ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Role of the enzymes derived from isolated bacteria *Pseudomonas aeruginosa*

Reem Zuhair Shinashal[*1], Eman Mohammed Hussein[2]

[1]College of Education for Girls, University of Mosul, Iraq
[2]College of Science, University of Al-Qadisiyah, Iraq

ABSTRACT

The study is included the effects of proteases enzymes producing by Pseudomonas aeruginosa, which isolated from keratitis and wounds. two hundred samples were collected, 150 samples of them were keratitis, and fifty samples were wounds. Seventeen isolates (8.2 %) were obtained and diagnosed as P. aeruginosa.13 isolates (8.5%) from keratitis and 4 isolates (8%) from wound swabs. All bacterial isolates were diagnosed by biochemical tests. The production of elastase was tested by using elastin agar. Thirteen isolates (76.5%) had the ability to producing elastase. The isolates P7 and P11from keratitis and P2 from wounds had the larger hydrolysis zone (10, 10, 8 millimetres) respectively. The production of alkaline protease was tested by using solid cultures all of them had the ability to produce of the enzyme, the isolates P12 isolated from keratitis had the larger hydrolysis zone (22 millimetres). Also, the production of the enzyme was tested in liquid cultures all isolates from keratitis had the ability to the production of the enzyme, the isolates P12 had the specific activity 97.469 U / mg, while 50% of the wounds isolates had the ability to produce alkaline protease.

*Corresponding Author

Name: Reem Zuhair Shinashal
Phone:
Email: reemzhyr1970@gmail.com

INTRODUCTION

Bacteria are widespread because they can grow in soil and water and on plant and animal tissues. They are opportunistic opportunists and are one of the most important Nosocomial pathogens and have the potential to cause disease in people who are sensitive to infection such as cancer, burns, And some diseases of the immune system (Huston *et al.*, 2004; Smith *et al.*, 2005) . The bacteria belong to the Pseudomonadaceae family, which includes the Pseudomonas and the Xanthomonas. The species of Pseudomonas includes three human pathogens: P.aeruginosa, P.mallei, and P. pseudomallei (Ehrlich, 2003). Bacteria are aerobic, non-composed of spinach, granular and gram-negative, mobile with a single polarity, a cell with a width of 0.5 (0.8 μm and a length of -1.5) (3.0 μm) and many ferment factors that include structural factors as well as enzyme components. The bacteria have the ability to tolerate a wide range of physical conditions including temperature, and this feature contributes to the enhancement of bacteria's ability as opportunistic pathogens, and bacteria prefer growth in wet environments, reflecting the presence of bacteria in soil and water (Ellen *et al.*, 2003). Bacteria have a highly adaptive capacity in environments that are not suitable for microorganisms' growth and may be virtually non-nutrient-capable, even in very low-nutrient-containing water such as tap water. Their growth was observed in distilled water, these bacteria are very limited, and this susceptibility is widespread due to the fact that bacteria possess many enzymes that regulate genes. Bac-

teria have the largest sequence of pairs of nitrogen bases. P. aeruginosa has more than 6 million base pairs and about 5,500 genes. While E. coli has 4.6 million pairs base and approximately 4,200 genes. This large number of genes indicates that bacteria have adaptability in many environments, and it has also been observed to have phenotypic heterogeneity, which divides the bacterial community into groups expressing the different surface compounds. In this strategy, bacteria can escape the host's immune defences (Chabeaud *et al.*, 2001).

The aim of the study is to Detection of the isolation of P. aeruginosa isolates isolated from eye injuries and wounds on the production of various proteolytic enzymes (for comparison of isolates). Purification of some types of enzyme proteases produced from these isolates and in part and using the methods of chromatography, gel filtration and ion exchange. And Study the role of enzymes partially cleared in corneal injury and ulceration using laboratory animal. Protease IV is one of the ferment factors that con- tribute to bacterial pathogens in keratitis (Malloy *et al.*, 2005) . Where enzyme production is associated with several animal models of tissue with tissue damage during corneal inflammation (Malloy *et al.*, 2005). There are many indications of the role of this enzyme in the pathogenesis of the bacterium of the eye. The isolates that cause the inflammation of the cornea in the experimental infection of the mice and rabbits have the productivity of this enzyme. The bacterial isolates that are unable to produce this enzyme are observed to reduce their virulence of the cornea, and the bacterial isolates that have recovered their ability to Enzyme production were able to cause injury. This enzyme has a high susceptibility to mucus analysis (Aristoteli and Willcox, 2003). Studies show that mutant bacterial isolates (non-enzyme- producing) show a decrease in virulence of the cornea and injection of plasmid, which encodes the enzyme into the P. pteridae bacteria, which is a non- pathogenic bacteria of the eye. This is caused by the increased virility of the cornea bacteria.

