Ultrastructure of Biofilms Formed by Bacteria from Industrial Processes

MARI RAULIO

Department of Food and environmental Sciences
Division of Microbiology
Faculty of Agriculture and Forestry
University of Helsinki

Academic Dissertation in Microbiology

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in 1041 at Viikki Biocenter 2, Viikinkaari 5, on December 22nd, 2010 at 12 o'clock noon.

Helsinki 2010

Supervisor: Prof. Dr. Mirja S. Salkinoja-Salonen
Division of Microbiology
Department of Food and Environmental Sciences
University of Helsinki,
Helsinki, Finland

Reviewers: Doc. Dr. Marja Tiirola
Department of Biological and Environmental Science
University of Jyväskylä
Jyväskylä, Finland

Doc. Dr. Leif Kronberg
Organic Chemistry
Åbo Akademi University
Turku, Finland

Opponent: Prof. Dr. rer. nat. Ulrich Szewzyk
Department of Environmental Microbiology
Technical University of Berlin
Berlin, Germany

ISBN 978-952-10-6737-2 (paperback)
ISBN 978-952-10-6738-9 (PDF)
Yliopistopaino
Helsinki, Finland 2010

Front cover: The images in clockwise order: 1. Epifluorescence micrograph of *D. geothermalis* microcolonies on titanium coupon stained with a nucleic acid specific dye. 2. SEM micrograph of the mixed species biofilm on a steel coupon.3. Confocal laser scanning micrograph of fluorescent *in situ* hybridisation of a process water sample from a paper mill. A probe targeted to eubateria was used (green fluorescence). Most bacteria adhered to a cellulose fibre. 4. Scanning electron micrograph of natural biofilm on the surface of steeped barley kernel. Fig shows slime covered bacterial cells and fungal hyphae. 5. Epifluorescence micrograph of mixed species biofilm (same as in fig. 2) stained with a nucleic acid specific dye on steel coupon. 6. Transmission electron micrograph of *Deinococcus geothermalis* cell adhered onto a glass surface.

“I hope you are getting on with your book better than I am with mine, which kills me with the labour of correcting, and is intolerably dull, though I did not think so when I was writing it. A naturalist's life would be a happy one if he had only to observe, and never to write.”

-Charles Darwin to Charles Lyell-
June 1. 1867

Contents:

List of original publications: 6
The author's contribution 6
Abbreviations 7
Definitions for terms 8
Abstract 10
Tiivistelmä 12
1 Introduction 14
1.1 Biofilms 14
1.1.1 A brief glance into the history of biofilm research 14
1.1.2 Biofilm mode of growth 15
1.1.3 Initial attachment 16
1.1.4 Attachment mechanisms of biofilm forming bacteria 18
1.1.4.1 Adhesion organelles 18
1.1.4.2 Exopolysaccharides 20
1.1.4.3 Extracellular DNA 21
1.2 Biofilm structures - industrial *vs.* pathogenic bacteria 21
1.2.1 Characteristics of selected biofilm forming bacteria 22
1.2.2 Biofilms in industrial settings - biofouling 28
1.2.3 Biofilms in paper machine environment 32
1.2.4 Biofilms on medical implants 35
1.2.5 Biofilms on malted barley kernel tissues 36
1.3 Antifouling – how to prevent or to remove unwanted biofilms 37
1.3.1 Surface parameters affecting the adherence of bacteria 37
1.3.2 Mechanisms occurring in nature 39
1.3.2.1 Lotus effect 39
1.3.2.2 Disturbance of quorum sensing 40
1.3.3 Engineering microbe repelling coatings 40
1.3.4 TiO_2 Photocatalysis: The fundamentals and microbicidal effect 45
1.4 Biofilm quantification 46
2 Aims of the study 48
3 Materials and methods 49
3.1 The methods used in this thesis 49
3.2 Methods other than those described in the papers I-IV 50
3.2.1 Deposits from a paper machine: a case study 50
3.2.1 Penetration of microbes into ceramic materials 50
3.2.2 Investigation of mixed species biofilms on coated steel surfaces 51
4 Results and Discussion 53
4.1 Means for preventing biofouling 53
4.1.1 Developing a method for quantification of biofilm on steel 53
4.1.2 How does coating of steel affect its tendency towards biofouling? 54
4.1.2.1 Adherence of microbes on steels coated with diamond like carbon or with fluoropolymers 54
4.1.2.2 Coating of steel with or without antimicrobial agents 57
4.1.3 Biofilms can be destroyed by photocatalysis 68
4.1.4 Use of starter cultures to modify biofilm structure in barley kernel tissues 72
4.2 Ultrastructural studies of bacteria adhered on abiotic surfaces using FESEM 76
4.2.1 Mechanisms of adhesion to biotic and abiotic surfaces by phylogenetically remote bacteria 76
4.2.2 Bacteria respond to the quality of an abiotic surface 82

4.3 Oxalate and silicate in deposits formed in a paper mill (a case study) 85
4.4 Do microbes penetrate ceramic coating materials? 88
4.5 A novel bacterial taxon, *Deinobacterium chartae* gen. nov. sp. nov., isolated from a paper machine 89
5 Conclusions 94
6 Acknowledgements 96
7 References 98

List of original publications:

I **Raulio M.**, Pore V., Areva S., Ritala M., Leskelä M., Lindén M., Rosenholm J. B., Lounatmaa K. and Salkinoja-Salonen M. 2006. Destruction of *Deinococcus geothermalis* biofilm by photocatalytic ALD and sol-gel TiO_2 surfaces. Journal of Industrial Microbiology and Biotechnology, 33: 261-268

II **Raulio M.**, Järn M., Ahola J., Peltonen J., Rosenholm J. B., Tervakangas S., Kolehmainen J., Ruokolainen T., Narko P. and Salkinoja-Salonen M. 2008. Microbe repelling coated stainless steel analysed by field emission scanning electron microscopy and physicochemical methods. Journal of Industrial Microbiology and Biotechnology, 35:751–760

III **Raulio M.**, Salkinoja-Salonen M., Wilhelmson A, and Laitila A. 2009. Ultrastructure of biofilms formed on barley kernels during malting with and without starter culture. Food Microbiology 26:437-443

IV Ekman J. V., **Raulio M.**, Busse H-J, Fewer D. P. and Salkinoja-Salonen M. 2010. *Deinobacterium chartae* gen. nov., sp. nov., an Extremely Radiation Resistant Biofilm Forming Bacterium Isolated From a Finnish Paper Mill. International Journal of Systematic and Evolutionary Microbiology, published on-line. doi: 10.1099/ijs.0.017970-0

In addition unpublished data and my data published in papers not included in this thesis are described (Keskinen *et al.*, 2006, Saarimaa *et al.*, 2006, Laitila *et al.*, 2010).

The author's contribution

I Mari Raulio designed and executed the experimental work, except for the preparation of the photocatalytical surfaces, interpreted the microbiological results, wrote the paper together with the other authors, and is the corresponding author

II Mari Raulio designed and executed the experimental work except for the physicochemical determinations, interpreted the microbiological results, wrote the paper together with the other authors and is the corresponding author

III Mari Raulio prepared the scanning electron microscopy samples and examined them, interpreted the images, wrote the paper together with the other authors and is the corresponding author

IV Mari Raulio planned and executed major part of the experimental work. She interpreted the results and wrote the article together with the first author.

Abbreviations

AFM	atomic force microscopy
ALD	atomic layer deposition
CoA	coenzyme A
CLSM	confocal laser scanning microscopy
CTMP	chemi-thermomechanical pulp
DLC	diamond like carbon
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
eDNA	extracellular DNA
EDS	energy dispersive X-ray spectrometry
EPS	extracellular polymeric substances
FESEM	field emission scanning electron microscope
FISH	fluorescence *in situ* hybridization
Gen. nov.	*Genus novum*
HAMBI	Culture Collection of the Faculty of Agriculture and Forestry, University of Helsinki
MIC	microbially influenced corrosion
MRSA	methicillin-resistant *Staphylococcus aureus*
PCD	programmed cell death
PCR	polymerase chain reaction
PIA	polysaccharide intercellular adhesin
PVC	polyvinyl chloride
ROS	reactive oxygen species
SEM	scanning electron microscope
S_{ku}	kurtosis
Sp. nov.	*Species nova*
Spp.	*Species* (plural)
S_{sk}	skewness
TEM	transmission electron microscope
TSA	tryptic soy agar
TSB	tryptic soy broth
TUT	Tampere University of Technology
VP-FESEM	variable pressure field emission scanning electron microscope

Definitions for terms

Abiotic surface	surface of non-living material
Anatase	one of the three natural crystalline forms of titanium dioxide, the other two being brookite and rutile
Band-gap	energy difference between the highest valence band and the lowest conduction band which is found in insulators and semiconductors. The band gap energy is the amount of energy required to move electrons from the valence band to the conduction band
Biofilm	a structured community of microbial cells enclosed in a self-produced polymeric matrix and adhered to an inert or living surface
Biofouling	undesired deposition of biomass (biofilms) on surfaces
Biotic surface	surfaces of living organisms
Brownian motion	the random movement of particles suspended in water
Conditioning film	within moments of placing a clean surface into a water source, a film begins to deposit consisting of substances adsorbed to the surface
Diffusion layer	a thin (µm), stationary layer of fluid on an immmersed surface which does not mix with the main body of the fluid. Also known as the stagnant layer or the boundary layer
Fimbria	a filamentous appendage on the surface of a bacterial cell
Genome	an organism's entire hereditary information, encoded in DNA or, for some viruses, in RNA
Gibbs free energy	the maximum amount of non-expansion work that can be obtained from an isothermal, isobaric thermodynamic system
Glycoconjugate	carbohydrates that are covalently linked with other chemical compounds, such as lipids or peptides (glycolipids and glycopeptides respectively)
Gushing	sudden over foaming of bottled beer
Electron hole (h^+)	the lack of an electron at a position where one could exist in an atom
Kurtosis, S_{ku}	surface parameter that describes the "peakedness" of the surface topography
Lectin	carbohydrate-binding proteins or glycoproteins that are highly specific for their sugar residues
Pellicle	biofilm structures that some bacterial species (*e.g. P. aeruginosa* and *Bacillus* spp.) are able to form at the air–liquid interface of stationary cultures
Periodic acid-Schiff	a staining method used to detect glycogen
Persister cell	the "persister" is a hypothetical cell state in which microorganisms are protected from antimicrobial insults
Photocatalysis	the acceleration of a photoreaction in the presence of a catalyst
Pilus	a hairlike appendage found on the surface of many bacteria. Pilus is required for bacterial conjugation and/or adhesion to a

	surface or to other bacteria. Type IV pili are known to generate movement called twitching motility
Planktic microorganism	free floating microorganism
Proteome	the expressed proteins in cells or an organism at a certain time under defined conditions
Quorum sensing	bacterial communication mechanism dependent on population density
Rutile	the most common natural crystalline form of TiO_2
Sessile microorganism	surface-bound microorganism
Skewness, S_{sk}	surface parameter that describes the asymmetry of the height distribution histogram
Substrate	enzymes catalyze chemical reactions involving the *substrate*
Substratum	a surface on which an organism grows or is attached
Wort	the sugar containing liquid extracted from the mashing process during the brewing of beer

Abstract

Microorganisms exist predominantly as sessile multispecies communities in natural habitats. Most bacterial species can form these matrix-enclosed microbial communities called biofilms. Biofilms occur in a wide range of environments, on every surface with sufficient moisture and nutrients, also on surfaces in industrial settings and engineered water systems. This unwanted biofilm formation on equipment surfaces is called biofouling. Biofouling can significantly decrease equipment performance and lifetime and cause contamination and impaired quality of the industrial product.

In this thesis we studied bacterial adherence to abiotic surfaces by using coupons of stainless steel coated or not coated with fluoropolymer or diamond like carbon (DLC). As model organisms we used bacterial isolates from paper machines (*Meiothermus silvanus, Pseudoxanthomonas taiwanensis* and *Deinococcus geothermalis*) and also well characterised species isolated from medical implants (*Staphylococcus epidermidis*). We found that coating of steel surface with these materials reduced its tendency towards biofouling: Fluoropolymer and DLC coatings repelled all four biofilm formers on steel. We found great differences between bacterial species in their preference of surfaces to adhere as well as their ultrastructural details, like number and thickness of adhesion organelles they expressed. These details responded differently towards the different surfaces they adhered to.

We further found that biofilms of *D. geothermalis* formed on titanium dioxide coated coupons of glass, steel and titanium, were effectively removed by photocatalytic action in response to irradiation at 360 nm. However, on non-coated glass or steel surfaces irradiation had no detectable effect on the amount of bacterial biomass. We showed that the adhesion organelles of bacteria on illuminated TiO_2 coated coupons were complety destroyed whereas on non-coated coupons they looked intact when observed by microscope.

Stainless steel is the most widely used material for industrial process equipments and surfaces. The results in this thesis showed that stainless steel is prone to biofouling by phylogenetically distant bacterial species and that coating of the steel may offer a tool for reduced biofouling of industrial equipment. Photocatalysis, on the other hand, is a potential technique for biofilm removal from surfaces in locations where high level of hygiene is required.

Our study of natural biofilms on barley kernel surfaces showed that also there the microbes possessed adhesion organelles visible with electronmicroscope both before and after steeping. The microbial community of dry barley kernels turned into a dense biofilm covered with slimy extracellular polymeric substance (EPS) in the kernels after steeping in

water. Steeping is the first step in malting. We also presented evidence showing that certain strains of *Lactobacillus plantarum* and *Wickerhamomyces anomalus*, when used as starter cultures in the steeping water, could enter the barley kernel and colonise the tissues of the barley kernel. By use of a starter culture it was possible to reduce the extensive production of EPS, which resulted in a faster filtration of the mash.

