JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

EFFECTS OF PSYCHOTROPIC DRUGS AS BACTERIAL EFFLUX PUMP INHIBITORS ON QUORUM SENSING REGULATED BEHAVIORS

Aynur Aybey, Alev Usta , Elif Demirkan*

Address(es): Aynur Aybey
[1] Uludağ University, Faculty of Arts and Sciences, Biology Department, 16059, Gorukle, Nilufer-Bursa, Turkey, phone number: +90 224 2941794.

*Corresponding author: aybeyaynur@gmail.com

doi: 10.15414/jmbfs.2014.4.2.128-131

ARTICLE INFO

Received 6. 10. 2013
Revised 21. 8. 2014
Accepted 22. 8. 2014
Published 1. 10. 2014

Regular article

OPEN ACCESS

ABSTRACT

Psychotropic drugs are known to have antimicrobial activity against several groups of microorganisms. The antidepressant agents such as duloxetine, paroxetine, hydroxyzine and venlafaxine are shown to act as efflux pump inhibitors in bacterial cells. In order to the investigation of the effects of psychotropic drugs were determined for clinically significant pathogens by using standart broth microdillusion method. The anti-quorum sensing (anti-QS) activity of psychotropic drugs was tested against four test pathogens using the agar well diffusion method. All drugs showed strong inhibitory effect on the growth of *S. typhimurium.* Additionally, quorum sensing-regulated behaviors of *Pseudomonas aeruginosa*, including swarming, swimming and twitching motility and alkaline protease production were investigated. Most effective drugs on swarming, swimming and twitching motility and alkaline protease production, respectively, were paroxetine and duloxetine; duloxetine; hydroxyzine and venlafaxine; paroxetine and venlafaxine; venlafaxine. Accordingly, psychotropic drugs were shown strongly anti-QS activity by acting as bacterial efflux pump inhibitors and effection on motility and alkaline protease production of *P. aeruginosa*.

Keywords: Anti-quorum sensing activity, efflux pump, motility, psychotropic drugs, *P. aeruginosa*

INTRODUCTION

Efflux bacterial systems involved in quorum sensing (QS) contribute to multidrug resistance as they expel different types of antibiotics and chemicals such as dyes, organic solvents, detergents, molecules needed for the cell to cell communication, biocides, and metabolic products. Hence understanding the mechanisms by which these pumps act and how to overcome its activity opens the door for restoring the antibacterial activity and constitute a promising target for novel antibacterial agents **(Vidal *et al.*, 2009; Dreier, 2007; Piddock, 2006; Schweizer, 2003)**. The continuous increase in the development of multidrug resistance by many pathogens has resulted in difficulties fighting many infectious diseases. In view of the fact that the majority of those multidrug resistant pathogens expresses and overproduces efflux pumps that are responsible for the expelling and extruding of the antibiotics from inside the cells, the new direction for other chemotherapeutics is the use of efflux pump inhibitors **(Page's and Amaral, 2009)**. Effection of bacterial quorum sensing (QS) systems could be an effective alternative in as much as they regulate a broad spectrum of cell functions, including virulence factor production and motility. Efflux bacterial systems are known related to quorum sensing. The recent research focus is to develop antipathogenic agents to control bacterial diseases by inhibiting QS **(Adonizio *et al.*, 2008; Al-Hussaini and Mahasneh, 2009)**.
The pathogenic bacterium *Pseudomonas aeruginosa* uses quorum sensing systems to regulate genes controlling virulence, motility and biofilm formation. *P. aeruginosa* has three modes of surface motility: swarming **(Overhage *et al.*, 2008; Verstraeten *et al.*, 2008)**, twitching **(Semmler *et al.*, 1999; Skerker and Berg, 2001)**, and swimming **(Murray and Kazmierczak, 2008)**. Twitching and swarming motilities are strongly linked with biofilm development and pathogenesis, and their mechanical components, the flagellum and type IV pili (TFP), have been demonstrated to be virulence factors **(Overhage *et al.*, 2008; Murray and Kazmierczak, 2008)**. Swarming, swimming and twitching are strongly related to disease **(Allison *et al.*, 1992; Elvers and Lappin-Scott, 2000)**. Bacterial proteases have the potential to destroy the structural and functional proteins that constitute host tissues as well as to destroy proteins important in host defense. Expression of many of the virulence factors (including *LasB* elastase, *LasA* protease, alkaline protease) in *P. aeruginosa* is controlled by quorum sensing (QS) system **(Venturi, 2006).** Antimicrobial activity was described in some psychotropic drugs **(Cederlund *et al.*, 1993; Kristiansen, 1990; Kristiansen, 1992).** The studies show that these psychotropic drugs, namely sertraline, fluoxetine and paroxetine have a significant antibacterial activity, mainly against several groups of pathogen bacteria and they may act as efflux pumps inhibitors since they also act on human cells and bacterial cells as pump inhibitors **(Munoz-Bellido *et al.*, 2002).** Psychotropic drugs are capable of inhibiting slime production in coagulase-negative staphylococci **(Mun˜oz Criado *et al.*, 1997),** and inhibit swarming in Proteus **(Mun˜oz Criado *et al.*, 1998).**
The aim of this study was to determine the anti-QS activity, effects of psychotropic drugs on efflux pump of clinically important pathogens and QS-controlled cellular functions including motility and alkaline protease production of *P. aeruginosa.*