METHODOLOGY

A total of 100 samples were collected from Mosul Teaching Hospital during the period from May 2018 to September 2018, and 100 samples of wound swabs were collected.

Isolation and purification of P. aeruginosa bacteria

Samples of keratitis and wound lesions were planted on the centre of blood agglutination, MacConkey agar and Agaromal agar. The dishes were incu-

bated at 37°C for 24 hours, allowing the development of bacteria on the centre of the trisugars agar and the development of bacteria at 42°C. Fol- lowing the implantation of the samples on the Pseudomonal agar medium, the developing isolates were taken to the centre and replanted on the centre of King A using the sterile carrier until pure isolates were obtained, incubated at 37°C for 24 hours. Physiological and biochemical tests were conducted based on scientific sources for the diagnosis of bacterial isolates (Collee *et al.*, 1996).

Preservation and maintenance of bacterial isolates

After ascertaining the physiological properties and some biochemical properties of the isolates, container tubes on Nutrient Agar Slant were reintroduced from the pure colonies on the center of King A and incubated at 37°C for 24 hours and were used as daily plantations and kept at 4°C until Con- duct diagnostic tests on them. For the first year or more, the BHIB was injected with 15% of the calcirol with bacterial isolates and incubated at 37°C for 24 hours and then kept at 20°C (Cruickshank *et al.*, 1975) .

Corneal infection of Rabbits by protease protease

BasalandLasApartiallypurified: Different concentrations of each enzyme were prepared. The basal protease enzyme was effectively present (10,7,5) units / ml, and the Tris-HCl locus was used with a concentration of 0.2 mLR with pH 8 as a control solution.The rabbit was then attended by 6 groups, anesthetized by ether; rabbit eye corneas were sterilized using a sterile needle (23G x1). Each concentration of each 40 μl enzyme was injected into the left eye of the narcotic rabbit while the right eyes of the narcotic were used as Group control. Enzymes were observed in injected horns 24 hours later, and the rabbit was killed. The role of enzyme protease basalis and LasA in the analysis of proteins Corneal in local rabbit.

Preparation of Rabbit eye corneas

The killing of a group of local rabbit healthy (non-infected) and poked her eyes and separated the cornea by and placed in Jaffna ceramic sterile, then was added to the solution buffer for these corneas by 2 mL of each cornea (buffer Tris- HCl concentration of 0.2 Mueller, a pH of 8 record in paragraph (5.5.1.2 d) For basal ganglia treated with protease enzyme, and addition of Tris-HCl with a concentration of 0.02 mpH 7.5 in paragraph (4.5.1.2 d) for Lasa-treated nuclei) The corneal tissues were then cut and subdivided under sterile conditions and each cornea was isolated separately and placed in a tubing with a cen-

trifugal spindle at 4000 ppm Per minute for 30 minutes to remove the remnants of tissue and fatty fiber then collect floes (crude extract Crude extract) for the purpose of his treatment with different concentrations of protease enzyme and basal Las A.

Treatment of crude extract with basal protease and LasA

The treatment of crude extract contained in different concentrations of en- zymes, where he attended the basal enzyme protease effectively (10, 7, 5) units / mL, and was attended by an enzyme LasA effectively (of 5, 3, 1) Unit / Ml. 0.5 mL of each enzyme was mixed with the 0.5 mL raw extract and incubated in the incubator at 37 ° C for 24 h. The reaction was suspended by placing the tubes in an ice bath for one hour.