Tiivistelmä

Mikrobit esiintyvät luonnossa etupäässä pintoihin kiinnittyneinä, useiden lajien muodostamina yhdyskuntina. Näitä liman verhoamia mikrobiyhteisöjä kutsutaan biofilmeiksi. Suurin osa mikrobilajeista pystyy muodostamaan biofilmejä. Biofilmejä esiintyy kaikilla pinnoilla, joilla on tarjolla tarpeeksi kosteutta ja ravinteita. Tällaisia pintoja on kaikkialla missä käsitellään vettä tai vesipitoisia nesteitä, kuten teollisuudessa ja vesijohtoverkossa. Biofilmin muodostus teollisuusprosesseissa voi heikentää laitteiden toimintakykyä ja lyhentää niiden käyttöikää, sekä aiheuttaa kontaminaatioita ja laadun heikenemistä tuotteisiin. Tästä syystä biofilmejä pyritään häätämään prosessilaitteista.

Tutkin väitöskirjassani bakteerien tarttumista pinnoitetuille ja pinnoittamattomille teräspinnoille. Käytin koe-eliöinä paperikoneiden biofilmeistä eristettyjä bakteerilajeja, *Meiothermus silvanus, Pseudoxanthomonas taiwanensis* ja *Deinococcus geothermalis*, sekä lajia, joka tekee biofilmejä ihmisen elimistöön sijoitetuille esineille (implantit), *Staphylococcus epidermidis*. Löysin bakteerilajien väliltä suuria eroja sekä niiden kyvyssä tarttua eri pinnoille, että itse bakteerien pinnan rakenteissa. Turtkimani bakteerit osasivat myös sopeuttaa solurakenteensa eri pinnoilla, mm. tarttumaelimien paksuus ja lukumäärät olivat erilaisia.

Käytin työssäni *D. geothermalis* -bakteerin muodostamia biofilmejä tutkiakseni, voidaanko ne irrottaa lasi- tai teräspinnoilta, jos pinnat pinnoitetaan titaanidioksidilla joka aktivoidaan 360 nm valolla. Tulokseni osoittivat, että näin saatiin aikaan biofilmiä tuhoava fotokatalyysi. Pinnoittamattomalla lasilla ja teräksellä biofilmin määrä pysyi muuttumattomana valaisusta riippumatta. Kun tutkin kenttäemissiopyyhkäisy-elektronimikroskoopilla biofilmejä TiO_2 pinnoitetuilla pinnoilla, havaitsin että valokäsitellyillä pinnoilla bakteerien tarttumaelimet olivat tuhoutuneet, mutta pinnoittamattomilla pinnoilla ne näyttivät ehjiltä.

Teräs on yleisin koneiden ja laitteiden materiaali monilla teollisuuden aloilla. Väitöskirjan tulokset kuitenkin näyttävät, että bakteerit tarttuvat hyvin juuri teräkseen. Samat tulokset saatiin hyvin erilaisilla, fylogeneettisesti kaukaisilla, bakteerilajeilla. Tulokset osoittivat myös, että teräksen alttiutta kerätä biofilmejä voidaan vähentää pinnoittamalla ja jos käytetään fotokatalyyttisesti aktiivista pinnoitetta, jo muodostuneetkin biofilmit voidaan poistaa valokäsittelyn avulla.

Tutkin myös eläviin solukkoihin tarttuvia mikrobibiofilmejä. Tutkimuskohteena oli ohran jyvän luonnollinen biofilmi. Havaitsin että myös nämä biofilmit rakentuivat mikrobeista, joilla oli tarttumaelimiä. Ulkonäöltään tarttumaelimet muistuttivat niitä, joita

olin löytänyt teräs- ja lasipinnoilta. Ohraa mallastettaessa kuivat jyvät liotetaan vedessä. Kun ohran jyvän biofilmiä tarkasteltiin elektronimikroskoopilla, havaittiin että likovaiheen aikana kuivien jyvien mikrobisto muuttui tiheäksi liman peittämäksi biofilmiksi. Tutkimukseni osoitti, että tätä limanmuodostusta voi vähentää lisäämällä likoveteen tiettyjä maitohappobakteereja (*Lactobacillus plantarum*) ja hiivoja (*Wickerhamomyces anomalus*). Nämä mikrobit pystyivät tunkeutumaan jyvän kuorikerroksen alle ja täyttämään pinnan, joka muuten olisi täyttynyt limaista biofilmiä tuottavilla mikrobeilla. Kun liman määrä väheni, niin mäskin suodatettavuus parani.

1 Introduction

1.1 Biofilms

1.1.1 A brief glance into the history of biofilm research

Henrici (1933) studied the microbiota of fresh water early in the 1930s, by immersing microscopic slides into the mud and, after a suitable time, examining the slides with a microscope. He was surprised by the number and the diversity of organisms adhered on it. He stated: "It is quite evident that for the most part the water bacteria are not free floating organisms, but grow upon submerged surfaces; they are of the benthos rather than the plankton". Few years later ZoBell and Allen (1935), studied the fouling of ship hulls and noticed that bacteria and other microbes are the primary biofouling organisms on submerged glass surface. The "film" formed on glass surface functioned as a foundation for larger organisms to adhere to. Later ZoBell (1943) found that many of the bacteria in sea water are sessile, growing attached to a solid surface and possessing specific organelles for such attachment.

Even though biofilms were first observed as early as 1930s, the significance of biofilm mode of growth was realized not until in 1970s, when Geesey *et al.* (1977 and 1978) noticed that most of the bacteria living in alpine streams were attached on river stones. The stream biofilms contained multiple bacterial species in spite of the lack of nutrients and great shear forces that removed the planktic bacteria.

The complexity of biofilm architecture was revealed later, when confocal scanning laser microscopy (CLSM) was applied for biofilm research in early 1990s (Lawrence *et al.*, 1991). For the first time, it was possible to look inside a thick, fully hydrated, live biofilm. Since that the biofilm related research and the number of published studies have increased exponentially (Fig. 1). The most studied model organism in biofilm research has been human pathogen *Pseudomonas aeruginosa*, a bacterium forming biofilm on cystic fibrosis patients' lungs causing chronic infection and most often death of the patient (May *et al.*, 1991, Singh *et al.*, 2000).

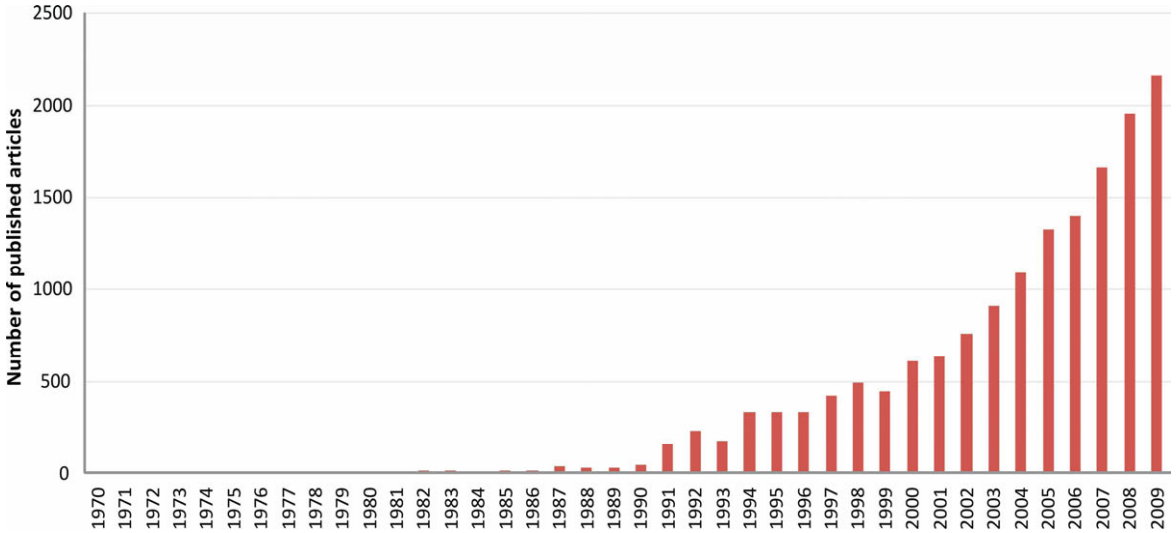

Fig. 1. Number of biofilm related papers published in peer reviewed journals in years 1970-2009. The articles were searched by the author from Web of Science with search words: biofilms or sessile bacteria* and with a time span of 1970 to 2010.

1.1.2 Biofilm mode of growth

Microorganisms have mainly been studied as planktic (*e.g.* free-swimming) cells previously, but the past decade has taught us that in natural habitats they exist predominantly as sessile multispecies communities. These surface attached highly structured matrix-enclosed microbial communities are known as biofilms. Most, if not all, bacterial species can form biofilms.

Biofilms occur in a wide range of environments, on every surface when supplied with moisture and nutrients. Biofilms can exist in natural ecosystems; like river stones and surfaces of a plant, or in ecosystems connected with disease; like surface of a tooth and the mucosal membranes of multicellular organisms, as well as in man-made ecosystem; like steel surfaces of industrial equipments, heat exchangers and water pipes (Costerton *et al.*, 1987 and 1999, Danhorn and Fuqua, 2007).

The biofilms usually consist of microcolonies formed by multiple microbial species embedded in an extracellular matrix. Composition of the extracellular matrix, *i.e.* extracellular polymeric substance (EPS), is as diverse as the biofilm forming microbial species. It is usually composed from polysaccharides, proteins, nucleic acids and cell components with concentrated minerals and nutrients from the surrounding environment (Reviewed by: Sutherland, 2001, Dunne, 2002, Allison, 2003). As the population in a biofilm grows, the individual microbial cells may differentiate and take on specific tasks enabling the formation of a defined architecture of shapes resembling mushrooms or towers connected by a network of water channels responsible for the transport of nutrients, oxygen and wastes (Stoodley *et al.*, 2002).

The complex structures of biofilms with a high level of differentiation among biofilm cells require cell-to-cell signalling, *i.e.* quorum sensing (Irie and Parsek, 2008). Watnick and Kolter (2000) compared natural biofilms to our cities: bacterial cells are living in a multispecies community; they stay or leave with purpose, share genetic material horizontally, and fill distinct niches within the biofilm.

When a bacterium starts to form a biofilm, it switches from single cell to multicellular lifestyle by up- and downregulating specific genes. Even in single species biofilms individual cells express their genes in a pattern that differs from cell to cell and from the planktic cells of the same species. Kolter and Greenberg (2006) suggested that biofilms hold microniches with varying gradients of nutrients due to metabolic activity of sessile cells. These small ecosystems create conditions for spontaneous mutations to occur. Different cell types in biofilm provide genetic diversity for adaptation to sudden environmental changes (Kolter and Greenberg, 2006).

For a human point of view, biofilms can be beneficial or detrimental. Biofilms are utilized in water purification (*e.g.* Nicolella *et al.*, 2000, Simpson, 2008) and bioremediation of hazardous substances in the environment (recently reviewed by Cao *et al.*, 2009). However, they cause inconvenience when formed on industrial surfaces (Wong, 1998, Coetser and Cloete, 2005) or when causing persistent infections in humans, animals or plants (Costerton *et al.*, 1999, Danhorn and Fuqua, 2007).

Bacteria in biofilms are far more resistant towards antimicrobial agents and physical stressors than their planktic counterparts. Many reviews have been published on the resistance properties of biofilm bacteria: *e.g.* Costerton *et al.*, 1987 and 1999, Mah and O'Toole, 2001, Stewart and William Costerton, 2001, Lewis, 2008. The mechanisms of the biofilm resistance have been reviewed by Mah and O'Toole (2001) and more recently Andersson and O'Toole (2008).

1.1.3 Initial attachment

When a clean abiotic surface is submerged in water, organic and inorganic molecules accumulate on the surface by diffusion or due to water flow. This surface phenomenon is called conditioning and the formed layer is a conditioning film. The formation of conditioning film may affect the adhesion of bacteria, since it alters the physicochemical properties of the surface, like surface energy, hydrophobicity or charge (Dunne, 2002).

Microbial colonization of solid-liquid interface and biofilm formation can be divided into sequences of events reviewed by Dunne, 2002, Stoodley *et al.*, 2002 and van Loosdrecht *et al.*, 1990 and illustrated in Fig. 2.