MATERIAL AND METHODS

Bacterial strains, growth and media conditions

Test pathogens including *Yersinia enterolitica* (ATCC 9610), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 35032) were maintained in 2% Luria Bertani (LB) broth (1% tryptone, 0.5% yeast extract, 0.5% (w/v) NaCl) and on LB agar (supplemented with 1% [w/v] agar) prior to inoculation into the motility assays. Swarm, swim, and twitch media were prepared to assess the corresponding type of motility. Swarm medium consisted of 8 g/L nutrient broth and 0.5% (w/v) agar supplemented with D-glucose (5 g/L, filter sterilized and added separately). Swim and twitch media consisted of LB broth supplemented with 0.3% and 1.0% (w/v) agar, respectively **(Wiegand *et al.*, 2008)**. Alkaline protease medium were consisted of LB broth supplemented with 1.5% (w/v) agar and 2% (w/v) skim milk. For minimal inhibitory concentration (MIC) assay were used Mueller Hinton broth (MHB). All culture strains were incubated at 37° C for 24 hours. Psychotropic drug stock solutions were prepared by dissolving duloxetine, paroxetine, hydroxyzine and venlafaxine in distilled water (100, 75, 50, 37.5, 25, 18.75, 12.5, 9.375, 6.25 mM), followed by heating to 100°C and filter sterilization using 0.2 µm pore size filters.

Minimal inhibitory concentration (MIC) assay

MIC was assessed using the standard broth microdilution protocol as described by **Wiegand *et al.* (2008)** with a change in incubation time from 20 hour to 24 hour. Overnight cultures were grown in Mueller Hinton broth (MHB) at 37°C and diluted to yield an inoculum of approximately 1×10^6 CFU/ml. Cultures were further diluted 1/100, and inoculated with 100, 75, 50, 37.5, 25, 18.75, 12.5, 9.375, 6.25 mM drug solutions in a polystyrene 96-well plates. Growth was done for 24 hour at 37°C. After growth, MIC value taken as the concentration that inhibited macroscopic growth was observed, and also determined by spectrophotometer.

Agar well diffusion assay

Agar well diffusion assay was used to detect anti-QS activity of the psychotropic drugs. Bacterial pathogens *Y. enterolitica* (ATCC 9610), *S. aureus* (ATCC 25923), *S. typhimurium* (ATCC 14028), *P. aeruginosa* (ATCC 35032) were used in this study. Cultures were grown and maintained on LB medium. Overnight cultures were prepared by diluting culture suspension in 0.9% saline to achieve an absorbance of 0.3 at 600 nm as determined by a spectrophotometer. A sterilized cotton swab was dipped in the diluted cultures and spread over the surface of the LB agar **(Nathan *et al.*, 1978)**. Wells were made in the inoculated agar medium under sterille conditions (7 mm in diameters), and 100 µl of each psychotropic drug determined MICs was loaded onto the well in LB plates. The plates were incubated 37°C for 24 hour and anti-QS was detected by measuring the clear zone in diameter.