Preparation of the separation gel at a concentration of 10%

Prepare a mixture of 36 mL of acrylamide-acrylic amide solution in paragraph 6.5.1.2a and 40 mL of solution for the separation gel in paragraph 6.5.1.2b and add to the mixture 4 μl of 15% ammonium sulfate solution in (6.5.1.2-e) and 100 μL of TEMED. Mix the mixture quickly and pour the gel into the mold for removal and leave to harden for 30 minutes. Placed in its place in the electric relay and connected to a cooled water bath at a temperature of 4 ° C. Then put the polarity buffer solution record in paragraph (c 6-5-1-2) in a reservoir positive electrode and the cathode and Aousela plate gel-mediated nomination papers have been linked to an electrical circuit between the electrical relay device and is equipped with power, and conducted an electric first deportation process to shed stream power 40 mA for 15 minutes.

Electrical deportation

I used the method of horizontal electric relay (Horizontal electrophoresis) where to add 100 Maikarolter of treatment models (crude extract of corneas treated with different concentrations of enzymes) / ml of buffer form record in paragraph (d 6-5-1-2-) in cracks (Slots) And then connect the power supply to 60 mA for 8 hours. Extract the gel and immerse in a kumasi dye solution prepared in (6.5.1.2 g) until blue beams are visible.

RESULTS AND DISCUSSION

Pseudomonas aeruginosa (12 eye swabs and 4 wound swabs) were obtained with isolation rate of 8.2%, and corneal lesions were obtained with 13 bacterial isolates. The isolating rate of keratitis patients was 8.5% which is less than what was mentioned in the local study conducted by Abadi

(2003), where she got a 10.5% isolation rate. They are also less than the authors Levey and Cohen (1996), noting that the percentage of isolating bacteria from patients with keratitis is 10%. In the United Kingdom, researcher Schaefer *et al.* (2001) points out that the percentage of isolating bacteria from patients with keratitis is 9%. In Australia, researchers report a high rate of isolation of these bacteria from cases of keratitis caused by contact lenses, which is 70% (Thakur *et al.*, 2001, 2004). Numerous studies of patients with bacterial keratitis indicate that bacteria are the most common and frequent cause of isolation from disease.

A total of 5 isolates were obtained from wound swabs (7% less than in local studies). Al-Douri (2003) obtained an isolation rate of 10.5%. It is also less than in Nigeria, where bacteria were isolated from surgical wounds by 33% and were the predominant bacteria in the microscopic lesions of wounds. It was also isolated by 30.2%. The bacteria were isolated by an approximation of 8.2%.

The percentages of isolation of these bacteria differ from one researcher to another, and the causes of variation are numerous at the time of collection of samples, size of samples taken, health and economic conditions, geographical location, variation in diagnostic methods and different sources of isolation.

Characterized using the phenotypic, biochemical and phylogenetic characteristics. When they developed on the Maconkey agglomeration, the colonies appeared in large size and pale, non-fermented color of lactose. Bacteria were also able to grow on Pseudomonal larvae, and the colonies appeared in medium size and whiteish-yellowish color. The bacteria grew in the center of KingA and were heterogeneous in their dye production, with the percentage of isolates producing pigment was 70.6%. The KingA center is a distinct center for Pseudomonas fluorescens that it cannot grow (Cruickshank *et al.*, 1975). Bacteria grew on the center of the blood. The colonies looked large with a slight rise. Bacteria were able to analyze the blood (b-hemolysin) fully. This is consistent with Qin *et al.,* (2003). It is also possible to distinguish between P. aeruginosa and P. fluorescens by growth at 42.4 ° C, where P. aeruginosa can grow at 42 ° C while P. fluorescens are not able to grow at this point but can grow at 4 ° C. P. aeruginosa bacteria growth at this degree and this is consistent with what he said (Iglewski, 2002).

The bacteria were given a positive result for the test of oxidase and catalase. When the bacterial cells were stained and stained with a grammole and examined under the light microscope, negative chromosome melanograms appeared.

The bacteria showed a negative result of the Vox-Proskauer test and are required for the red-type test. When the bacteria were developed on the TSI medium, growth appeared on the surface of the medium. The color of the medium did not change, and the presence of gas and H2S was not observed. The bacteria were able to consume jackets and showed their mobility. The bacteria also showed a positive result for the test of urease and galenase enzymes. The results of diagnostic tests for all bacteria are consistent with (Holt *et al.*, 1994).