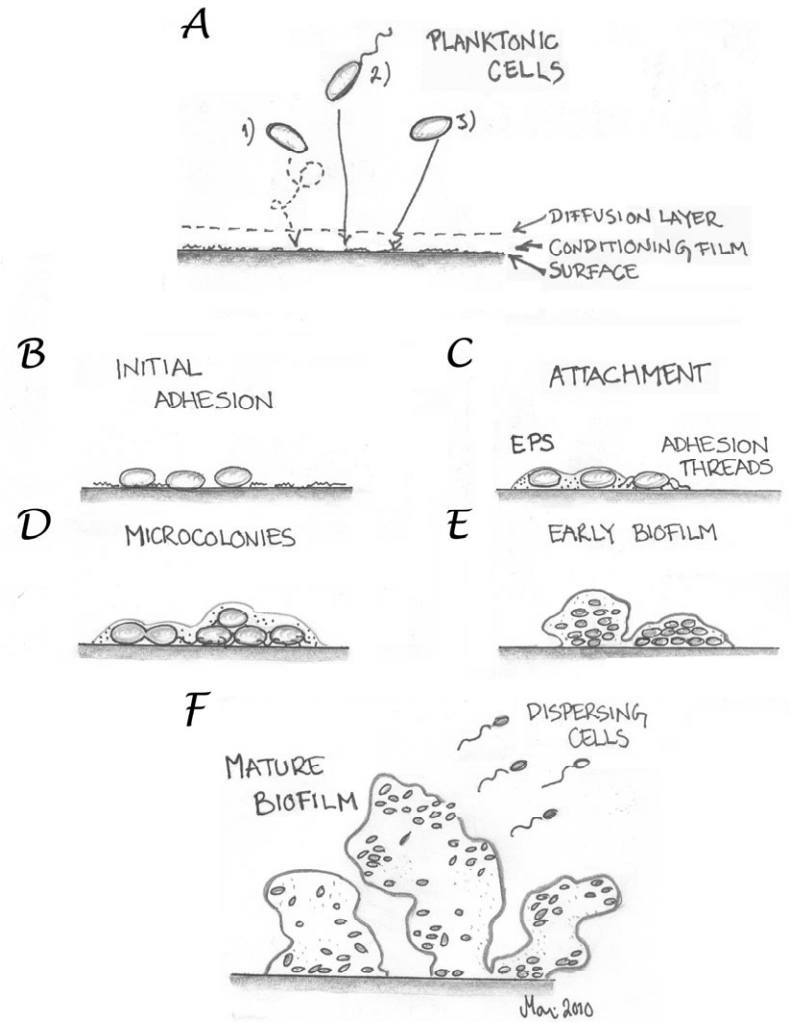

Fig. 2. Schematic drawing showing the attachment of planktic cells and sequential stages of biofilm formation. (Modified from van Loosdrecht *et al.* and 1990, Costerton, 2007).

Panel A, Transportation of the planktic cells to a surface by: 1) Brownian motion, which is random movement of particles in water, 2) active movement of a motile flagellated bacterium, 3) convective transport with the current in the fluid. Panel B, reversible initial adhesion. Forces involved are Van der Waals forces, electrostatic forces and hydrophobic interactions. Panel C, irreversible attachment with adhesion organelles or EPS. Irreversibly attached bacteria will no longer detach from the surface unless strong shear force is used. Panel D, surface growth by cell division and formation of microcolonies. Panel E, early architecture of biofilm. Panel F, three dimensional structure of mature biofilm and dispersing cells. Single cells or aggregates dispersed from a biofilm can colonise new locations. Dispersion can be driven by shear forces or enzymatic degradation of EPS (Hall-Stoodley *et al.*, 2004).

Interactions between microorganisms and the substratum in natural or industrial aquatic environments are complex. Since multiple factors affect cell attachment it is difficult or impossible to make an exact model for biofilm formation. Many factors contribute to the initial attachment of a bacterial cell to a surface: characteristics of the bacterial species involved (motility and EPS production; see chapters 1.1.4.1 and 1.1.4.2, respectively), characters of the surface being colonised (roughness, charge and hydrophobicity of the surface; see chapter 1.3.1) and physical and chemical parameters of the environment (such as the hydrodynamics and nutrient availability; see chapter 1.2 and Stoodley *et al.*, 1998.

Environmental influence on biofilm development was recently reviewed by Goller and Romeo (2008).

1.1.4 Attachment mechanisms of biofilm forming bacteria

1.1.4.1 Adhesion organelles

The irreversible attachment of bacterial cells to an abiotic surface is often mediated by adhesins, surface macromolecules produced by bacteria (An and Friedman, 1998). Adhesins can be associated with specialized surface structures or appendages of bacterium. These appendages, flagelli, pili or fimriae and curli, have been identified from several bacterial species (Table 1).

Watnick and Kolter showed that fimbriae and flagelli accelerate the biofilm formation of *Vibrio cholerae*, but that these organelles are not required for attachment to an abiotic surface (Watnick and Kolter, 1999). It was also reported that *V. cholerae* uses divergent pathways for biofilm formation on biotic and abiotic surfaces: toxin-coregulated pilus is used for colonising the intestine, but mannose-sensitive hemagglutinin fimbriae for colonising an abiotic surface (Watnick *et al.*, 1999, Thelin and Taylor, 1996, Tacket *et al.*, 1998). O'Toole and Kolter reported that both flagellar motility and type IV pili were required for proper biofilm formation of *P. aeruginosa* when glucose was used as the carbon source. The mutants not expressing type IV pili, formed a monolayer of cells on abiotic surface, but did not develop microcolonies, whereas the non-motile mutants were not able to adhere on the surface at all (O'Toole and Kolter, 1998a). This was partly confirmed later by Klausen *et al.* (2003a) who reported that the flagelli and type IV pili did not affect *P. aeruginosa* initial attachment or biofilm formation, but the wild type strain formed biofilms with different structures than flagellum and type IV pili mutants. They also showed that when citrate was used as the carbon source the *P. aeruginosa* formed flat biofilm with no microcolonies and that the initial attachment was independent on flagellar motility or on type IV pili (Klausen *et al.*, 2003a). Later Klausen *et al.* (2003b) proposed that *P. aeruginosa* biofilm structure development is dependent on migration driven by type IV pili. Merritt *et al.* (2007) showed that the influence of flagelli and motility on biofilm formation by *Agrobacterium tumefaciens* depended on culture conditions. Non-motile mutants were not able to form biofilm under static conditions, whereas under flow they formed a denser biofilm than wild type. A mutant having a non-functioning flagellum was unable to form biofilm under either condition (Merritt *et al.*, 2007). This was confirmed by Houry *et al.* who found that neither the flagellum nor motility were required for biofilm formation by *Bacillus cereus* in a flow

cell, but on microtiter plate and in glass tubes motility contributed to biofilm formation (Houry *et al.*, 2010).

Flagellar motility has been shown to be required for bacteria to attach on biotic surfaces (*e.g.* Smit *et al.*, 1989, De Weger *et al.*, 1987).

It thus seems that bacterial adhesion organelles, such as flagelli and fimbriae, may contribute to the initial attachment as well as to the biofilm architecture, but are not required for either of these. Motility may help the bacterial cell to penetrate the diffusion layer of water on the surface and in that way help the initial attachment to occur.

Table 1. Adhesion organelles and envelope structures involved in abiotic surface colonization by bacteria

Putative adhesion organelle or adhesin	Reported from	Reference
Flagelli	*Salmonella enterica* ser. Typhimurium, *Vibrio cholerae, Pseudomonas aeruginosa, P. fluorescens, Escherichia coli, Agrobacterium tumefaciens*, *Cronobacter sakazakii, Bacillus cereus*	Watnick and Kolter, 1999, O'Toole and Kolter, 1998a, Merritt *et al.*, 2007, Houry *et al.*, 2010, Kim and Wei, 2009, Korber *et al.*, 1994, Pratt and Kolter, 1998, Hartmann *et al.*, 2010
Type IV pili	*V. cholerae, Deinococcus geothermalis, P. aeruginosa, Ralstonia solanacearum, V. parahaemolyticus, Clostridium perfringens, Shewanella oneidensis*	Saarimaa *et al.*, 2006, O'Toole and Kolter, 1998a, Watnick *et al.*, 1999, Barken *et al.*, 2008, Kang *et al.*, 2002, Shime-Hattori *et al.*, 2006, Varga *et al.*, 2008, Thormann *et al.*, 2004
Type I pili	*E. coli*	Pratt and Kolter, 1998
Type 3 pili	*Klebsiella pneumoniae*	Di Martino *et al.*, 2003
Thin pili	*Acinetobacter baylyi*	Gohl *et al.*, 2006
S. maltophilia fimbriae 1 (SMF-1)	*Stenotrophomonas maltophilia*	de Oliveira-Garcia *et al.*, 2003
Curli	*E. coli*	Vidal *et al.*, 1998, Pawar *et al.*, 2005
Mat fimbriae (*E. coli* common pilus)	*E. coli*	Lehti *et al.*, 2010
Conjugative fimbriae	*E. coli*	Ghigo, 2001
Thin aggregative fimbriae (curli like)	*Salmonella enterica* ser. Enteritidis	Austin *et al.*, 1998
Bap adhesin	*Staphylococcus aureus, Salmonella enterica* ser. Enteritidis, *Burkholderia cepacia*	Cucarella *et al.*, 2001, Latasa *et al.*, 2005, Huber *et al.*, 2002
Esp adhesin	*Enterococcus faecalis*	Toledo-Arana *et al.*, 2001
Teichoic acid	*Staph. aureus*	Gross *et al.*, 2001

1.1.4.2 Exopolysaccharides

Exopolysaccharides have been suggested to be the main component of biofilm extracellular polymeric substance (EPS) or slime layer, which not only surrounds and protects the biofilm bacteria, but also anchors them onto the surface (Dunne, 2002). Once a bacterium adheres onto the surface, biotic or abiotic, it surrounds itself with polysaccharides to form microcolonies (Costerton *et al.*, 1981). However, polysaccharide production is not required for initial attachment contrary to the development of the biofilm architecture (Sutherland, 2001). So the primary function of biofilm matrix is in the maintenance and persistence of the attached bacteria (Allison, 2003).

Production of exopolysaccharides has been shown to be essential for building the biofilm architecture by *Vibrio cholerae* (Watnick and Kolter, 1999), as well as many other *Vibrio* spp. (reviewed by Yildiz and Visick, 2009), *E. coli* (Danese *et al.*, 2000) and for the formation of pellicles by *Bacillus subtilis* (Nagorska *et al.*, 2010).

There is evidence that alginate, probably the most studied exopolysaccharide, is not essential for biofilm formation in *Pseudomonas aeruginosa* (Stapper *et al.*, 2004) and was not a major constituent of the extracellular matrix of two strains of this species (Wozniak *et al.*, 2003). Nevertheless, it was previously shown that overproduction of acetylated alginate leads to significant architectural and morphological changes in *P. aeruginosa* biofilms (Stapper *et al.*, 2004, Hentzer *et al.*, 2001). Besides alginate, the *P. aeruginosa* biofilm matrix contains other exopolysaccharides as well (Wozniak *et al.*, 2003, and a review by Ryder *et al.*, 2007).

Peltola *et al.* (2008a) studied the architecture of *Deinococcus geothermalis* E50051 biofilms on glass and on stainless steel with CLSM and large number of different fluorescently tagged lectins. The deinococcal biofilm had a non-slimy appearance and the microcolonies seemed to contain exopolysaccharides mainly internally. Nevertheless, the type strain of *D. geothermalis* has been shown to produce a capsule-like structure surrounding the cells (Ferreira *et al.*, 1997). Bacterial capsule consists usually of polysaccharides and polypeptides forming a covering layer outside the bacterial cell wall separated from extracellular slime. Bacterial capsules occur both in Gram-negative and Gram-positive bacteria. It has been suggested that cell surface polysaccharides and proteins act as bacterial adhesins (Review by An and Friedman, 1998).

1.1.4.3 Extracellular DNA

It has been known for a long time, that extracellular DNA (eDNA) is a component of biofilm EPS (Sutherland, 2001). It has been commonly considered to be derived from lysed cells. Whitchurch *et al.* (2002) found that extracellular DNA is required for the initial establishment of *P. aeruginosa* biofilms. They suggested that eDNA was not derived from lysed cells but rather from membrane vesicles. Barken *et al.* (2008) reported that quorum sensing-controlled eDNA release is required for the formation of mushroom-shaped multicellular structures in *P. aeruginosa* biofilms.

Allesen-Holm *et al.* (2006) suggested that eDNA functions as a compound connecting cells to each other in *P. aeruginosa* biofilms. Also other results suggest a structural role for eDNA (Flemming *et al.*, 2007). Some bacteria produce substantial quantities of eDNA through a mechanism that is thought to be independent on cell lysis and involves the release of small vesicles from the outer membrane. DNA stabilises the biofilm structure. Furthermore, recent findings suggest its role in gene transfer within biofilms (Vlassov *et al.*, 2007).

Qin *et al.* (2007) reported that the extracellular DNA is as a major component required for the initial attachment and the early phase of biofilm development of *Staphylococcus epidermidis*. They also showed that eDNA was generated in *Staph. epidermidis* populations through lysis of a subpopulation of the bacteria, and that the eDNA promoted biofilm formation of the remaining population. Extracellular DNA has also been shown to have role in biofilm formation of following bacterial species: *Streptococcus pneumoniae* (Moscoso *et al.*, 2006), *Streptococcus mutans* (Perry *et al.*, 2009), *Staphylococcus aureus* (Izano *et al.*, 2008), *Listeria monocytogenes* (Harmsen *et al.*, 2010), *Neisseria meningitidis* (Lappann *et al.*, 2010), *Enterococcus faecalis* (Thomas *et al.*, 2008), *Haemophilus influenzae* (Jurcisek and Bakaletz, 2007) and *Bacillus cereus* (Vilain *et al.*, 2009).

Adhesion of bacteria to an abiotic surface has been reviewed by many authors *e.g.* An and Friedman, 1998, Pratt and Kolter, 1999, Wimpenny *et al.*, 2000, Lejeune, 2003. Biofilm matrix was recently reviewed by Flemming and Wingender, 2010.

1.2 Biofilm structures - industrial *vs.* pathogenic bacteria

Due to the wide variety of environments in which biofilms are found and the microbial species forming them, it is impossible to generalize about their structure or physiological activities, as indicated by Sutherland (2001). Every biofilm has its unique characteristics depending on the microbial species present and conditions prevailing.

