL).
Tableau 1. Activité antimicrobienne des extraits de plantes et de l'oleuropéine contre *P. atrosepticum exprimée en* MICs et CI50 (mg/ml)

Samples	IC_{50} (mg/mL)	MIC (mg/mL)
Oleuropein	0.2	nd
Olive leaf extract acetone	2.4	9.8
Olive leaf extract ethanol	4.9	nd
Carob leaf extract acetone	1.5	6.2
Carob leaf extract ethanol	2.2	9.0

Production of plant cell wall degrading enzymes

The potential effect of the acetone and ethanol extracts on the production of pectate lyase was tested in conditions generating, or not, the formation of pectate lyase inducers. Pectate lyase production was highly induced when bacteria were grown in medium supplemented with both PGA and $CaCl_2$ (Table 2). Both acetone and ethanol extracts of *O. europaea* leaves weakly induced pectate lyase activity. No significant effect was observed with oleuropein. In contrast, the acetone and ethanol extracts of *C. siliqua* leaves strongly inhibited the pectate lyase activity (Table 2).

Table 2. Effect of leaf extracts and oleuropeine on the production of pectatelyases by *P. atrosepticum*.
Tableau 2. Effet des extraits de feuilles et de l'oleuropéine dans la production de pectate lyase par *P. atrosepticum*.

Samples	Pectate lyase production (µmol min^{-1} mg^{-1})		
	Concentration of extract (mg/ml)	Non induced condition (M63Y medium)	Induced condition (M63Y medium + PGA, $CaCl_2$)
Control	0	0.0069±0,001^{b}	2.15±0,0081^{b}
Oleuropein	0.1	0.0076±0,001^{b}	2.21±0,008^{b}
Olive leaf extract acetone	0.6	0.0091±0,001^{ab}	3.59±0,065^{a}
Olive leaf extract ethanol	0.6	0.0143±0,000^{a}	3.54±0,079^{a}
Carob leaf extract acetone	0.6	0.0018±0,000^{c}	0.02±0,004^{c}
Carob leaf extract ethanol	0.6	0.0023±0,000^{c}	0.14±0,005^{c}

DISCUSSION

Antibacterial activity of plant extracts

The extracts exhibited antibacterial activity against *P. atrosepticum* strain CFBP-3584 (Table 1) with IC_{50} values ranging from 0.2 and 4.9 mg/mL. Oleuropein inhibited *P. atrosepticum* at a similar rate to those of oleuropein acid hydrolysate on *Erwinia carotovora* (IC_{50}~ 0.18 mg/mL) due to the antibacterial activity of elenolic acid (Fleming *et al*., 1973).
MICs for acetone leaf extracts of *C. siliqua* and *O. europaea* (6.2 and 9.8 mg/ml, respectively) were lower than their corresponding ethanol extracts as described by Meziani *et al.* (2015). For ethanol extract of olive leaf, MIC is higher than the detection limit of 10 mg/mL. No MIC data for *P. atrosepticum* were available in literature to our knowledge. Nissen *et al*. (2010) reported MICs of essential oils (>0.2%v/v) of three industrial hemp varieties tested for *P. carotovorum* susbsp