Production of Proteases Enzymes

The results showed that 76% of the wound isolates had the ability to produce the enzyme. The decomposition area around the bacterial growth was very clear, and the bacterial isolation gave P2 the largest degradation area (8 ml). The isolated bacterial isolates from the keratitis patients producing this enzyme were 76.5%. It was observed that the bacterial isolates P11 and P7 gave the largest decomposition area (10 mm).

These results are consistent with studies that confirm that bacterial isolates isolated from wounds have high enzyme productivity. Hamood *et al.*, (1996) indicates that the enzyme is produced at high levels by most bacterial isolates isolated from pulmonary, urinary and wound injuries, while Lomholt *et al.* (2001) reported that isolated bacterial isolates from keratitis The productivity of the enzyme is estimated at 51% and its high efficacy is shown.

In a study of 150 bacterial isolates (isolated in the United States of America) isolated from various clinical sources, including corneal inflammation, these isolates all had the gene responsible for producing the enzyme, but four failed to express gene expression (Caballero *et al.*, 2004). While our results agree with another study showing that the ratio of enzyme production of isolated bacterial isolates from different clinical sources is between 98- 73%. One study indicates that the amount of enzyme production from isolated bacterial isolates from different clinical sources has been set in the plant's transgenic range (115-9.86) μg / mL (Callaghan *et al.*, 1996) .

Investigation of the enzyme using the steel medium

The bacterial isolates from keratitis and wound were all shown to have the ability to produce the enzyme, where the decomposition area around the bacterial growth was very clear, and the bacterial isolation showed P12 Was isolated from keratitis, the largest area of decomposition (22 mm) followed by P16 isolates isolated from keratitis, where a decomposition area (16 mm) was shown. P3 iso-

lates isolated from the wounds showed a decomposition zone (15 mm).

Al-Douri (2003) reported that 25% of the bacterial isolates showed a zone of degradation of the skim milk, and the largest decomposition of 15 ml of isolated bacterial isolation was obtained from burns. This difference may be due to the diversity of sources of isolation on which the bacteria were isolated from different clinical sources (e.g., burns, ear, wounds, blood, pus, etc.) Bacteria isolated from different clinical sources.

Investigation of the enzyme using the liquid medium

The amount of protein produced by these isolates was measured using Bradford (1976) method and by reference to the standard curve of bovine serum albumin (Figure 1) and calculate the specific efficacy (unit/milligram) of the enzyme.

The results showed that half of the bacterial isolates isolated from wounds (50%) had low enzymatic productivity (30.230-16.1) milligram, while the remaining half of these bacterial isolates showed high productivity ranging between 90- 77.1082 units/milligrams.

Bacterial isolates isolated from keratitis showed nine isolates (69.2%) with low enzymatic activity. These bacterial isolates exhibited a specific enzyme efficacy (41.665-22.348) (unit/milligram). Four bacterial isolates (30.8%) showed high enzymatic activity. P12 isolates showed the highest enzymatic efficacy and were of good efficacy (97.469 units/milligrams) followed by P14 isolates with a specific efficacy of 90.1 (mg/mg). The bacterial isolates P15 and P5 had a specific efficacy of 89.444 and 79.072 (units/mg), respectively, so P12 isolation was the best in the production of the enzyme.

These results are not consistent with those obtained by al-Douri (2003). Their study indicated that the highest enzyme activity was bacterial isolation from burns (738.8 mg/kg). The enzyme production of isolated bacterial isolates from keratitis may be due to the role of the enzyme in bacterial pathogenesis. Valenzim is not only a factor causing tissue damage but also is a factor of settlement in the eyes of the laboratory rabbit at injury.