The basic biofilm architecture model of microcolonies, sometimes called ”mushrooms”, with water channels and voids in between, have been discovered from single species *in vitro* model systems as well as the tooth plaque in human (Wood *et al.*, 2000) and stream biofilms (Battin *et al.*, 2003).

Industrial environments differ from those in nature. Waters in industrial processes are often oligotrophic whereas the media used for culturing pathogenic bacteria for medical purposes are nutrient-rich. The bacterial species present in industrial processes are other than those in foods or clinical specimens. Substrate concentrations will also affect the biofilm structure.

Fluid shear and flow rates are high in industrial systems. Fluid shear influences the density and the tensile strength of the biofilm (Stoodley *et al.*, 1998, Reviewed by Stoodley *et al.*, 2002). It was shown that *P. aeruginosa* as well as mixed species biofilm became elongated into the downstream direction and formed filamentous streamers under high flow and increased shear stress (Stoodley *et al.*, 1999). Mattila *et al.* (2002) reported that under high flow the biofilms formed on-site on stainless steel in paper machine remained flat and dense. This result is in agreement with those reported by Liu and Tay (2001). Battin *et al.* (2003) reported that the architecture of stream biofilms was affected by flow velocity as well. The biofilms that developed under slower velocity were thicker and had higher areal coverage than their counterparts exposed to higher velocities (Battin *et al.*, 2003).

The factors influencing the biofilm structure has been reviewed by Wimpenny *et al.*, 2000.

1.2.1 Characteristics of selected biofilm forming bacteria

Although biofilms in nature are usually formed by multiple microbial species, most of the biofilm research today in laboratories has been conducted with few strains of human pathogens like *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis* and *Vibrio cholerae* (Fig. 3). These strains have often a long history of laboratory maintenance and therefore they have undergone countless subculturings leading to unintended adaptation. The conventional laboratory growth conditions are a selective disadvantage for biofilm forming phenotype. For *P. aeruginosa* it has been shown that great proportion of genes present in wild isolates may have been lost during the subculturings (Costerton, 2007). Similar adaptation has been shown for *E. coli* and *Staph. aureus* (Cooper *et al.*, 2003, Somerville *et al.*, 2002). The history of three laboratory reference strains (*i.e. E. coli* K12, *P. aeruginosa* PAO1 and *Staph. aureus* COL) commonly

used in biofilm research and the important genomic differences between these strains and clinical isolates of the same species was reviewed by Fux *et al.* (2005).

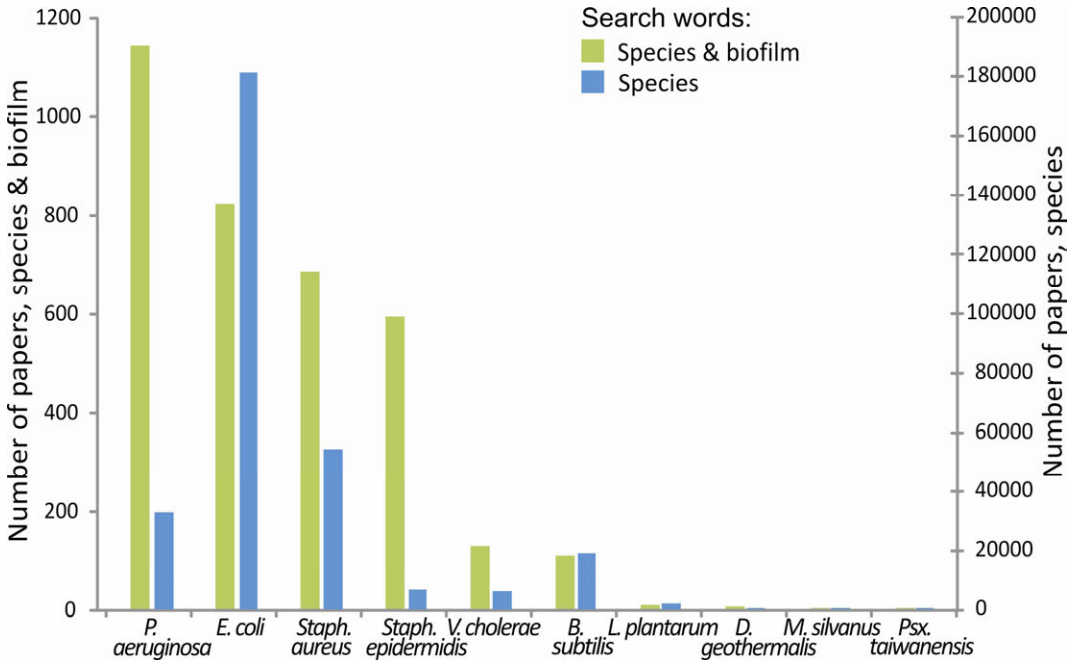

Fig. 3. Number of papers published in peer reviewed journals relating to certain biofilm forming bacteria. The articles were searched by the author from PubMed with the search words: *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Vibrio cholerae, Bacillus subtilis, Lactobacillus plantarum, Deinococcus geothermalis, Meiothermus silvanus* or *Pseudoxanthomonas taiwanensis* and biofilm and limiting the search to the title and the abstract.

Not all of the knowledge presently available of biofilms is applicable to biofilms in industrial settings or bacterial species recently isolated from an industrial environment. Biological characteristics of biofilm forming bacterial species belonging to diverse phyla are summarised in Tables 2 a and b.

Table 2. Characteristics of selected bacterial species on which biofilm studies have been reported in the literature. Table 2 a; the bacterial species used as model organisms in this thesis: *Deinococcus geothermalis, Meiothermus silvanus, Pseudoxanthomonas taiwanensis, Staphylococcus epidermidis* and *Lactobacillus plantarum*. Table 2 b; the bacterial species most often used in biofilm research: *Vibrio cholerae, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis.*

Table 2 a	*Deinococcus geothermalis*	*Meiothermus silvanus (syn. Thermus silvanus)*	*Pseudoxanthomonas taiwanensis*	*Staphylococcus epidermidis*	*Lactobacillus plantarum*
Phylum/class	*Deinococcus-Thermus*	*Deinococcus-Thermus*	*Gammaproteobacteria*	*Firmicutes*	*Firmicutes*
Metabolism	Aerobe, chemoorganotrophic	Strict aerobe, can use nitrate as terminal electron acceptor	Strict aerobe, heterotrophic	Facultative anaerobe	Facultatively heterofermetative
Cell wall	The envelope consisted of a three-layered asymmetric cytoplasmic membrane surrounded by a gram-positive-like cell wall with an innermost highly electron-dense layer and a corrugated outer surface. Growth on solid medium was shown to produce a fibrous periodic acid-Schiff positive capsular layer surrounding and interconnecting adjacent cells.	An envelope consisted of a three layer symmetric cytoplasmic membrane and a cell wall with an inner, electron-dense thin layer, which presumably represens the peptidoglycan. An outer corrugated layer was connected to the peptidoglycan by irregularly spaced invaginations.		Cell wall teichoic acid: glycerol, glucose and N-acetylglucosamine	Teichoic acid: ribitol or glycerol
Flagelli	0	0	0	0	0
Fimbriae	Type IV pili				
G + C mol%	65.9	63.6	69.9-70.1	33.5 ± 0.2	44-46
Cell morphology	Spherical Ø 1.2 to 2.0 µm, non-motile, nonsporing, Gram stains positive	Gram-neg., rods of 0.5 to 0.8 µm wide, various lengths and short filaments, nonsporing	Gram-neg., rods 0.5-0.8 µm × 0.9-1.4 µm, non-motile, nonsporing	Gram-pos. spherical Ø 0.5 to 1.0 µm, in pairs and tetrads. Non-motile, nonsporing	Gram-pos. rods, 0.7 to 1.0 × 3.0 to 8.0 µm, singly or in short chains. Non-motile, nonsporing
Pigment	Pink, orange	Red	Yellow, brown	None	

	Deinococcus geothermalis	*Meiothermus silvanus*	*Pseudoxanthomonas taiwanensis*	*Staphylococcus epidermidis*	*Lactobacillus plantarum*
Peptidoglycan type	A3β (L-Orn-Gly2-3)	A3β (L-Orn-Gly2-3)	ND	A3α (L-Lys-Gly4-5, L-Ser0.7-1.5)	A1γ (m-Dpm-direct)
Respiratory quinones	Menaquinone 8	Menaquinone 8	ND*	Menaquinone 7	None
Isolation sites	Hot springs and runoffs at Italy and Portugal; paper machines	Hot springs and runoffs at Vizela, Portugal and Geysir geothermal area, Iceland; paper machines	Chi-ban Hot Springs in eastern Taiwan; paper machines	Human skin and cutaneous ecosystem, indwelling medical devices as opportunistic pathogen	Dairy products, silage, pickled vegetables, sour dough, cow dung, sewage and human mouth, intestinal tract and stools
Other	Gamma radiation resistant, primary biofilm former from paper machines	Found from several defects (holes and spots) in paper products	Primary biofilm former from paper machines, unusual denitrification reaction, reducing nitrite, but not nitrate, with the production of N_2O only	Presently recognized as an opportunistic pathogen	Used for the production of fermented foods. Certain strains are used as probiotics
Reference	Saarimaa *et al.*, 2006, Battista and Rainey, 2001, Ferreira *et al.*, 1997	Tenreiro *et al.*, 1995, Nobre *et al.*, 1996, Nobre and da Costa, 2001, Ekman *et al.*, 2007	Lipski and Stackebrandt, 2005, Chen *et al.*, 2002	Kloos and Schleifer, 1986, Schleifer and Kloos, 1975	Kandler and Weiss, 1986, Pederson, 1936, Brooijmans *et al.*, 2009

ND = Not detected

*) Ubiquinone 8 is found from several species of *Pseudoxanthomonas, e. g. Psx. broegbernensis* (Finkmann *et al.*, 2000), *Psx. mexicana* and *Psx. japonensis* (Thierry *et al.*, 2004) *Psx. sacheonensis* (Lee *et al.*, 2008), *Psx. dokdonensis* (Yoon *et al.*, 2006)

Table 2 b	*Vibrio cholerae*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Bacillus subtilis*
Phylum	*Gammaproteobacteria*	*Firmicutes*	*Gammaproteobacteria*	*Gammaproteobacteria*	*Firmicutes*
Metabolism	Facultative anaerobe, chemoorganotrophic, reduction of NO_3^- to NO_2^-, acid but no gas is formed from fermenting glucose	Facultative anaerobe, chemoorganotrophic	Aerobe, respiratory metabolism with oxygen as terminal electron acceptor but can use nitrate as alternative electron acceptor.	Aerobe and facultatively anaerobe, both respiratory and fermentative type of metabolism, chemoorganotrophic. Acid and gas are formed from fermentable carbohydrates	Aerobe, chemoorganotrophic, reduction of NO_3 to NO_2
Cell wall	Lipopolysaccharide (LPS) characteristic to Gram-neg. bacteria.	Capsule. Cell wall teichoic acid: ribitol with either α- or β-glycosidically liked *N*-acetyl-D-glucosamine residues.	Cell wall and membranes characteristic of Gram-neg. bacteria. No capsule. Two LPS O-polysaccharide species in outer membrane: hydrophobic and hydrophilic.	Outer membrane lipopolysaccharide (LPS) characteristic to Gram-neg. bacteria.	Paracrystalline cell wall surface layer (S-layer)
Flagelli	1	0	1	Peritrichous 5-10	Peritrichous
Fimbriae	Mannose-sensitive haemagglutinin type IV pili (MSHA), toxin co-regulated pili (TCP), chitin-regulated pili (ChiRP)		Type IV pili	Type 1 pili. More than 30 fimbriae described in *E. coli*	
G + C mol%	47-49	32-36	67.2	48.5-52.1	42.9-43.1
Cell morphology	Gram-neg. rods 0.5-0.8 µm × 1.4-2.6 µm straight or curved, motile, nonsporing	Gram-pos. spherical Ø 0.5 to 1.0 µm, singly or in pairs. Non-motile, nonsporing	Gram-neg. rods 0.5 µm × 1.5-2.5 µm, motile, nonsporing	Gram-neg. rods 1.1-1.5 µm × 2.0-6.0 µm, singly or in pairs, motile, nonsporing	Gram-pos. rods 0.7-0.8 µm × 2-3 µm, motile, endospore forming
Pigment	Some strains produce melanin like brown pigment	Triterpenoid carotenoid, (gray with yellowish tint, yellow-orange, orange)	Pyocyanine, pyorubin, chlororaphin, oxiphenazin, the *Pseudomonas* blue protein, pyoverdine	Orange-red pigment on tryptophane containing media	Brown, red, orange, black

	Vibrio cholerae	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Bacillus subtilis*
Peptidoglycan type	A1γ (*m*-Dpm-direct)	A3α (L-Lys-Gly_{5-6})	A1γ (*m*-Dpm-direct)	A1γ (*m*-Dpm-direct)	A1γ (*m*-Dpm-direct)
Respiratory quinones	Ubiquinone 8	Menaquinone 7 and 8	Ubiquinone	Ubiquinone, menaquinone and demethylmenaquinone	Menaquinone 7
Optimum T°C	37	30 to 37	37	37	37
Isolation sites	Water: sewage, brackish water, estuaries, coastal inlets, polluted streams, rivers, ponds, lakes and tissues of crustacean	Nasal membranes, skin, perineum, gastrointestinal tract and genital tract of warm-blooded animals	Soil, water, clinical specimens	Lower part of the intestine of warm-blooded animals	Endospores are widespread. Vegetative cells in degrading organic materials
Other	Some serotypes cause severe diarrhea, cholera, and other serotypes cause gastrointestinal diseases in varying severity	Pathogen, causes number of infectious in human	Some strains produce alginate (*e. g.* mucus). Opportunistic pathogen	Colonises mammalian intestine as a harmless commensal, but certain strains are pathogenic for humans and animals.	Some strains produce heat stable toxin amylosin
Reference	Yildiz and Visick, 2009, Farmer III *et al.*, 2005, Casutt *et al.*, 2010	Kloos and Schleifer, 1986	Palleroni, 2005, Hugh and Leifson, 1964, Heilmann, 1974	Scheutz and Strockbine, 2005, Unden and Bongaerts, 1997, Benjamin and Tamhane, 1966, Schleifer and Kandler, 1972	Claus and Berkely, 1986, Apetroaie-Constantin *et al.*, 2009

1.2.2 Biofilms in industrial settings - biofouling

Biofilms are the predominate mode of growth for microbes in industrial settings and engineered water systems as well as in natural ecosystems. Biofouling is a term describing unwanted biofilm formation on equipment surfaces. This can occur in wide range of man-made environments and can significantly decrease equipment performance and lifetime and cause contamination and impaired quality of the products. Since biofilms are more resistant against external forces than free swimming cells, they are more difficult to eradicate from industrial processes by biocides or cleaning agents. Therefore they create diverse problems (Table 3). In heat exchangers biofilms decrease the efficiency of heat transfer by forming an insulating layer and in pipelines they cause fluid frictional resistance and induce corrosion (MIC, a book by: Borenstein, 1994). In ship hulls the marine biofilms reduce speed and increase the fuel consumption of the ships. In drinking water industry biofilms may provide a habitat for many pathogenic bacteria (for reviews see Coetser and Cloete, 2005 and Flemming, 2002). In food industry biofilms are common sources of contamination, since they are attached and grow on food processing equipments, food contact surfaces and pipelines and are not always removed by routine cleaning procedures. This may lead to serious hygienic problems and food spoilage. Significance of biofilms in food industry has been reviewed by Kumar and Anand, 1998.