carotovorum. Benzylacetophenone (an antibiotic produced by an entomopathogenic bacterium) MIC was higher than 10 mg/mL for *P. carotovorum* subsp *atrosepticum* (Ji *et al*., 2004).
All extracts showed antibacterial activity against *P. atrosepticum* but acetone extract in the present work demonstrated an interesting potential for developing an antimicrobial product. The sensitivity of *P. atrosepticum* to acetone and ethanol extracts was probably due to different amount and classes of active phytochemicals. Polyphenolic compounds with different structures such as flavonoids and tannins are known to inhibit the growth of microorganisms (Chung *et al.*, 1998). The antimicrobial action of phenols is related to their ability to denature proteins, and they are generally classified as surface-active agents. Those polyphenolics compounds caused leakage of cytoplasmic constituents such as proteins, glutamate, potassium and phosphate from bacteria inducing disruption of peptidoglycan layer or damaging cell membrane. Phenolic toxicity also includes enzyme inhibition, possibly through reactions with sulfhydryl groups or through nonspecific interactions with the proteins (Cowan, 1999).
The olive leaf is known to be resistant to microbial attacks and much research has focused on the antimicrobial activity of compounds contained in olive extracts (Paudel *et al*., 2011). The phenolic compounds of olive leaf extracts are effective antibacterial agents against several microorganisms that may be causal agents of human intestinal and respiratory tract infections (Pereira *et al*., 2007). The strong growth inhibition shown by oleuropein against *P. atrosepticum* (IC_{50} of 0.2 mg/mL) was consistent with the antimicrobial activity previously reported against various pathogens (Korukluoglu *et al*., 2006; Long *et al*., 2010).
The acetone extract of *C. siliqua* leaves exerted the best antibacterial activity (Table 1). This extract had the highest total phenolic content containing polyphenols known to possess efficient antibacterial activity (Velioglu *et al*., 1998). The antimicrobial activity of gallotannins and their structure activity-relationship have been described earlier (Tian et *al*., 2009). *P. atrosepticum* inhibition by carob leaf extracts may also be related to the general antimicrobial properties of tannins through enzyme inhibition/inactivation by forming complex with enzymes and other microbial proteins, and/or inhibiting electron transport (Chung *et al.*, 1998).

Production of plant cell degrading enzymes

The soft rot symptom caused by *P. atrosepticum* is due to the secretion of enzymes able to degrade the plant cell components. Both acetone and ethanol extracts of *O. europaea* leaves weakly induced pectate lyase activity (Table 2).
Lyon and McGill (1989) reported that several phenolic compounds inhibit bacterial growth, but only a few inhibit the activity of *P. carotovorum* pectic enzymes, polygalacturonases and pectate lyases. Thus, they could play a role in bacterial growth, production and activity of pectate lyases. Different plant extracts cause an increased induction of *Dickeya dadantii* pectate lyases in the presence of polygalacturonate, a phenomenon called hyperinduction (Bourson *et al*., 1993). Ferulic acid is reported to provoke such hyperinduction (Hassan & Hugouvieux-Cotte-Pattat, 2011). The increase in pectate lyase production induced by olive leaf extract could be the result of such hyperinducing compounds. To our knowledge, this study is the first report of a strong inhibition of pectate lyase production with plant extracts, namely those of carob leaves (Table 2).
Pectate lyase is inhibited by gallic and tannic acids that reduce growth and virulence of *P. chrysanthemi* with tannic acid being more effective on enzyme inactivation than gallic acid (Zaidi-Yahiaoui *et al*., 2008). Gallotannins and gallic acid derivatives were only identified in acetone extract of carob leaves corresponding to the gallic acid dominated polyphenolic profile of carob fibre (Owen *et al*., 2003); this specific activity is most likely due to this class of compounds. Antilisterial activity has also been reported for methanol extract of carob leaves rich in gallic acid derivatives and myricitrin (Aissani *et al*., 2012).

CONCLUSION

This study describes the *in vitro* antimicrobial activity of extracts from leaves of *O. europaea* cultivar *aimel* (olive) and *C. siliqua* (carob), typical Mediterranean trees commonly found in Algeria, against the pectinolytic bacterium *P. atrosepticum*. These plants were selected based on their high polyphenolic content in carob and olive leaves. The acetone extracts of these leaves exhibited potent antimicrobial activity contributed by the presence and abundance of polyphenols,

flavonoids and tannins in considerable quantities. Our results showed that oleuropein has the potential to limit the *in vitro* multiplication of *Pectobacterium* species causing soft rot on potato tubers.

Olea europaea leaf extracts have a slight inducing effect on the production of pectate lyases by *P. atrosepticum*. In contrast, the leaf extracts of *C. siliqua* displayed strong inhibition and may be a good source of pectate lyase inhibitor. Carob leaf extract is therefore a potential tool in managing potato soft rot diseases due to *Pectobacterium* species by its capacity to inhibit both bacterial growth and the production of the main virulence factor.

BIBLIOGRAPHIE

Aissani N., Coroneo V., Fattouch S. & Caboni P., 2012. Inhibitory effect of carob (*Ceratonia siliqua*) leaves methanolic extract on *Listeria monocytogenes*. *Journal of Agricultural and Food Chemistry*, 60, 9954-9958.