The results are also inconsistent with Engel *et al.* (1998) noting that bacterial isolates isolated from all wounds are highly effective for the enzyme. As well as inconsistent with Lomholt *et al.* (2001), whose study indicated that isolated bacterial isolates from wounds, corneal inflammation, exhalation, blood, lung, and oedema all have high enzyme efficacy.
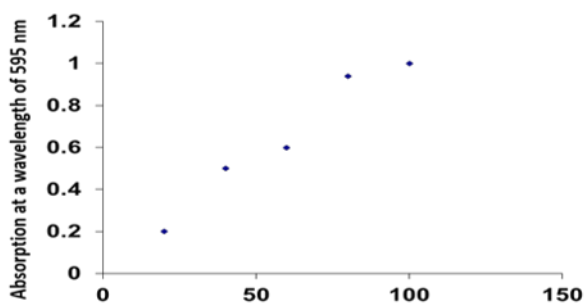
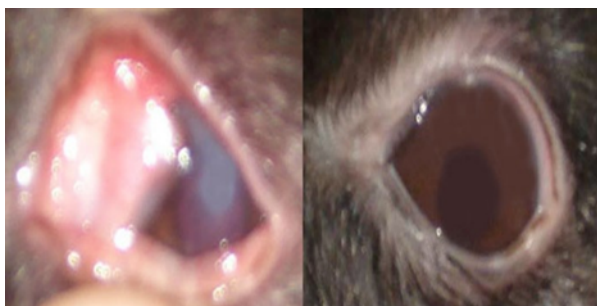**Figure 1: The standard curve for bovine serum albumin**

Figure 2: Horns of rabbit eyes injected with the protease enzyme partially purified from P. aeruginosa

Eyes of Rabbits

Various concentrations (10,7,5) (unit / mL) of 40 μl of B-protease partially purified from the isolation of P. aeruginosa P12 were injected into rabbit eye corneas. Clinical changes were observed on treated corneas after 24 hours of injection; Corneal injury is evaluated based on Burns *et al.,* (1990). The results indicate that all concentrations have led to the emergence of clinical symptoms that increase with increasing effectiveness of the enzyme form compared with the control group.

Different concentrations (3.5.1) (unit / mL) of 40 μl of LasA enzyme purified from P. aeruginosa P6 isolates were injected into rabbit eye corneas. Clinical changes were observed on treated corneas 24 hours after injection and corneal injury. The results showed that the concentration of 1 unit / mL did not show any disease on the treated rabbit horn (Figure 2), while the concentration (5.3) (unit/ml) showed clinical signs of cor- neal injury, (5/3-milliliters) The effects of disease are more severe than the pathogenic effects of the concentration method (3 units / mL).

The findings correlate with injecting P. aeruginosa proteases with a concentration of 1 μl / mL in the cornea that caused damage to the cornea 18 hours after injection. The results also agree with the findings of Kreger and Gray (1978). The researchers concluded that the injected protease at a concentra-

tion of 0.5 (unit / mL) led to a small central cornea in the cornea and developed into the corneal lesion when injected with pure protease (1 unit / mL) and when injected with pure protease at a concentration of 2 (unit/ml), it resulted in necrosis Intensive liquefaction necrosis in the cornea, and all of these results were observed 4 to 6 hours after injections of 40 to 30 μl purified protease.

The findings agree with the findings of Preston *et al.* (1997). The study confirmed the role of the LasA enzyme in the corneal injury by injection of the enzyme (5 micrograms) in the cornea. It was noted that the effects of tissue that led to damage in the cornea, the researcher and his group damage the cornea when injecting the purified enzyme with mutagenic bacterial isolation (non-producing enzyme) have no susceptibility to the injury, which indicates the role of this enzyme in causing the injury.

The findings also agree with the findings of Stern *et al.* (1982). The results of the study indicate that the addition of the enzyme protease (5 μg) with the bacterial isolation (non-producing enzyme) to the corneas of the eyes of laboratory animals led to a similar injury Caused by non-mutant bacterial isolation (i.e., enzyme-producing) demonstrating the role of this enzyme in corneal injury. Our results also agree with several studies suggesting that injecting the enzyme (20 micrograms) in the cornea of the laboratory animals has resulted in severe corneal damage. These studies indicate that the enzyme is a major factor in corneal injury (Howe and Iglewski, 1984; Preston *et al.*, 1997; Nagano *et al.*, 2001).