Table 3. Biofilm problems in selected industrial settings.

Industrial process	Biofilm problem	Bacterial species found from	
Paper industry	Slimes on machine surfaces affect machine runnability and can induce process failure. The slimes dropping from machine surfaces can cause defects (spots, holes) in end products. Microbially induced corrosion (MIC). Contaminations in end products.	Biofilms on paper machine surfaces	Reference
		Burkholderia cepacia, B. coagulans, Deinococcus geothermalis, Meiothermus silvanus, Pseudoxanthomonas taiwanensis, Bacillus cereus, Brevundimonas vesicularis, Cytophaga sp.*, Enterobacter* sp.*, Klebsiella pneumonia, Paenibacillus stellifer, Starkeya* sp., *Rubellimicrobium thermophilum*	Ekman *et al.*, 2007, Väisänen *et al.,* 1998, Peltola *et al.,* 2008b, Kolari *et al.,* 2003, Rättö *et al.,* 2005, Denner *et al.,* 2006
		End-product defects	
		Meiothermus spp.	Ekman *et al.*, 2007, Haapala *et al.*, 2010
		Slimes on corrosion pits of stainless steel	
		Sulphate reducing bacteria	Thorpe, 1987, Uutela *et al.*, 2003
		End-products	
		Bacillus cereus, B. mycoides, B. thuringiensis, B. globisporus, B. licheniformis, B. megaterium, B. circulans, B. pumilus, B. subtilis, B. flexus, Paenibacillus polymyxa, P. macerans, P. pabuli, P. stellifer	Väisänen *et al.,* 1991, Pirttijärvi *et al.*, 1996, Raaska *et al.*, 2002, Suominen *et al.*, 2003
Malting and brewing	EPS production by bacteria on barley kernel during malting may restrict grain germination and disturb wort separation in brewing process. Using *Fusarium* infected barley malt may cause alterations in wort composition, beer gushing as well as mycotoxin production. The proteases produced by mold may affect color, flavor, texture, and foaming of beer.	EPS production	
		Pseudomonas spp., *Enterobacter* spp.	Laitila *et al.*, 2007a, Laitila *et al.*, 2006
		Mycotoxin, protease and gushing factor production	
		Fusarium spp.	Wolf-Hall, 2007

Industrial process	Biofilm problem	Bacterial species found from	
Dairy	Contamination of end-products which may cause food borne illnesses to the consumers and decreased life-time of the product	Biofilms on dairy plant surfaces	
		Staphylococcus epidermidis, Bacillus spp., *Lactobacillus* spp., *Streptococcus* spp., *Lactococcus* spp., *Staphylococcus* spp., *Shigella spp., Escherichia coli , Enterobacter aerogenes, Citrobacter* spp., *Flavobacterium* spp., *Proteus* spp., *Listeria monocytogenes*	Wong, 1998, Sharma and Anand, 2002, Jaglic *et al.*, 2010
Food-processing	Contamination of products which may cause food borne illnesses to the consumers and decreased life-time of the product	Biofilms on beef-processing plant surfaces	
		Shewanella putrefaciens, Pseudomonas putida, P. anguilliseptica, Arthrobacter spp., *Staphylococcus xylosus, Staph. capitis, Staph. sciuri, Micrococcus caseolyticus, Bacillus subtilis, B. pumilus, Kocuria varians, Aerococcus viridans, Staphylococcus equorum, Listeria monocytogenes*	Marouani-Gadri *et al.*, 2009, Leroy *et al.*, 2009, Gamble and Muriana, 2007, Kathariou, 2002
		Biofilms on raw vegetable processing line	
		Vibrio diazotrophicus, Serratia plymuthica, Panthoea agglomerans	Van Houdt *et al.*, 2004
		Biofilms in poultry processing plants	
		Listeria monocytogenes	Kathariou, 2002, Ojeniyi *et al.*, 2000
Industries using heat exchangers	Biofouling causes efficiency loss of the heat exchanger, clogging of the cooling circuit pipes and may cause MIC. Bacteria can oxidize soluble Mn^{2+} to insoluble MnO_2	Thermo-fluid heat exchanger in nuclear power plant	
		Iron oxidizing bacteria and sulphate reducing bacteria	Rao *et al.*, 2009
		Lamellar heat exchanger in Baltic seawater after pressure wash	
		Shewanella sp., *Pseudomonas* sp., *Flavobacterium* sp., *Aeromonas* sp.	Kuosmanen *et al.*, 2006

Industrial process	Biofilm problem	Bacterial species found from	
Drinking water industry	The drinking-water distribution systems and bottom of drinking-water reservoirs are colonised by biofilms which might affect the water's quality, *e.g.* taste, odour and colour. The biofilms can provide a habitat for pathogenic micro-organisms. Nitrification by bacteria can contribute to the depletion of monochloramine and result in the formation of nitrate.	Biofilms	
		Pseudomonas vesicularis, Flavobacterium spp., *Burgholderia cepacia, P. pickettii, P. stutzeri, Alcaligenes* spp., *Acinetobacter* spp., *Moraxella* spp., *Agrobacterium radiobacter, Arthrobacter* spp., *Corynebacterium* spp., *Bacillus* spp., *Enterobacter agglomerans*	LeChevallier *et al.*, 1987
		Pathogenic bacteria in biofilms	
		Legionella spp., *Aeromonas* spp., *Pseudomonas aeruginosa*. *Campylobacter jejuni, C. coli, Mycobacterium intracellulare, M. avium, M. lentiflavum, M. gordonae*	Szewzyk *et al.*, 2000, Falkinham, 1996, Falkinham *et al.*, 2001, Sen and Rodgers, 2004, Tsitko *et al.*, 2006
		Nitrifiers	
		Nitrosomonas spp., *Nitrosospira* spp., *Nitrobacter* spp.,	Berry *et al.*, 2006

1.2.3 Biofilms in paper machine environment

Paper mills provide ideal conditions for bacteria to grow and form biofilms. The dissolved and colloidal substances, such as polysaccharides and wood extractives, are released into the process waters during pulping and are transferred to paper machine, where they provide energy sources and nutrients for microbial growth. In addition to wood components, the process waters contain papermaking additives such as fillers, retention aids and fixatives. The conditions, *i.e.* warm water (40 to 55°C) and near neutral or slightly alkaline pH, prevailing in the paper mahine, are suitable for microbes. Planktic bacteria, even in high numbers (10^6 ml^{-1} of white water), do not necessarily adversely influence machine operation or the quality of the end-product, but biofilms, *i.e.* slimes and stickies, accumulating on steel surfaces of the machinery are known to cause problems in runnability, process failure, and contaminations and defects (spots, holes) in end products (Ekman *et al.*, 2007, Haapala *et al.*, 2010, Sanborn, 1933, reviewed by Blanco *et al.*, 1996 and Kanto Öqvist, 2008). Therefore, frequent down-time for cleaning of the machine is needed. The paper machine slimes have been show to consist of bacterial cells and EPS produced by bacteria as well as wood fibres, emulsified pitch and inorganic material (Väisänen *et al.*, 1994, Lindberg *et al.*, 2001).

Not all bacteria can initiate a biofilm formation in paper machine environment (Kolari *et al.*, 2001). Therefore, it is important to identify the primary biofilm formers in order to design antifouling strategies for paper machines. The genera *Deinococcus, Meiothermus, Pseudoxanthomonas* and *Rubellimicrobium,* have been identified as primary biofilm formers by methods requiring cultivation (Kolari *et al.*, 2003, Denner *et al.*, 2006, Kolari *et al.*, 2002). However, it has been shown that the biofilms are colonised also by unculturable bacteria and that nitrogen fixing bacteria might have a role in formation of paper machine biofilms (Lahtinen *et al.*, 2006). The bacteria initiating biofouling in two paper mills were studied by Tiirola *et al.* (2009) using heterogeneity analysis of PCR-amplified 16S rRNA genes (LH-PCR), a method not requiring cultivation, for profiling bacteria attaching on stainless steel in process water. They found that primary biofilm formers in these machines were from the genera *Rhodobacter, Tepidimonas* and *Cloacibacterium.* These genera have been described from paper machine previously as well (Kolari *et al.*, 2003, Suihko and Skyttä, 2009, Prince *et al.*, 2009).

Coloured biofilms are especially harmful when occurring on paper machines (Kolari *et al.*, 2003), since they cause spots in the end-products spoiling the quality (Ekman *et al.*, 2007) (Fig. 4). Bacterial species forming pigmented biofilms in paper machines have been isolated,

characterized and quantitated in many studies (Ekman *et al.*, 2007, Väisänen *et al.*, 1998, Peltola *et al.*, 2008b, Kolari *et al.*, 2003, Denner *et al.*, 2006, Kolari *et al.*, 2001, Oppong *et al.*, 2000).

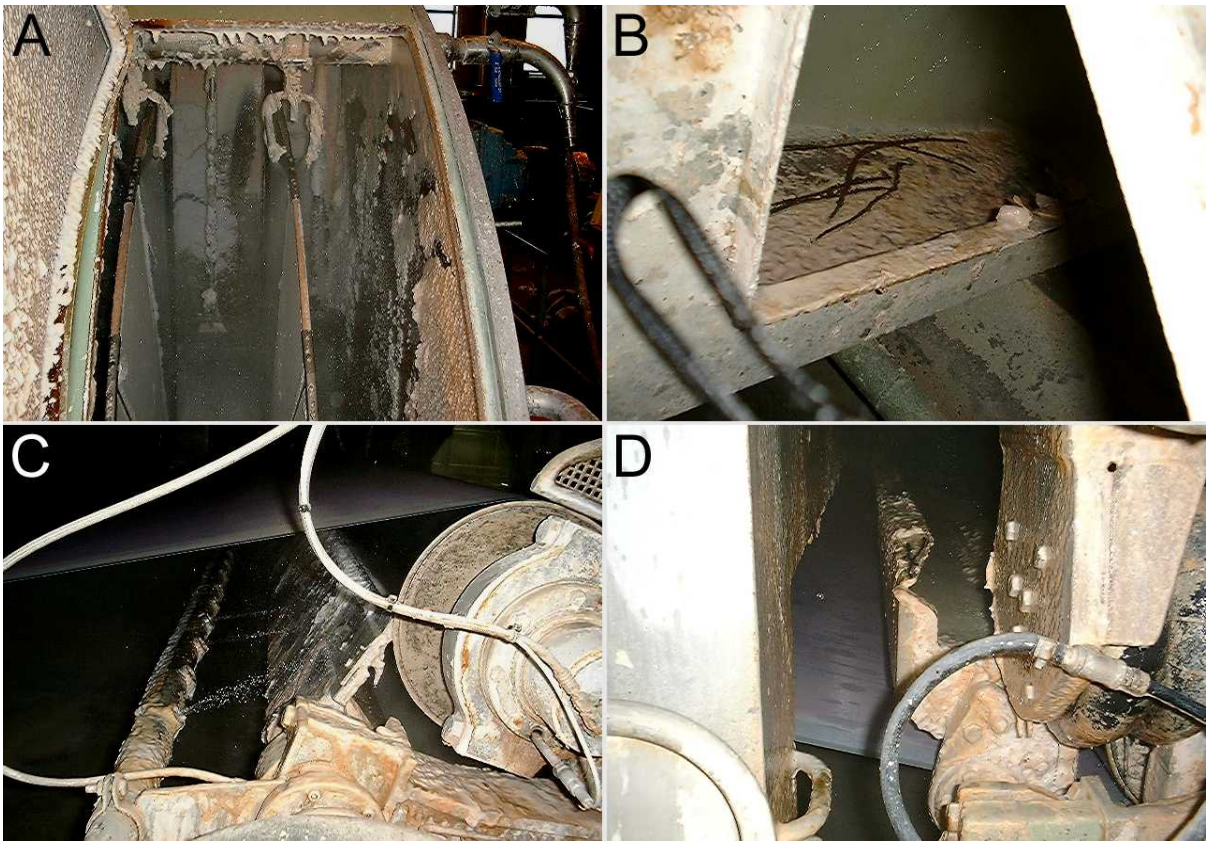

Fig. 4. Colourful biofilms on paper board machine surfaces. Panel A, disc filter; panel B; duct of a wire pit; panel C, doctor blade of the leading roll on wire section; panel D, splash area in the wire section. Courtesy of Juhana Ahola.