Bourson C., Favey S., Reverchon S., Robert-Baudouy J., 1993. Regulation of the expression of a *pelA: uidA* fusion in *Erwinia chrysanthemi* and demonstration of the synergistic action of plant extract with polygalacturonate on pectate lyase synthesis. *Journal of Genetic Microbiology*, 139, 1-9.

Chung K.T., Wong T.Y., Wei C.I., Huang Y.W. & Lin Y., 1998. Tannins and human health: a review. *Critical Review of Food Science and Nutrition*, 38, 421-464.

Cowan M.M., 1999. Plant products as antimicrobial agents. *Clinical Microbiological Review*, 12, 564-582.

Fleming H.P., Walter Jr. W.M. & Etchells J.L., 1973. Antimicrobial properties of oleuropein and products of its hydrolysis from green olives. *Applied Microbiology*, 26, 777-782.

Gardan L., Gouy C., Christen R. & Samson R., 2003. Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. *International Journal of Systematic Evolution and Microbiology*, 53, 381-391.

Gracia-Garza J.A., Blom T.J., Brown W. & Allen W., 2002. Pre- and post-plant applications of copper-based compounds to control *Erwinia* soft rot of calla lilies. *Canadian Journal of Plant Pathology*, 24, 274-280.

Hassan S. & Hugouvieux-Cotte-Pattat N., 2011. Identification of two feruloyl esterases in *Dickeya dadantii* 3937 and induction of the major feruloyl esterase and of pectate lyases by ferulic acid. *Journal of Bacteriology*, 193, 963-970.

Hugouvieux-Cotte-Pattat N., Condemine G., Nasser W. & Reverchon S., 1996. Regulation of pectinolysis in *Erwinia chrysanthemi*. *Annual Review of Microbiology*, 50, 213-257.

Ji D., Yi Y., Kang G.H., Choi Y.H., Kim P., Baek N.I. & Kim Y., 2004. Identification of an antibacterial compound, benzyldeneacetone, from *Xenorhabdus nematophila* against major plant-pathogenic bacteria. *FEMS Microbiology Letter*, 239, 241-248.

Kivçak B., Mert T. & Ozturk H.T., 2002. Antimicrobial and cytotoxic activity of *Ceratonia siliqua* L. extracts. *Turk Journal of Biology*, 26, 197–200.

Korukluoglu, M., Sahan, Y., Yigit, A., Karakas, R., 2006. Antifungal activity of olive leaf (*Olea europaea* L.) extracts from the Trilye region of Turkey. *Ann. microbial.* 56 (4), 359-362.

Latour X., Faure D., Diallo S., Cirou A., Smadja B., Dessaux Y. & Orange N., 2008. Lutte contre les maladies bactériennes de la pomme de terre dues aux *Pectobacterium* spp. (*Erwinia carotovora*). *Cahier Agriculture*, 17, 355-360.

Long, H.S., Tilney, P.M., Van Wyk, B.-E., 2010. The ethnobotany and pharmacognosy of *Olea europaea* subsp. *africana* (*Oleaceae*).South Afr. J. Bot. 76, 324-331.

Lyon G.D., McGill, F.M., 1989. Inhibition of polygalacturonase and polygalacturonic acid lyase from *Erwinia carotovora* by phenolics *in vitro*. Potato res. 32, 267-274.

Ma B., Hibbing M.E., Kim H.-S., Reedy R.M., Yedidia I., Breuer J., Breuer J., Glasner J.D., Perna N.T. & Kelman A., 2007. Host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya*. *Phytopathology*, 97, 1150-1163.

Makris D.P. & Kefalas P., 2004. Carob pods (*Ceratonia siliqua* L.) as a source of polyphenolic antioxidants. *Food Technology and Biotechnology*, 42, 105-108.

Mazid M., Khan T. A. & Mohammad F., 2011. Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine*, 3, 232-249.

Mills A.A.S., Platt H.W. & Hurta R.A.R., 2004. Effect of salt compounds on mycelial growth, sporulation and spore germination of various potato pathogens. *Postharvest Biology and Technology*, 34, 341-350.