The effects of the enzyme protease-base in the cornea treatment effectively proteins (10, 7, 5) units / mL where he appeared entirely biodegradable for pack the cornea proteins, compared to proteins of the cornea Tris-HCl treatment at 0.2 μl pH 8 as a control. The forms show an increase in the intensity of protein degradation and its oxidation by increasing the effectiveness of the enzyme. The results also show above the effectiveness of the enzyme the LasA in the cornea proteins, which showed a similar effect of what happened with the enzyme protease basement where he also appeared denaturation and decomposition of the packages cornea proteins treatment effectively (of 5, 3, 1) Unit / mL , respectively, compared to proteins of the cornea treatment Badari Tris- HCL concentration of 0.02 Mueller with a pH of 7.5, but the severity of his analysis of these proteins were less than it is in the enzyme protease-base.

These results confirm what has been reached in the

experience of injury corneas eyes of rabbits Palansemen when different clinical injuries ap- peared in the intensity of corneas eyes, and these experiences together confirmed that the enzymes of the Proteases of the bacterium P. aeruginosa role important as a virulence factor in the events of injury inflammation of the cornea.

The results are consistent with studies that suggest that the enzyme protease-base role effective in necrosis Altmiei (Liquefactive Necrosis) rapid vain cornea, which is characterized by infection of bacterial cornea, as the isolates bacterial mutagens enzyme cannot predict events such as so damaged by bacterial corneal injury, so referred To the enzyme as an essential ferment factor in the pathogenesis of the bacterium of the cornea, regardless of whether or not the bacteria have the efficacy of Elastin (Elastolytic Activity) or lack of efficacy (Lomholt *et al.*, 2001; Hobden, 2002).

CONCLUSION

Bacterial isolates isolated from keratitis showed the ability to produce the protease, while 75% of the isolates isolated from the wounds showed high enzymatic activity. Bacterial isolates isolated from keratitis are produced by the LasD enzyme and are highly effective in measuring bacterial isolates isolated from wounds. The enzyme protease base in the incidence of infection of the cornea as the injection of the enzyme to the darkness in two thirds to the entire surface of the cornea with a moderate swelling. The treatment of the extract of corneal proteins in the enzyme to decompose most of these proteins, where the increase of degradation by increasing the effectiveness of the enzyme. The enzyme LasA has a role in the development of the cornea, as the injection of the enzyme into the darkness in two-thirds of the surface of the cornea with a slight swelling. The treatment of the extract of the corneal proteins of the enzyme to the degradation of corneal proteins, but the effects less severe than in the enzyme protease base.

REFERENCES

Aristoteli, L. P., Willcox, D. P. 2003. Mucin degradation mechanisms by distinct Pseudomonas aeruginosa isolates in vitro. *Infect. Immun*, 71(10):5565–5575.

Caballero, A., Thibodeaux, B., Marquart, M., Traidej, M., Callaghan, R. O. 2004. Pseudomonas keratitis: proteaseIVgene conservation, distribution, and production relative to virulence and other Pseudomonas protease. *Invest. Ophthalmol. Vis. Sci*, 45:522–530.

Callaghan, R. J. O., Engel, L. S., Hobden, J. A., Callegan, M. C., Green, L. C., Hill, J. H. 1996. Pseudomonas keratitis the role an uncharacterized exoprotein, protease IV, in corneal virulence. *Invest. Ophthalmol. Vis. Sci*, 37(4):534–543.

Chabeaud, P., Groot, A. D., Bitter, W., Tommassen, J., Heulin, T., Achouak, W. 2001. Phase-variable expression of an operon encoding extracellular alkaline protease, a serine protease homolog, and lipase in Pseudomonas brassicacearum. *J. Bacteriol*, 183(6):2117–2120.

Collee, J. G., Miles, R. S., Watt, B. 1996. *Test for the identification of bacteria in Practical Medical Microbiology*.

Cruickshank, R., Duqnid, J. P., Marmion, B. P., Swain, R. H. 1975. *The Practice of Medical Microbiology*. Churchill Living Stone, London.

Ehrlich, R. L. 2003. The critical link: Pseudomonas aeruginosa. *Heal. Peop. Hael. Comm*, 7(12):1–17.

Ellen, J. L., Cowell, B. A., Evans, D. J., Fleiszig, M. J. 2003. A contribution of exs A-regulated factors to corneal infection by cytotoxic and invasive Pseudomonas aeruginosa in murine scarification model. *Invest. Ophthalmol. Vis. Sci*, 44:3892–3898.