Motility and alkaline protease production

Motility assays were undertaken in petri dishes. The swarming, swimming and twitching motilities, and alkaline protease production of *P. aeruginosa* were performed using swarm, swim, twitch and alkaline protease plates. The MIC concentration of each psychotropic drug was added to plates prior to a 30–45 min drying period. For the swimming, swarming, twitching assays and inhibition of alkaline protease production, the bacterial cells were gently inoculated using a toothpick at the center of the agar surface **(De´ziel *et al.*, 2003)** and the plates were incubated at 25°C for 16 hour, 37°C for 16–18 hour, 30°C for 16 hour and 37°C for 24 hour, respectively, and halo diameters were measured. Datas from swarming, swimming, twitching assays and alkaline protease production were used as control against zero inhibition exhibited on each media by no drug.

RESULTS AND DISCUSSION

Growth inhibition following psychotropic drugs treatment of pathogens, MICs were determined. MIC assay results were obtained with different concentrations of the duloxetine, paroxetine, hydroxyzine and venlafaxine. Respectively, it was determined as 75 mM, 100 mM, 75 mM, 100 mM for *Y. enterolitica*; 37.5 mM, 75 mM, 75 mM, 100 mM for *S. aureus*; 75 mM, 100 mM, 100 mM, 100 mM for *S. typhimurium*; 37.5 mM, 37.5 mM, 37.5 mM, 37.5 mM for *P. aeruginosa*. For further studies, these values were used for anti-QS activity against each pathogen bacteria, and also 37.5 mM concentration of the each psychotropic drugs was used on *P. aeruginosa* motility and alkaline protease production.

Four psychotropic drugs (duloxetine, paroxetine, hydroxyzine and venlafaxine) selected for their anti-QS activity were tested against four pathogens on LB plates. QS inhibition was detected by a clear zone, and the diameter of zone of inhibition was measured. As shown on table 1, duloxetine (30 mm) and venlafaxine (30 mm) on *Y. enterolitica*, hydroxyzine (35 mm) and venlafaxine (35 mm) on *S. typhimurium*, duloxetine (32 mm) on *S. aureus* and venlafaxine (33mm) on *P. aeruginosa* were effective. All drugs showed strong inhibitory effect on the growth of *S. typhimurium* (Fig 1[b]). As seen figure 1, maximum inhibition zone for duloxetine was showed *S. aureus* > *S. typhimurium*= *Y. enterolitica*> *P. aeruginosa;* for hydroxyzine was *S. typhimurium*> *S. aureus*> *P. aeruginosa*= *Y. enterolitica;* for venlafaxine was *S. typhimurium*> *P. aeruginosa*> *Y. enterolitica*> *S. aureus;* for paroxetine was *S. typhimurium*> *P. aeruginosa*> *Y. enterolitica*= *S. aureus.*

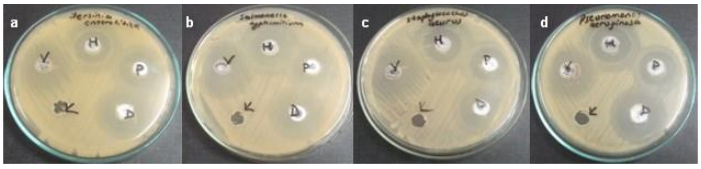

Figure 1 Anti-QS activities of four psychotropic drugs. (a) *Yersinia enterolitica*, (b) *Salmonella typhimurium*, (c) *Staphylococcus aureus*, (d) *Pseudomonas aeruginosa*. K:Control, D:Duloxetine, H:Hydroxyzine, P:Paroxetine, V:Venlafaxine.

Table 1 Inhibition zones of psychotropic drugs against *Y. enterolitica*, *S. typhimurium*, *S. aureus*, *P. aeruginosa*. (*Most effective drug inhibition zones).

	İnhibition zones (mm)			
	Duloxetine	**Paroxetine**	**Hydroxyzine**	**Venlafaxine**
Y. enterolitica	30*	23	21	30*
S. typhimurium	30	30	35*	35*
S. aureus	32*	23	25	29
P. aeruginosa	26	24	24	33*

Duloxetine, paroxetine, venlafaxine and hydroxyzine decreased the three types of motility of *P. aeruginosa* were observed in distinct decreases in halo diameter in the presence of drug concentrations at 37.5 mM (Fig 2 [1,2,3]). When comparing the percent inhibiton effect of psychotropic drugs on swarming, twitching and swimming motility, it was seen much greater effect of drugs on swarming and twitching (Fig 3).