Väisänen *et al.* (1998) identified the species *Deinococcus geothermalis* as a producer of pink biofilms on the wire section of paper machines. This species was capable of adhering to stainless steel and long-term growth at elevated temperatures (50°C). *D. geothermalis* is also known for its radiation resistance (Ferreira *et al.*, 1997), a property connected to desiccation resistance, and organic solvent-tolerance (Kongpol *et al.*, 2008). Later Kolari *et al.* (2003) recurrently found this species among 95 pink-, red-, orange- or yellow pigmented biofilm forming isolates from several paper and board machines.

D. geothermalis, Meiothermus silvanus and *Pseudoxanthomonas taiwanensis* (the latter was earlier thought to represent *Thermomonas* spp.), the species found from coloured biofilms by Kolari *et al.* (2003), and *Tepidimonas* spp. have been isolated and described from geothermal springs before (Ferreira *et al.*, 1997, Tenreiro *et al.*, 1995, Chen *et al.*, 2002, Moreira *et al.*, 2000, Chen T. L. *et al.*, 2006). The findings indicate hot springs as the natural

habitat of many paper machine bacteria. Runoff areas of geothermal springs are often coloured by pigmented biofilms (Fig. 5).

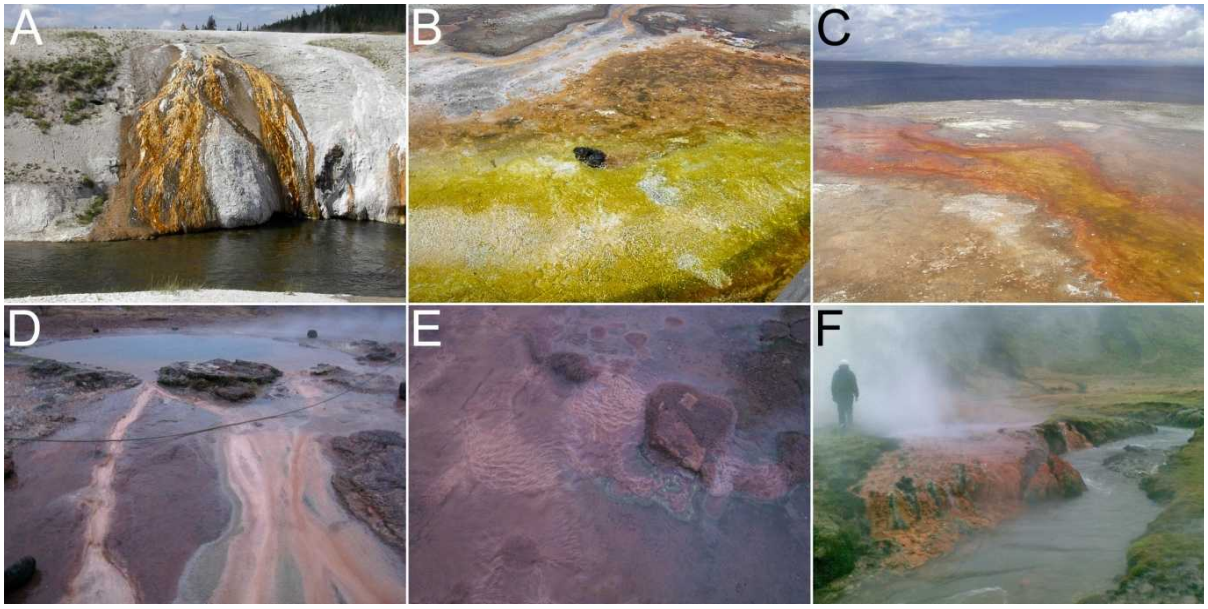

Fig. 5. Colourful biofilms growing in the runoff areas of hot springs. Panels A to C, Yellowstone National Park, USA; Panels D and E, Geysir geothermal area, Iceland; Panel F, Reykjadalur valley, Dalasel-Klambragil area, Iceland. Photos: Mari Raulio.

Ekman *et al.* (2007) used quantitative PCR to study the prevalence of *Meiothermus* spp. and Peltola *et al.* (2008b) of *D. geothermalis* in deposits, slimes and end products in paper industry. These studies showed that *Meiothermus* spp. are common biofoulers in paper industry and often occur in large quantities in the deposits from paper machines. They also demonstrated the connection between coloured microbes and end-product defects. *Meiothermus* was reported as the dominant bacterial genus also by other authors in a Canadian paper machine (Prince *et al.*, 2009). Haapala *et al.* (2010) found *Meiothermus* spp. from coloured biofilms collected from surfaces and from the end product of a paper machine producing newsprint. *D. geothermalis* was found at many machines but always as a minor constituent. It was detected in deposits in splash areas of the wire section from machines where *Meiothermus* spp. was the dominant constituent. Peltola *et al.* (2008b) suggested that *D. geothermalis* operated as a pedestal for other bacteria to adhere and grow into a biofilm. *Psx. taiwanenis* has been reported from various samples in four studied paper mills (Suihko *et al.*, 2004) and from pulp of two paper mills (Suihko and Skyttä, 2009). *Pseudoxanthomonas* sp. was reported from pulps and slimes (Desjardins and Beaulieu, 2003) as well as from the headbox of a paper machine in a Canadian mill (Prince *et al.*, 2009) and later from newsprint producing machine where it was common throughout the machine (Haapala *et al.*, 2010).

It is clear that the diversity of bacteria colonizing a paper machine is extensive, and in addition the population fluctuates according to raw materials and biocide treatments as well as changing conditions. However, only a part of this large community of microbial species is capable of colonizing clean surface and causing problems. This suggests that designing antifouling methods targeted against the primary biofilm formers, not necessarily aiming to kill them, could be a rational aim.

1.2.4 Biofilms on medical implants

Biofilms were estimated to be associated with 65% of reported nosocomial infections (Mah and O'Toole, 2001). Biofilms are a common cause of persistent infections, such as dental caries, valve endocarditis, otitis media, periodontitis, cystic fibrosis, chronic wounds, and a frequent reason for the failure of biomedical devices, such as prosthetic joints, catheters, heart valves and cardiac pacemakers. Because of the high resistance of bacteria in biofilms to antimicrobial agents, implant infections can often be remediated only by surgical removal of the implant (reviewed by Costerton *et al.*, 1999 and 2005, McCann *et al.*, 2008).

The common human skin bacterium *Staphylococcus epidermidis*, once considered harmless, has become one of the most important pathogens causing chronic infection in immunocompromised, immunosuppressed and long-term hospitalized hosts. It is also the leading cause of infections of implanted medical devices (reviewed by Costerton *et al.*, 1995, Götz, 2002, McCann *et al.*, 2008, Rohde *et al.*, 2010). In *Staph. epidermidis*, as well as other staphylococci, the major component of the EPS is a polysaccharide intercellular adhesin (PIA), a polysaccharide composed of β-1,6-linked *N*-acetylglucosamines with partly deacetylated residues, whose synthesis is mediated by the *ica* operon (Mack *et al.*, 1996). PIA has been shown to be a major virulence factor and to contribute to biofilm formation of *Staph. epidermidis* (Izano *et al.*, 2008, Mack *et al.*, 1994). The cells are embedded in PIA (Fig. 6), which is a barrier against immune defence of the host and against treatments with antibiotics (reviewed by Costerton *et al.*, 2005, Götz, 2002, Rohde *et al.*, 2010).

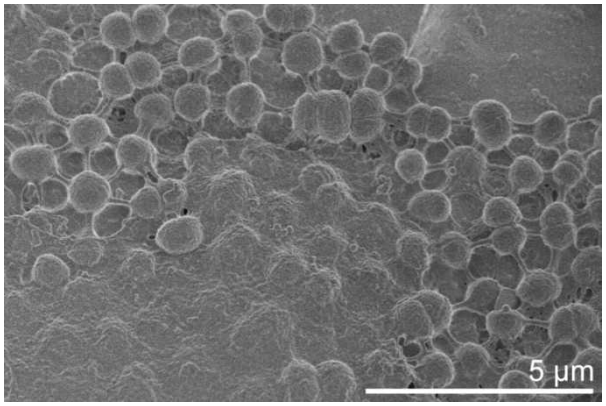

Fig. 6. Scanning electron micrograph of *Staphylococcus epidermidis* O-47 (PIA^+) attached on steel surface. The slimy appearance of biofilm is due to a polysaccharide intercellular adhesin (PIA) produced by these bacteria. Courtesy of Juhana Ahola.

1.2.5 Biofilms on malted barley kernel tissues

Malted barley is used for the production of beer and distilled spirits as well as an ingredient for various food products. Malting exploits the biochemical reactions of grain germination as enzymatic release of nutrients, such as fermentable sugars, needed for yeast growth in beer fermentation. The barley kernel is naturally colonised by microorganisms. The malting process is consisting of two metabolically active groups: the microbial community and the barley kernel.

Malting consists of three steps: steeping, germination and kilning. Steeping of barley, *i.e.* soaking in water, induces germination of the kernel but also favours microbial growth and is therefore considered to be the critical step in malting with respect to microbial activity (Laitila *et al.*, 2006a, Noots *et al.*, 1999, Kelly and Briggs, 1992, O'Sullivan *et al.*, 1999). Microbial activity remains high during the germination stage, but the removal of water that takes place during kilning slows down the microbial activity. Nevertheless, kilning appears to have little impact on the viable counts of bacteria or fungi. Generally the counts are higher in the finished malt than in the dry kernels (Laitila *et al.*, 2006a and 2007a, reviewed by Noots *et al.*, 1999).

The diversity of microbial species depends on the barley cultivar, climate, soil as well as the conditions of harvesting, storage and transport. Various types of bacteria, yeast and filamentous fungi are present (Laitila *et al.*, 2006a and 2007a, Noots *et al.*, 1999, Petters *et al.*, 1988). The microorganisms present during the malting significantly affect the grain germination, performance of malting and brewing as well as the quality of the final product (Laitila *et al.*, 2006b and 2007b, Noots *et al.*, 1999, Doran and Briggs, 1993, Kelly and Briggs, 1992, Lowe and Arendt, 2004, Van Campenhout *et al.*, 1998, Van Campenhout *et al.*, 1999). Depending on the species present, these impacts may be beneficial or harmful for the malting and brewing processes. Therefore it is important to know the composition of microbial communities in barley kernels. Malt-derived lactic acid bacteria and certain fungi

offer a potential tool as biocontrol agents. The well characterised microbes can be applied as starter cultures into the steeping water to control the growth of undesirable indigenous microbial contaminants (Laitila *et al.* 2006, Laitila, 2007 and reviewed by Lowe and Arendt, 2004).

It appears that microbes in barley kernels are present as biofilms, which are remarkably resistant against desiccation as well as antimicrobial agents (Laitila, 2007).

1.3 Antifouling – how to prevent or to remove unwanted biofilms

Wet industrial processes provide environments for formation of microbial biofilms. Open processes are impossible to keep sterile. Designing materials or coatings effectively repelling towards all kinds of microbial colonization on long term basis has proven to be equally impossible. Therefore novel methods for antifouling are needed.

In paper industry microbial growth is usually controlled by dispensing biocides and slime removing chemicals into the process. In addition, mechanical cleaning of the machinery is necessary (reviewed by Blanco *et al.*, 1996). The procedures used in industrial cleaning are not necessarily effective against most tenacious primary biofilm formers, like *D. geothermalis* (Kolari *et al.*, 2002). The efficacy of biocides is diminished by the appearance of biocide resistant microbial species and also causes concern for damage to the environment when discharged in wastewaters. Kolari *et al.* (2003) reported that by the usage of biocides, such as methylene bisthiosyanate (MBT) or 2,2-dibromo-3-nitrilopropionamide (DBNPA), at concentrations inhibiting planktic bacteria actually promoted the transition of the colonisers into biofilm mode of growth.

In processes like food manufacturing or in systems in open connection to marine or fresh water environment, control of biofouling with chemical biocides is not possible. Engineering surfaces and coating materials to repel microbial adhesion or using physical methods (*e.g.* photocatalysis, electrochemical polarization) rather than chemicals is therefore of great interest for antifouling. The countermeasures against biofouling should be separately tailored for each individual process situation.

1.3.1 Surface parameters affecting the adherence of bacteria

More than three decades ago, Fletcher and Loeb (1979) studied the influence of substratum characteristics on the attachment of marine bacteria. They concluded that both electrostatic and hydrophobic interactions were involved in bacterial attachment. They also found that larger numbers of bacteria attached to hydrophobic than to hydrophilic surfaces.

The substrata found to be most repellent towards marine bacteria were hydrophilic and negatively charged. After this study a considerable number of papers have been published in this area, but no agreement on the surface parameters to repel microbial adhesion has been achieved. Also, comparison of the results is complicated, since the bacterial strains, conditions and detection methods vary between the studies.