Meziani S., Oomah B.D., Zaidi F., Simon-Levert A., Bertrand C., Zaidi-Yahiaoui R., 2015. Antibacterial activity of carob (*Ceratonia siliqua* L.) extracts against phytopathogenic bacteria *Pectobacterium atrosepticum*. *Microbial Pathogenesis*, 78, 95-102.

NCCLS, 2006. National Committee for Clinical Laboratory Standards). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobicaly. *Approved Standard, NCCLS Document M7-A7, 7th ed*. Vilanova, PA.

Nissen L., Zatta A., Stefanini I., Grandi S., Sgorbati B., Biavati B. & Monti A., 2010. Characterization and antimicrobial activity of essential oils of industrial hemp varieties (*Cannabis sativa* L.). *Fitoterapia,* 81, 413-419.

Owen, R., Haubner, R., Hull, W., Erben, G., Spiegelhalder, B., Bartscha, H., 2003. Isolation and structure elucidation of the major individual polyphenols in carob fibre. *Food Chem. Toxicol*. 41, 1727-1738.

Paudel S., Magrati T. & Lamichhane J.R., 2011. Antimicrobial activity of wild olive crude extracts *in vitro*. *International Journal of Pharma Sciences and Research*, 2, 110-113.

Pereira A.P., Ferreira I.C.F.R., Marcelino F., Valentão P., Andrade P.B., Seabra R., Estevinho L., Bento B., Pereira J.A., 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. *cobrançosa*) leaves. *Molecules.* 12 (5), 1153-1162.

Ranalli A., Contento S., Lucera L., Di Febo M., Marchegiani D. & Di Fonzo V., 2006. Factors affecting the contents of iridoid oleuropein in olive leaves (*Ole aeuropaea* L.). *Journal of Agricultural and Food Chemistry*, 54, 434-440.

Rauscher G.M., Smart C.D., Simko I., Bonierbale M., Mayton H., Greenland A. and Fry. W.E., 2006. Characterization and mapping of $R_{pi\text{-}ber}$, a novel potato late blight resistance gene from *Solanum berthaultii*. *Theoretical and Applied Genetics* , 112, 674-687.

Samson R., Legendre J.B., Christen R., Achouak W. & Gardan L., 2005. Transfer of *Pectobacterium chrysanthemi* (Brenner et *al.,* 1973) and *Brenneria paradisiacal* (Hauben et al., 1998) to the genus Dickeya gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiacal* comb. nov. and delineation of four novel species: *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. *Int. International Journal of Systematic and Evolution Microbiology*, 55, 1415-1427.

Sudjana A.N., D'Orazio C., Ryan V., Rasool N., Ng N., Islam J., Riley T.V. & Hammer K.A., 2009. Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. *International Journal of Antimicrobial Agents*, 33, 461-463.

Tardy F., Nasser W., Robert-Baudouy J. & Hugouvieux-Cotte-Pattat N., 1997. Comparative analysis of the five major *Erwinia chrysanthemi* pectate lyases: enzyme characteristics and potential inhibitors. *Journal of Bacteriology*, 179, 2503-2511.

Tian F., Li B., Ji B., Zhang G. & Luo Y., 2009. Identification and structure–activity Relationship of gallotannins separated from *Galla chinensis*. *LWT-Food Science and Technology*, 42, 1289-1295.

Toth I.K., Bell K.S., Holeva M.C. & Birch P.R.J., 2003. Soft rot erwiniae: From genes to genomes. *Molecular Plant Pathology*, 4, 17-30.

Velioglu Y.S., Mazza G., Gao L. & Oomah B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, 54, 4113-4117.

Wuyts N., De Waele D. & Swennen R., 2006. Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminate* grandenaine) roots. *Plant Physiology and Biochemistry*, 44, 308-314.

Zaidi-Yahiaoui R., Zaidi F. & Bessai A.A., 2008. Influence of gallic and tannic acids on enzymatic activity and growth of *Pectobacterium chrysanthemi* (*Dickeya chrysanthemi* bv. *chrysanthemi*). *African Journal of Biotechnology*, 7, 482-486.