Swarming, swimming and twitching motility assays showed decreases at 37.5 mM MIC concentration of each drug and for *P. aeruginosa*. It was determineted that for swarming, swimming and twitching motilities, respectively, the most effective drugs were paroxetine and duloxetine (Fig 2 [1b, 1d]), duloxetine (Fig 2 [2b]), paroxetine and venlafaxine (Fig 2 [3d,3e]). But significantly, swarm and twitch halo diameters of *P. aeruginosa* were very nearly inhibited in the presence of all drugs (Tab 2). As seen figure 3, swimming motility showed less inhibitory results than swarming and twitching motility, due to a lack of consistent replicates across three biological repeats. Also we observed smaller clear zones on alkaline protease plates that drug concentrations at 37.5 mM significantly effected alkaline protease production (Fig 2 [4]). The most effective drug on alkaline protease production was determineted as venlafaxine (Fig 2 [4d], Fig 3).

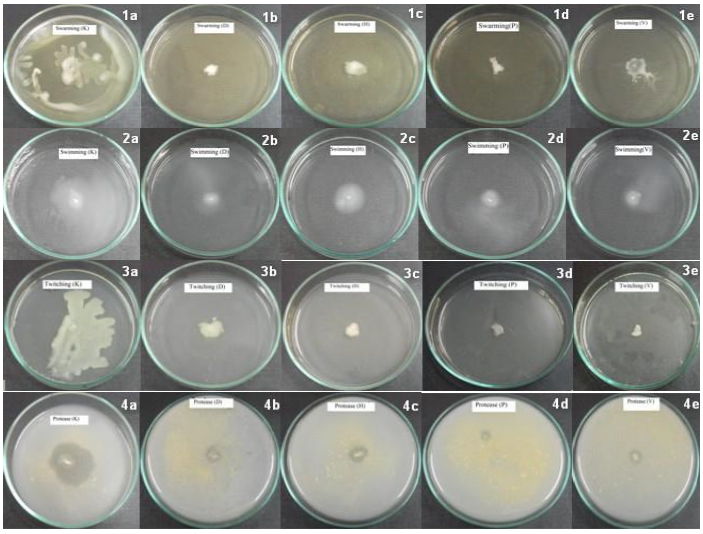

Figure 2 Inhibition of *P. aeruginosa* swarming (1), swimming (2), twitching (3) motility and alkaline protease activity (4) by psychotropic drugs (a:control, b:duloxetine, c:hydroxyzine, d:paroxetine, e:venlafaxine).

Table 2 *Pseudomonas aeruginosa* swarming, swiming, twitching motility and alkaline protease zones in the presence of psychotropic drugs.

***Pseudomonas aeruginosa* motility and alkaline protease zones (mm)**				
	Swarming	**Swimming**	**Twitching**	**Alkaline protease**
Control	60 ± 0	40 ± 0	50 ± 0	20 ± 0
Duloxetine	4 ± 1	4 ± 2	6 ± 1,5	4 ± 0,5
Paroxetine	3 ± 1	7 ± 0,5	5 ± 0,5	3 ± 1
Hydroxyzine	5 ± 2	8 ± 1	3 ± 1	4 ± 1
Venlafaxine	5 ± 2	6 ± 1,5	2 ± 0,5	2 ± 0,5

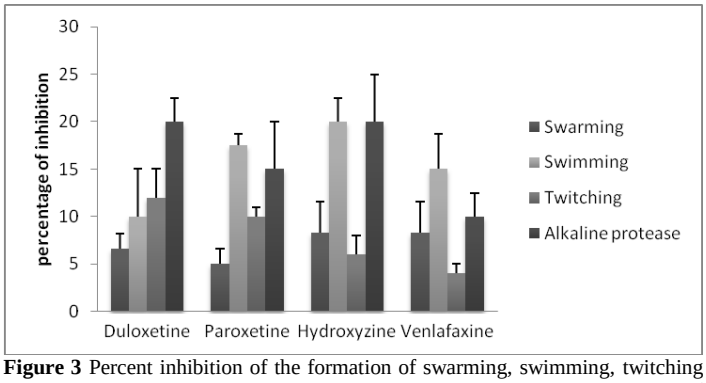

Figure 3 Percent inhibition of the formation of swarming, swimming, twitching motility and alkaline protease activity halo by *P. aeruginosa* in the presence of psychotropic drugs. (Values are normalized to no inhibition exhibited by the no drugs controls. The average ±SD for three samples is shown in each column).