Yet, some major trends were seen. Goller and Romeo (2008) reviewed the surface parameters influencing initial attachment of bacteria and noted that surface roughness and hydrophobicity were important. In agreement with Fletcher and Loeb, Goller and Romeo stated that bacteria typically adhered to hydrophobic surfaces more eagerly than on hydrophilic surfaces. As an example they used clinical isolates of *Staphylococcus epidermidis*. *Listeria monocytogenes* was mentioned as an exception.

Gottenbos *et al.* (2001) studied the adhesion and surface growth of *Staphylococcus aureus, Staph. epidermidis, Pseudomonas aeruginosa* and *Escherichia coli* on positively and on negatively charged poly(methacrylate) surfaces. As expected, they found that bacteria adhered more on the positively charged surface, but interestingly, the surface growth of the two Gram-negative species was inhibited on these surfaces. They suggested that this is due to strong binding through electrostatic interaction.

In general rough surfaces, offering a large surface area, are colonised more easily than smooth surfaces, but bacteria can attach even to smooth surfaces. The surface roughness has been reported to be a dominant factor in the plaque formations (Morgan and Wilson, 2001, Charman *et al.*, 2009). Arnold and Bailey (2000) studied the attachment of a mixed species bacterial population on stainless steels with different surface roughness and found that significantly fewer bacterial cells attached on electropolished stainless steel, which was the least rough surface, than on the other treated surfaces. This result was confirmed by Mattila *et al.* (2002). Inconsistent result was reported by Mitik-Dineva *et al.* (2009), who found that *E. coli*, *P. aeruginosa* as well as *Staph. aureus* attached significantly more on modified glass surface than on non-modified glass. The modified glass surface was significantly smoother according to data obtained from the determination of four roughness parameters (*i.e.* the average surface roughness, the root mean square, the maximum roughness and the 10-point average roughness). However, glass is hydrophilic wheras steel is hydrophobic surface, which may explain this inconsistancy.

It has also been suggested that there is an optimal roughness, or surface topography, for bacterial adherence. It has been hypothesized that if the crevises on the surface are similar size to microbial cell, the adhesion is more efficient than on a rougher or on a smoother

surface. The large crevices would offer protection from shear forces and offer an increased area for attachment providing more contact points (reviewed by Verran and Boyd, 2001 and Whitehead and Verran, 2006). On the other hand, it has been reported that specific micro-topography decrease bacterial adherence (Allion *et al.*, 2006). Medilanski *et al.* (2002) found that four bacterial species from three different phyla adhered least onto steel with scratches of the size corresponding to the width of the bacterial cells. Bacteria fitted these scratches in longitudinal orientation only. Three of the strains were found to align in the scratches.

As a conclusion, the role of the surface roughness to microbial adhesion is still under debate, but most of the studies show that smoother surface attracts fewer bacteria.

After decades of searching a surface or a surface coating repellent to microbial adhesion, it seems likely that there is no such surface parameter which would prevent adhesion of all microorganisms in all environments. It is difficult to predict adherence of bacteria in natural waters, containing non-defined dissolved components that may alter the substratum. Microorganisms may possess different surface characteristics, making the situation even more unpredictable. Microorganisms are able to adapt to a new environments and new surfaces, making the surfaces prone to colonisation. In addition, the conditioning layer forming also on antibacterial surfaces may change characteristics of the surface and allow biofilm formation when under heavy bacterial load (Chiang *et al.*, 2009).

However, a multible approach, surface parameters that reduce microbial attachment combined with control of biofilm formation or with enhancement of the removal of biofilm may offer a great progress in many industrial settings.

1.3.2 Mechanisms occurring in nature

1.3.2.1 Lotus effect

In some Eastern cultures, the lotus plant (*Nelumbo nucifera,* Fig. 7 A) is a symbol of purity. Lotuses prefer to grow in muddy waters but the leaves remain clean. This phenomenon is nowadays commonly called lotus effect. The lotus effect refers to the very high water repellency (superhydrophobicity) exhibited by the leaves of the certain aquatic plants. On the lotus leaf (Fig. 7) the epidermal cells coated with tubular epicuticular waxes create a structured surface with high water repellency (non-wettability) and reduced particle adhesion generating a self-cleaning mechanism (Koch and Ensikat, 2008, Lafuma and Quere, 2003, Barthlott and Neinhuis, 1997). The adhesion of contaminating particles, including pathogenic spores and conidia, onto the leaf is reduced due to surface structure and the particles are removed completely by water droplets rolling off the surface. There would be

many applications for self-cleaning materials mimicking this natural phenomenon. However, it has been reported that the self-cleaning effect is not achieved with submerged surfaces in nature (Flemming, 2002).

Fig. 7. Panel A, lotus leaf with water droplets (http://mi9.com/c22p1g2008f1i25647/lotus-leaf/); panel B, computer graphic of lotus leaf surface with water droplets and dirt particles. http://en.wikipedia.org/wiki/File:Lotus3.jpg.

1.3.2.2 Disturbance of quorum sensing

Several marine organisms, mostly bacteria or cyanobacteria, block quorum-sensing signals to control the growth and biofilm formation of other bacteria (Dobretsov *et al.*, 2009). Hentzer *et al.* (2002) found that a synthetic halogenated furanone, derivative of the secondary metabolites produced by an Australian macroalga (*Delisea pulchra*), permeated into the biofilm matrix and interfered with quorum-sensing in *Pseudomonas aeruginosa*. The compound did not affect the growth or the initial attachment of *P. aerugnosa* to the substratum, but only the architecture of the biofilm. It also enhanced the detachment, which led to a loss of bacterial biomass from the substratum. Later Hentzer *et al.* (2003) reported that this furanone also inhibited the expression of virulence factors in *P. aeruginosa*.

After this discovery, quorum sensing inhibition has been under intense investigation. In future prospects the quorum-sensing blockers will be applied as anti-microbial agents to control biofilm-associated infections (reviewed by Njoroge and Sperandio, 2009). Biotechnological applications of quorum-sensing inhibitors have resulted in more than 100 published patents worldwide. The applications include industrial as well as therapeutic usage (reviewed by Dobretsov *et al.*, 2009).

1.3.3 Engineering microbe repelling coatings

Engineering surfaces with certain physical parameters (topography, charge *etc.*) to repel microbial adhesion with no direct antimicrobial action is very challenging. In industrial

processes the complexity of microbial populations with multiple adhesion mechanisms and possibility of fast mutations, as well as the changing conditions make the situation very complex. Even for a single microbial strain and a surface, the environmental stimuli can change the relative importance of the mechanisms of adhesion and characteristics of the surface. There is no single material feature or microbial characteristic that would completely describe or control the microbial adhesion. (Lichter *et al.*, 2009)

Lichter *et al.* (2009) introduced three general strategies to limit colonization of material surfaces. The first strategy is based on repellency of bacterial adhesion by modifying the surface characteristics; *e.g.* hydrophobicity or topography. In this strategy the focus is not in killing but in inhibition of the adhesion.

The second strategy is based on surfaces impregnated with antimicrobial agents and leaching of this agent into the surrounding solution. This "release-killing" kills bacteria not only on the surface, but also nearby. For example silver-ions are known to disrupt the permeability barrier function of the cell membrane. Serious disadvantage in this strategy is that the diffusion of antimicrobial agent into the environment may cause emergence of resistant strains. In addition, when antimicrobial agent is released gradually into the surrounding solution, the effect fades when leaching antimicrobial agent is exhausted (Lichter *et al.*, 2009).

The third strategy is based on contact killing. Antibiotic functional groups are linked to a long polymeric chain, which is anchored covalently to the surface of a material. The polymeric chain allows the antimicrobial moieties to permeate into, and kill, the microbial cell. Immobilized cations have been studied for contact killing surfaces. It is suggested that long cationic polymers penetrate the cell membrane or induce cation exchange that disrupts the membrane integrity and induce cell lysis. As an advantage to this strategy, antimicrobial agent is not released into the environment and the risk of generating resistance to the compound is unlikely (Lichter *et al.*, 2009, Lewis and Klibanov, 2005, Murata *et al.*, 2007).

Examples of studies describing surfaces or coatings limiting or inhibiting bacterial adhesion are listed in Tables 4 and 5.

Table 4. Antimicrobial agents used in coating materials for biocide leaching or contact killing strategies

Strategy	Substance	Model organism	Application	Reference
Contact killing				
Immobilised cations	Quaternary ammonium: 2-(dimethylamino)ethyl methacrylate	*Escherichia coli*	Industrial, medical, community and private settings	Murata *et al.*, 2007
	N-alkylated poly(4-vinylpyridine)	*Staphylococcus aureus, Staph. epidermidis, E. coli, Pseudomonas aeruginosa*	Consumer and medical products	Lewis and Klibanov, 2005, Tiller *et al.*, 2001
	Polyelectrolyte multilayers of poly(allylamine hydrochloride) and poly(sodium 4-styrene sulfonate)	*Staph. epidermidis, E.coli*		Lichter and Rubner, 2009
	Polyelectrolyte layers of *N,N*-dodecyl,methyl-polyethylenimine	*Staph. aureus, E.coli*, A/WSN (H1N1) virus	Items handled by people (*e.g.*, doorknobs, keyboards,)	Wong *et al.*, 2010
	Polyelectrolyte multilayers of hyaluronic acid and chitosan	*Staph. aureus*	Titanium based implant materials	Chua *et al.*, 2008
Release killing				
Silver ions	Ag–SiO_2 thin films	*E. coli, Staph. aureus*	Antibacterial glass	Jeon *et al.*, 2003
	Ag nanoparticles	*E. coli*	Biomedical devices	Podsiadlo *et al.*, 2005, Lee *et al.*, 2005
	Ag-Pd	*E. coli*	Medical devices, water distribution systems, food production facilities	Chiang *et al.*, 2009
	Ag-DLC	*Staph. epidermidis*	Biomedical devices	Katsikogianni *et al.,* 2006
Leaching biocides	Triclosan-incorporated polymer	*E. coli, Bacillus thuringiensis*	hospital use as fabric seat covers, tables, chairs, and clothing	Kalyon and Olgun, 2001
	Polymer-encapsulated ClO_2 + Zinc chloride	*B. subtilis, Staph. aureus, E. coli*		Li *et al.*, 2009

	Substance	Model organism	Application	Reference
	Polyurethane coated with AgCl and benzalkonium chloride	*Staph.aureus, Staph. epidermidis, E.coli, P. aeruginosa, Serratia marcescens, Candida albicans*	Central venous catheter	Li *et al.*, 1999
	Furanone (3-(1'-bromohexyl)-5-dibromomethylene-2(5H)-furanone) -coated polymers	*Staph. epidermidis*	Biomedical devices	Hume *et al.*, 2004, Baveja *et al.*, 2004
Leaching antibiotics	Antibiotics rifampin and a sparfloxacin	*Staph. epidermidis*	Ventricular catheter	Kohnen *et al.*, 2003
	Polyelectrolyte multilayers incorporating gentamicin	*Staph. aureus*	Biomedical devices	Chuang *et al.*, 2008
	Ciprofloxacin, gentamycin, fosfomycin and flucloxacillin incorporated into polyurethanes	*Staph. aureus, Klebsiella edwardsii*	Biomedical devices	Schierholz *et al.*, 1997

Table 5. Antifouling surfaces/coatings based on surface modification

Strategy	Antifouling approach / Surface modification	Model organism	Application	Reference
Surface roughness /topography	SiO_2 mesoporous materials, SiO_2-CaO-P_2O_5 glass and biphasic magnetic bioceramics	*Staph. epidermidis, Staph. aureus*	Bone and dental implants, implantable drug delivery systems	Kinnari *et al.*, 2009
	Ti thin films	*Staph. aureus, P. aeruginosa*	Orthopedic implants	Ivanova *et al.*, 2010
	Nano-scale rough Ti surfaces	*Staph. aureus, Staph. epidermidis, and P. aeruginosa*	Orthopedic implants	Puckett *et al.*, 2010
	Etched glass surfaces with nano-scale roughness	*E. coli, P. aeruginosa, Staph. aureus*		Mitik-Dineva *et al.*, 2009
	Stainless steel with different surface finish	*Desulfovibrio desulfuricans, P. aeruginosa, P. putida, Rhodococcus sp.*		Medilanski *et al.*, 2002

	Antifouling approach / Surface modification	Model organism	Application	Reference
Surface charge	Surface charge Poly(methacrylate)	*Staph. epidermidis, Staph. aureus, E.coli, P. aeruginosa*	Implants	Gottenbos *et al.*, 2001
Surface free energy / hydrophobicity	Perfluoroalkoxy alkane (PFA), polytetrafluoroethylene (PTFE), Ni–P–PTFE, Ni–P and Ni–1% Al coatings	*P. aeruginosa*	Medical devices	Pereni *et al.*, 2006
	Fluoropolymers, DLC	*Deinococcus geothermalis, Pseudoxanthomonas taiwanensis, Meiothermus silvanus, Staph. epidermidis*	Industrial applications	Raulio *et al.*, 2008,
	Fluoropolymers	*Lactobacillus paracasei, Pseudomonas fluorescens, Serratia marcescens, Pichia anomala* (yeast)	Brewery	Preliminary results in Storgårds *et al.* 2007
Altering chemical composition	Ion implantation of steel with SiF^{3+}	*Staph. epidermidis, Staph. aureus*	Biomedical devices	Zhao *et al.*, 2008
	Ti and Ti-6Al-7Nb	*Staph. aureus* (MRSA)	Metal implants	Harris *et al.*, 2007
Photocatalysis	Ti and Ti-Ag nanoparticles (release killing and photocatalysis)	*D. geothermalis*		Keskinen *et al.*, 2006
	TiO_2	*E. coli, Burkholderia cepacia, P. aeruginosa, B. subtilis*		Li and Logan, 2005
	TiO_2	*D. geothermalis*	Industrial applications	Raulio *et al.*, 2006

1.3.4 TiO_2 Photocatalysis: The fundamentals and microbicidal effect

Photocatalysis by a semiconductor is a technology in which activation of the catalytic solid occurs through absorption of photons with an irradiation wavelength greater than the semiconductor band-gap. Anatase crystal form of titanium dioxide (TiO_2) is a semiconductor with a band gap of ≥3.2 eV. When TiO_2 particles are irradiated with near UV light (λ >350 nm), holes (h^+) and hydroxyl radicals ($OH^\bullet$) are generated in the valence band, and electrons and superoxide anions (O_2^-) are generated in the conduction band. The electrons possess the reducing power of the conduction band energy and the holes have the oxidizing power of the valence band energy. The oxidation power of the holes in the TiO_2 valence band can mineralize any organic compound by participating in a series of oxidation reactions leading to carbon dioxide, water, and mineral acids (Fujishima *et al.*, 2000, Linsebigler *et al.*, 1995, Mills and Le Hunte, 1997, Josset *et al.*, 2008).