Engel, L. S., Hill, J. M., Moreau, J. M., Green, L. C., Hobden, J. A., Callaghan, R. J. O. 1998. Pseudomonas aeruginosa protease IV produced corneal damage and contributed to bacterial virulence. *Invest. Ophthalmol. Vis. Sci*, 39:662–665.

Hobden, J. A. 2002. Pseudomonas aeruginosa proteases and corneal virulence. *DNA Cell Biol*, 21(5-6):391–396.

Holt, J. G., Krieg, N. B., Stabley, J. T., Williams, S. T. 1994. Bergey's manual of determinative bacteriology.

Howe, T. R., Iglewski, B. H. 1984. Isolation and characterisation of alkaline protease - deficient mutants of Pseudomonas aeruginosa in vitro and in a mouse eye model. *Infect. Immun*, 43(3):1058–1063.

Huston, W. M., Potter, A. J., Jennings, M. P., Rello, J., Hauser, A. R., Mcewan, G. 2004. Survey of ferroxidase expression and siderophore production in clinical isolates of Pseudomonas aeruginosa. *J. Clin. Microbiol*, 42(6):2806–2809.

Iglewski, B. 2002. Real-time identification of Pseudomonas aeruginosa directs from clinical samples using a rapid extraction method and polymerase chain reaction. *J. Clin. Lab. Anal*, 15:131–137.

Kreger, A. S., Gray, L. D. 1978. Purification of Pseudomonas aeruginosa proteases and micro-

scopic characterisation of pseudomonal prote-ase-induced rabbit corneal damage. *Infect. Immun*, 19(2):630–648.

Lomholt, J. A., Poulsen, K., Kilian, M. 2001. Epidemic population structure of Pseudomonas aeruginosa: evidence for a clone that is pathogenic to the eye and that has a distinct combination of virulence factors. *Infect. Immun*, 69(10):6284–6295.

Malloy, J. L., Veldhuizen, R. A. W., Thibodeaux, B. A., Callaghan, R. J. O., Wright, J. R. 2005. Pseudomonas aeruginosa protease IV degrades surfactant proteins and inhibits surfactant host defense and biophysical functions. *Am.J. Phy. Lun. Cell. Mol. Phy*, 288:409–418.

Nagano, T., Long, H. J., Nakamura, M., Kumagai, N., Abe, M., Nakazawa, T., Nishida, T. 2001. The stimulatory effect of pseudomonal elastase on collagen degradation by cultured keratocytes. *Invest. Ophthalmol. Vis. Sci*, 42:1247–1253.

Preston, M. J., Seed, P. C., Toder, D. S., Iglewski, B. H., Ohman, D. E., Gustin, J. K., Goldberg, J. B., Pier, G. B. 1997. A contribution of proteases and LasR to the virulence of Pseudomonas aeruginosa during corneal infection. *Infect. Immun*, 65(8):3086–3090.

Schaefer, F., Bruttin, O., Zagrafas, L., Crosier, Y. 2001. Bacterial keratitis: a prospective clinical and microbiological study. *Br. J. Ophthalmol*, 85:842–847.

Smith, E. E., Sims, E. H., Spencer, D. H., Kaule, R., Olson, M. V. 2005. Evidence for diversifying selection at the pyoverdine locus of Pseudomonas aeruginosa. *J. Bacteriol*, 187(6):2138–2147.

Stern, G. A., Weitzenkorn, D., Valenti, J. 1982. Adherence of Pseudomonas aeruginosa to the mouse cornea epithelial-stromal adherence. *Arch. Ophthalmol*, 100:1956–1958.

Thakur, A., Barrett, R. P., Hobden, J. A., Hazlett, L. D. 2004. Caspase-1 inhibitor reduces the severity of Pseudomonas aeruginosa keratitis in mice. *Invest. Ophthalmol. Vis. Sci*, 45(9):3177–3184.

Thakur, A., Kyd, J., Xue, M., Willcox, D. P., Cripps, A. 2001. Effector mechanisms of protection against Pseudomonas aeruginosa keratitis in immunized rats. *Infect. Immun*, 69(5):3295–3304.