Effects of psychotropic drugs on microorganisms is not limited to antimicrobial activity. They also act as efflux pump inhibitors **(Munoz-Bellido *et al.*, 2002)** and modify bacterial metabolism, as shown in studies on slime and swarming inhibition **(Mun˜oz Criado *et al.*, 1997)**. Our results show that psychotropic drugs have anti-QS properties that are mediated through their inhibition of efflux pumps that extrude the noxious QS signal before it reaches its intended target. Inhibition of QS activity is not cause by inhibition of growth; it can also be done by disrupsion of QS signalling systems without killing bacteria by using psychotropic drugs. **Hentzer *et al.* (2002)** demonstrated the use of a furanone compound as a QSI compound and have focussed on the effect the furanone exerts on the *las* quorum sensing system. Furanone compounds have seemed to hold promise as AHL-antagonists and for development of novel non-antibiotic, anti-pathogenic agents, which interfere with bacterial cell-to-cell communication and render bacteria less virulent. Also, the focus of a study was the BpeAB-OprB pump. It would be prudent to study the roles of *B. pseudomallei* resistance-nodulation-division efflux pumps in the efflux of acyl-HSLs. In *P. aeruginosa*, reduced synthesis of signal molecules was associated with overexpression of the MexAB-OprM and MexEF-OprN efflux pumps **(Kohler *et al.*, 2001)**, while reduced production of quorum-sensing regulated products was associated with an impaired MexGHIOpmD pump **(Aendekerk *et al.*, 2002)**. Inhibition of these efflux pumps would be therapeutically beneficial, because it attenuates virulence by preventing quorum sensing **(Inoue *et al.*, 2008).** As expected in our study, psychotropic drugs inhibit the efflux pump of a pathogenic bacterium and may be exploited for the prevention of QS responses of infecting bacteria.

P. aeruginosa is a model organism, but this could potentially extend to other organisms that undertake motility, such as *Escherichia coli*, *Vibrio parahaemolyticus*, *Serratia marcescens*, *Salmonella enterica* and *Proteus mirabilis* **(Copeland *et al.*, 2009).** *P. aeruginosa* is known to exhibit movement on surfaces by three types of motilities: swimming, swarming, and twitching. It has been observed that the non-antibiotic psychotropic drugs such as duloxetine, paroxetine, venlafaxine and hydroxyzine hinders motility of *P. aeruginosa*. But the inhibition of swimming motility was not as drastic as that of swarming and twitching motility. The psychotropic drugs might have some effects on flagella-related processes, namely, flagella biosynthesis, rotation, and chemotaxis, which may lead to a decrease in swimming and swarming activities. Also twitching motility, which is dependent on functional type IV pili **(Mattick, 2002)**, was significantly affected by the addition of drugs, this result indicated that twitching inhibition by the drugs was caused by defects in pili-related functions. Since bacterial motility necessary for proper biofilm formation was impaired in a concentration-dependent manner, these data suggest that the suppressive effect of psychotropic drugs on flagella-related and pili-related motility. In swimming and twitching motilities, the cells move independently, but swarming requires the bacteria to effectively work together via a process termed "quorum-sensing," involving bacteria sensing the extracellular signals produced by other bacteria **(Tremblay *et al.*, 2007)**. Given the importance of swarming motility for conferring biofilm development **(O'Toole *et al.*, 2000; Toutain *et al.*, 2004)** and antibiotic resistance **(Caiazza *et al.*, 2005; Butler *et al.*, 2010)**, on going research in our laboratory is aimed at investigating the effect of psychotropic drugs as efflux pump inhibitors. It was found that there was an inhibitory effect of drugs on swarming, swimming, and twitching motility in *P. aeruginosa*, but we cannot conclude whether or not drugs preferentially targets one mechanism or motility system in *P. aeruginosa*. It was found that a BCFA, anteiso-C15:0, inhibits swimming, swarming, colony wetness, and biofilm formation in *P. aeruginosa* **(Inoue *et al.*, 2008)**. Also, the swarming motility of *P. aeruginosa* was blocked by cranberry proanthocyanidins and other tannin-containing materials **(O'May and Tufenkji, 2011)**. As cell motility are proven instrumental in biofilm formation, it was investigated whether or not salicylic acid affected the motility of *P. aeruginosa*. There was also a direct inhibitory effect of salicylic acid on swarming, swimming, and twitching motility in both PAO1 and PA14 lab strains **(Chow *et al.*, 2011)**. The motility in this present study illustrated the effect as 37.5 mM MIC concentrations of drugs had significant effects in decreasing swarming, swimming and twitching motility for *P. aeruginosa*.

P. aeruginosa secretes alkaline protease and two elastases (A and B) that have been characterized as exoenzymes and virulence factors **(Suter, 1994)**. Proteases are important in tissue damage caused by *P. aeruginosa* infection and *P. aeruginosa* as a pathogen use proteases to cross proteinaceous barriers within the host contributes to bacterial virulence. Production of proteases (elastase, *LasA* protease and alkaline protease) in *P. aeruginosa* is undercontrolled *las* quorum sensing system including biosynthesis of AHL molecule and the expression of *lasA* (lasA protease) and *apr* (alkaline protease) **(Pesci et al., 1997)**. The alkaline protease activity at 37.5 mM MIC concentrations of drugs has been shown by smaller clear zone. QS system can be interfered with inhibition AHL molecule biosynhesis by QS inhibitors **(Kumar *et al.*, 2011)**. Therefore, we decided to use psychotropic drugs such as duloxetine, paroxetine, hydroxyzine and venlafaxine for their QS inhibitory activity.

CONCLUSION

Psychotropic drugs have effected bacterial efflux pump related to QS and suppressed alkaline protease production and swarming, swimming and twitching motility by blocking efflux pump and QS system. Thus, psychotropic drugs such as duloxetine, paroxetine, hydroxyzine and venlafaxine will be played active role as an alternative antimicrobial therapeutic on the treatment of infectious diseases.

REFERENCES

ADONIZIO, A.L., KONG, K.F. AND MATHEE, K. 2008. Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by South Florida plant extracts. *Antimicrobial Agents Chemotherapy*, 52, 198–203, http://dx.doi.org/10.1128/AAC.00612-07

AENDEKERK, S., GHYSELS, B., CORNELIS, P. AND BAYSSE, C. 2002. Characterization of a new efflux pump, MexGHI-OpmD, from *Pseudomonas aeruginosa* that confers resistance to vanadium. *Microbiology*, 148, 2371–2381.

AL-HUSSAINI, R. AND MAHASNEH, A.M. 2009. Antimicrobial and anti-quorum sensing activity of different parts of *Laurus nobilis* extracts. *Jordan Medical Journal*, 43(4), 286–298.

ALLISON, C., COLEMAN, N., JONES, P.L. AND HUGHES, C. 1992. Ability of *Proteus mirabilis* to invade human urothelial cells is coupled to motility and swarming differentiation. *Infection and Immunity*, 60, 4740–4746.

BUTLER, M. T., WANG, Q. F.AND HARSHEY, R. M. 2010. Cell density and mobility protect swarming bacteria against antibiotics. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 3776–3781, http://dx.doi.org/10.1073/pnas.0910934107

CAIAZZA, N. C., SHANKS, R. M. Q. AND O'TOOLE, G. A. 2005. Rhamnolipids modulate swarming motility patterns of *Pseudomonas aeruginosa*. *Journal of Bacteriology*, 187, 7351–7361, http://dx.doi.org/10.1128/JB.187.21.7351-7361.2005

CEDERLUND, H. AND MARDH, P.A. 1993. Antibacterial activity of non-antibiotic drugs. *Journal of Antimicrobial Chemotheapy*, 32, 355–65, http://dx.doi.org/10.1093/jac/32.3.355

CHOW, S., GU, K., JIANG, L. AND NASSOUR, A. 2011. Salicylic Acid Affects Swimming, Twitching and Swarming Motility in *Pseudomonas aeruginosa*, resulting in Decreased Biofilm Formation. *Journal of Experimental Microbiology and Immunology*, 15, 22 – 29.

COPELAND, M. F., AND WEIBEL, D. B. 2009. Bacterial swarming: a model system for studying dynamic self-assembly. *Soft Matterial*, 5, 1174–1187, http://dx.doi.org/10.1039/B812146J

DE´ZIEL, E., LEPINE, F., MILOT, S. AND VILLEMUR, R. 2003. *rhlA* is required for the production of a novel biosurfactant promoting swarming motility in *Pseudomonas aeruginosa*: 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs), the precursors of rhamnolipids. *Microbiology*, 149, 2005–2013, http://dx.doi.org/10.1099/mic.0.26154-0

DREIER J. 2007. Active drug efflux in bacteria. R.A. Bonomo and M. Tolmasky (ed.), Enzyme-mediated resistance to antibiotics Washington, DC, ASM Press, 235–64 p. http://dx.doi.org/10.1128/9781555815615.ch15

ELVERS, K.T. AND LAPPIN-SCOTT, H.M. 2000. Encyclopedia of Microbiology. J.Lederberg (ed.), San Diego, California, Academic Press.

HENTZER, M., RIEDEL, K., RASMUSSEN, T.B., HEYDORN, A., ANDERSEN, J.B., PARSEK, M.R., RICE, S.A., EBERL, L., MOLIN, S., HØIBY, N., KJELLEBERG, S., GIVSKOV, M. 2002. Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound. *Microbiology*, 148, 87–102.

INOUE, T., SHINAKI, R. AND FUKUI, K. 2008. Inhibition of swarming motility of *Pseudomonas aeruginosa* by branched-chain fatty acids. *FEMS Microbiology Letters*, 281, 81–86, http://dx.doi.org/10.1111/j.1574-6968.2008.01089.x

KRISTIANSEN, J.E. The antimicrobial activity of psychotherapeutic drugs and their stereo-isomeric analogues. [Ph.D. Thesis]. 1990. University of Copenhagen. Dan Med Bull, 37, 165–82.

KRISTIANSEN, J.E. 1992. The antimicrobial activity of non-antibiotics. *Acta Pathologica, Microbiologica et İmmunologica Scandinavica*, 100, 7–14.
KOHLER, T., VAN DELDEN, C., CURTY, L. K., HAMZEHPOUR, M. M. AND PECHERE, J. C. 2001. Overexpression of the MexEF-OprN multidrug efflux system affects cell-to-cell signaling in *Pseudomonas aeruginosa*. *Journal of Bacteriology*, 183, 5213–5222, http://dx.doi.org/10.1128/JB.183.18.5213-5222.2001
KUMAR, M.R., SATHYABAMA, S., RAMATHILAGAM, R.D., PRIYADARISINI, V.B. 2011. Anti-QS activity of medicinal plants and detection of N-acyl homoserin lactone signal molecules. *International Journal of Integrative Biology*, 11, 21–5.
MATSOMOTA, K. 2004. Role of bacterial proteases in pseudomonal and serratial keratitis. *Biological Chemistry*, 385(11), 1007–16. http://dx.doi.org/10.1515/BC.2004.131
MATTICK, J.S. 2002. Type IV pili and twitching motility. *Annual Reviews Microbiology*, 56, 289–314, http://dx.doi.org/10.1146/annurev.micro.56.012302.160938
MUNOZ-BELLIDO, J.L., MUNOZ-CRIADO, S., GARCI`A-RODRI`GUEZ, J.A. 2002. Antimicrobial activity of psychotropic drugs: Selective serotonin reuptake inhibitors. *International Journal of Amtimicrobial Agents*, 14, 177–180, http://dx.doi.org/10.1016/S0924-8579(99)00154-5
MUN˜OZ CRIADO, S., FAJARDO, M., GUTIE´RREZ, M.N., MUN˜OZ BELLIDO, J.L., GARCI`A RODRI`GUEZ, J.A. 1997. Psychiatric drugs inhibit slime production in *Staphylococcus epidermidis*. (International Congress of Chemotherapy), Sidney, Australia.
MUN˜OZ CRIADO, S., MUN˜OZ BELLIDO, J.L., ALONSO MANZANARES, M.A., GUTIE´RREZ ZUFIAURRE, M.N., GARCI`A-RODRI`GUEZ, J.A. 1998. Psychotropic drugs inhibit swarming in *Proteus* spp. and related genera. *Clinical Microbiology Infection*, 4(8), 447–449, http://dx.doi.org/10.1111/j.1469-0691.1998.tb00393.x
MURRAY, T. AND KAZMIERCZAK, B. 2008. *Pseudomonas aeruginosa* exhibits sliding motility in the absence of type IV pili and flagella. *Journal of Bacteriology*, 190(8), 2700–8, http://dx.doi.org/10.1128/JB.01620-07
NATHAN, P., LAW, E.J., MURPHY, D.F. AND MACMILLAN, B.G. 1978. A laboratory method for selection of topical antimicrobial agents to treat infected burn wounds. *Burns*, 4, 177–187, http://dx.doi.org/10.1016/S0305-4179(78)80006-0
O'MAY, C., AND TUFENKJI, N. 2011. The Swarming Motility of *Pseudomonas aeruginosa* Is Blocked by Cranberry Proanthocyanidins and Other Tannin-Containing Materials. *Applied and Enviromental Microbiology*, 77(9), 3061–3067, http://dx.doi.org/10.1128/AEM.02677-10
O'TOOLE, G., KAPLAN, H. B. AND KOLTER, R. 2000. Biofilm formation as microbial development. *Annual Reviews Microbiology*, 54, 49–79, http://dx.doi.org/10.1146/annurev.micro.54.1.49
OVERHAGE, J., BAINS, M., BRAZAS, M.D., HANCOCK, R.E.W. 2008. Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. *Journal of Bacteriology*, 190(8), 2671–2679, http://dx.doi.org/10.1128/JB.01659-07
PAGE`S, J.M. AND AMARAL, L. 2009. Mechanisms of drug efflux and strategies to combat them: challenging the efflux pump of gram-negative bacteria. *Biochimica et Biophysica Acta*, 1794, 826- 33, http://dx.doi.org/10.1016/j.bbapap.2008.12.011
PESCI, E.C., PEARSON, J.P., SEED, P.C., IGLEWSKI, B.H. 1997. Regulation of las and shl Quorum Sensing in *Pseudomonas aeruginosa*. *Journal of Bacteriology*, 179, 3127–3132.
PIDDOCK, L.J. 2006. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clinical Microbiology Reviews*, 19, 382–402, http://dx.doi.org/10.1128/CMR.19.2.382-402.2006
SCHWEIZER, H.P. 2003. Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeuroginosa* and related bacteria: unanswered questions. *Genetics and Molecular Research*, 2, 48–62.
SEMMLER, A.B.T., WHITCHURCH, C.B., MATTICK, J.S. 1999. A re-examination of twitching motility in *Pseudomonas aeruginosa*. *Microbiology*, 145(10), 2863–73.
SKERKER, J.M., BERG, H.C. 2001. Direct observation of extension and retraction of type IV pili. *Proceedings of the National Academy of Sciences of the United States of America*, 98(12), 6901–4, http://dx.doi.org/10.1073/pnas.121171698
SUTER, S. 1994. The role of baderial proteases in the pathogenesis of cystic fibrosis. *American Journal of Respiratory and Critical Care Medicine*, 150, 118–122, http://dx.doi.org/10.1164/ajrccm/150.6_Pt_2.S118
TOUTAIN, C. M., CAIAZZA, N. C. AND O'TOOLE, G. A. 2004. Molecular basis of biofilm development by pseudomonads. M. Ghannoum and G. A. O'Toole (ed.), Microbial biofilms. ASM Press, Washington, DC, 43–63 p. http://dx.doi.org/10.1128/9781555817718.ch4
TREMBLAY, J., RICHARDSON, A. P., LEPINE, F. AND DEZIEL, E. 2007. Self-produced extracellular stimuli modulate the *Pseudomonas aeruginosa* swarming motility behaviour. *Enviromental Microbiology*, 9, 2622–2630, http://dx.doi.org/10.1111/j.1462-2920.2007.01396.x
VENTURI, V. 2006. Regulation of quorum sensing in *Pseudomonas*. *FEMS Microbiology Reviews*, 30, 274–291, http://dx.doi.org/10.1111/j.1574-6976.2005.00012.x
VERSTRAETEN, N., BRAEKEN, K., DEBKUMARI, B., FAUVART, M., FRANSAER, J., VERMANT, J. 2008. Living on a surface: Swarming and biofilm formation. *Trends Microbiology*, 16(10), 496–506, http://dx.doi.org/10.1016/j.tim.2008.07.004
WIEGAND, I., HILPERT, K. AND. HANCOCK, R.E.W. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3,163–175, http://dx.doi.org/10.1038/nprot.2007.521