Photocatalytic activity by water suspended TiO_2 particles is known to have microbicidal action (Matsunaga *et al.*, 1985, Ireland *et al.*, 1993, Wei *et al.*, 1994, Maness *et al.*, 1999, Huang *et al.*, 2000, Sunada *et al.*, 2003a). Its effectiveness in killing microorganisms in aqueous environment is well documented and also has numerous practical applications, but its mode of action is still under debate. Matsunaga *et al.* (1985) were the first to report on the microbicidal effects of TiO_2 photocatalysis reaction. They proposed that the oxidation of terminal sulfhydryl groups of intracellular coenzyme A, leading to inhibition of respiration and death of the cells, is the main bacterial killing mechanism of photocatalysis. Later Maness *et al.* (1999) showed that reactive oxygen species (ROS) generated on the irradiated TiO_2 surface attack unsaturated phospholipids in *E. coli*, which subsequently causes a breakdown of the cell membrane structure and therefore is a primary cause of cell death. Huang *et al.* (2000) proposed a detailed mechanism for the bactericidal effect of TiO_2 photocatalytic reaction. The initial oxidative damage takes place on the cell wall, where the TiO_2 photocatalytic surface makes first contact with intact cells. After the protection by the cell wall is lost, the oxidative damage expands into the underlying cytoplasmic membrane progressively increasing the cell envelope permeability, and subsequently allows the free efflux of intracellular contents that leads to cell death. With further illumination, the cells completely decompose (Huang *et al.*, 2000, Sunada *et al.*, 2003a). The presently accepted view is that $OH^\bullet$ produced by TiO_2 upon illumination is the primary killing agent, but that other ROS generated in the photocatalysis by TiO_2, such as O_2^- and H_2O_2, may be partly responsible for inactivation of bacteria (Cho *et al.*, 2005). Because of the short half life of $OH^\bullet$ and its low diffusion potential, bacterial cells to be oxidized must be close to the site

where $OH^{\bullet}$ is generated. Gogniat *et al.* (2006) showed that adsorption of bacteria on TiO_2 is essential for the bactericidal effect of photocatalysis. Whole cells of Gram-positive and Gram-negative bacteria, viruses, as well as bacterial and fungal spores have been completely mineralized to carbon dioxide via photocatalysis with TiO_2 (Jacoby *et al.*, 1998, Wolfrum *et al.*, 2002).

There is a growing interest in the use of TiO_2 thin film coatings as a method of keeping surfaces free of microbes. Such self-disinfecting surfaces would be of particular value for various applications where sterile surfaces are essential, such as in hospitals. The effectiveness of the TiO_2 thin films in inactivation of water suspended organisms has been demonstrated in various studies (Sunada *et al.*, 2003a, Kikuchi *et al.*, 1997, Amézaga-Madrid *et al.*, 2002, Kühn *et al.*, 2003, Sunada *et al.*, 2003b).

Nearly all studies on photooxidation of bacteria are conducted with water suspended model organisms, most frequently *Escherichia coli*. The potential of photocatalytic TiO_2 against biofilm formation or destroying existing biofilm has received little attention so far. Li and Logan (2005) studied *Escherichia coli*, *Burkholderia cepacia* and *Pseudomonas aeruginosa* adhered on TiO_2 coated glass illuminated at 254 (UVC) and 340 nm (UVA). They achieved up to 50 % and 30-70 % removal at 340 nm and at 254 nm respectively. Moreover, successful photocatalytic oxidation of natural biofilms on surfaces coated with rutile crystal form of TiO_2 has been reported (Rajagopal *et al.*, 2006).

1.4 Biofilm quantification

To collect quantitative data from the biofilm density on different substrata, a method is needed for counting the adhered bacteria independent on possible presence of extracellular materials like slime. Examples of methods used for biofilm quantification in the literature are presented in Table 6.

Table 6. Examples of methods used for quantification of bacterial biofilms in the literature. The table lists methods that were used to evaluate the impact of abiotic surfaces or conditions on the tendency to accumulate biofilm.

Method	Species	References
Fluorescence staining & scanning fluorometry	*Bacillus coagulans, B. cereus, B. amyloliquefaciens, B. pumilus, B. licheniformis, Deinococcus geothermalis*	Mattila *et al.*, 2002, Kolari *et al.*, 2001
Microscopic imaging & automated enumeration software	*Staphylococcus epidermidis, Staph. aureus, Streptococcus oralis, Strept. mutans, Proteus mirabilis,*	Charman *et al.*, 2009, Harris *et al.*, 2007, Cerca *et al.*, 2005, Stickler *et al.*, 2006, Vacheethasanee *et al.*, 1998, Aykent *et al.*, 2010. Reviewed by Beyenal *et al.*, 2004
Staining & microscopic counting	*B. stearothermophilus, B. thermoleovorans, B. flavothermus, B. licheniformis, B. coagulans, B. pumilus, Staph. aureus, Escherichia coli,*	Fletcher and Loeb, 1979, Parkar *et al.*, 2001, Pompermayer and Gaylarde, 2000
Counting from SEM micrographs	*Staph. aureus, Staph. epidermidis, Pseudomonas aeruginosa, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedi*	Arnold and Bailey, 2000, Ivanova *et al.*, 2010, Kodjikian *et al.*, 2003, Kuula *et al.*, 2004
Removing bacteria by ultrasonication or vortexing & plate count method	*Salmonella* spp., *Listeria monocytogenes, Staph. aureus, Staph. epidermidis, Salmonella enteritidis,*	Chua *et al.*, 2008, Kinnari *et al.*, 2009, Zhao *et al.*, 2008, Sinde and Carballo, 2000, Shi *et al.*, 2006, Chen W. *et al.*, 2006, Giaouris and Nychas, 2006, Katsikogianni *et al.*, 2006
Crystal violet staining & absorbance measurement	*E. coli*, *D. geothermalis, Meiothermus silvanus, M. ruber, Vibrio cholerae, Pseudomonas fluorescens, E. coli, B. cereus, Strept. agalactiae*	Watnick and Kolter, 1999, Houry *et al.*, 2010, Pratt and Kolter, 1998, Pawar *et al.*, 2005, Kolari *et al.*, 2003, Van Houdt *et al.*, 2004, Kolari *et al.*, 2001, Silagyi *et al.*, 2009, O'Toole and Kolter, 1998b, Rinaudo *et al.*, 2010
ATP measurement		Mattila *et al.*, 2002
Dry-weight determination	*Staph. epidermidis*	Cerca *et al.*, 2005
Quantitative PCR	*L. monocytogenes*	Guilbaud *et al.*, 2005

2 Aims of the study

The aim of this thesis was to study the ultrastructure and biological functionalities of biofilms formed on biotic and abiotic surfaces by phylogenetically distant micro-organisms.

Further detailed aims of this thesis were:

1. Describe the interactions in mixed species biofilms on biotic and abiotic surfaces (paper III, this thesis).

2. Study the responses of biofilm forming bacteria towards engineered novel surfaces (papers I-II).

3. Explore the responses of biofilms towards photocatalytic TiO_2 coatings (paper I).

4. Document the ultrastructure of natural microbial community on and within barley kernels and its responses to malting with and without a starter culture (paper III).

5. Quantitatively analyse the formation of biofilms on abiotic materials (paper I-II).

6. Characterise known and novel biofilm forming taxons within the sparsely studied phylum *Deinococcus-Thermus*. Members of this phylum are important foulers of paper machine steel surfaces (paper IV).

7. Use identical methods to examine the biofilms formed by selected phylogenetically distant microbes (paper I-III, this thesis).

8. Observe the interactions between selected phylogenetically distant microbial species and different abiotic materials (paper II).

3 Materials and methods

3.1 The methods used in this thesis

The methods used in this thesis are compiled in Table 7.

Table 7. The methods used in this thesis. Detailed descriptions of the methods can be found from the original publications or in the chapter 3.2.

Method	Description
Microscopy methods	
Fluorescence microscopy	Paper I, Paper IV, this thesis
Light microscopy, phase contrast	Paper IV, this thesis
Scanning electron microscopy	Papers I- IV, this thesis
Transmission electron microscopy, thin sections	Paper IV
Transmission electron microscopy, negative staining	Paper IV
SEM-EDS elemental analysis	This thesis
Fluorescence detection methods	
Staining live biofilms with fluorochromes	Paper I, Paper II, this thesis
Quantitative scanning fluorometry	Paper I, Paper II, this thesis
Other methods	
Studying the resistance of a bacterium to ionizing radiation	Paper IV
Cultivation of biofilms on abiotic surfaces	Paper I, Paper II, this thesis
Staining endospores with malachite green	Paper IV, this thesis
Gram-staining	Paper IV, this thesis
Analysis of maximum and minimum growth temperature of a bacterium	Paper IV
Whole bacterial cell fatty acid analysis	Paper IV, this thesis
Studying the production of gas and acid from carbohydrates	Paper IV
Detection of oxidase activity of a bacterium	Paper IV
Isolation of pure bacterial cultures	This thesis

3.2 Methods other than those described in the papers I-IV

3.2.1 Deposits from a paper machine: a case study

Two petrified deposits were receiveded from a paper mill using recycled fibre. Deposit A was scales from the disperger of a recycled fibre plant (75°C, pH 10). Deposit B was from a vacuum pump of the paper machine (45°C, pH 7.8). Pieces of the deposits were placed in tubes with 1 ml 1 M HCl and boiled in water bath for 15 min. The obtained extract was neutralised with NaOH and measured for oxalic acid using an enzyme assay (R-Biopharm 755699, Darmstadt, Germany). The deposits did not dissolve noticeably.

Untreated pieces of the deposits were used to inoculate R2 agar. The agar designed for isolations of oxalic acid producing bacteria contained: half strength R2A agar, $CaCl_2$ (0.3 g l^{-1}), yeast extract (0.25 g l^{-1}), Victoria Blue B -stain (0.75 mg l^{-1}, Sigma-Aldrich, St. Louis, MO, USA), pulp (pine wood cellulose and CTMP 1:4; ~0.5 w/v %) and agar (4.5 g l^{-1}).

Oxalic acid production by the obtained isolates was investigated by aerobic cultivation in R2 broth amended with soft wood pulp (~0.5 w/v %) and sodium pyruvate (0.6 g l^{-1}) for 7 d, 45°C, 160 rpm. The oxalic acid content of the culture was measured using the enzyme assay as above.

For electron microscopy, pieces of air dried deposits were coated with Au and then inspected by SEM (JEOL JSM-840A, Tokyo, Japan). The elemental compositions were analysed using SEM with energy dispersive X-ray spectroscopy (SEM-EDS, JEOL JSM-840A Röntec Edwin Winshell).

3.2.1 Penetration of microbes into ceramic materials

We investigated the penetration of bacteria through ceramic materials intended for coating of press cylinders at paper machine. The materials were received from the Ceramic materials laboratory, Technical University of Tampere (TUT). The ceramic sheets were with five different compositions: 1) TiO_2; 2) CrO_3; 3) Al_2O_3; 4) 87% Al_2O_3, 13% TiO_2; 5) 75% CrO_3, 25% TiO_2. The sheets were disinfected in 70% v/v ethanol, glued with silicon onto coupons of autoclaved stainless steel and submerged in a medium consisting of sterilized white water (volume 1 l, from a paper machine) yeast extract (0.1 g l^{-1}), starch (1 g l^{-1}), sodium pyruvate (0.3 g l^{-1}), sodium thiosulphate (0.3 g l^{-1}) and calcium chloride (0.5 g l^{-1}). This medium was seeded with 0.5% v/v of an overnight culture (in R2 broth) of an oxalic acid producing strain of *Meiothermus silvanus* ox-13 (Hambi 2510) and with *Deinococcus geothermalis* E50051, and incubated at 45°C (130 rpm). One-half of the medium was replaced by fresh medium each two days. After 17 d the coupons were removed and the

ceramic sheets separated carefully by cutting with sterile scalpel. Both the ceramic and the steel surfaces were stained with acridine orange and examined with epifluorescence microscope (emission filter λ 450-490 nm).

The steel coupons were boiled for 15 min in 1 M HCl and the oxalic acid concentrations were measured using an enzyme assay.

3.2.2 Investigation of mixed species biofilms on coated steel surfaces

The bacterial strains used are listed in Table 8. The strains were grown on R2 agar plates for 2 d at 45°C. The target surfaces (Table 9) were acid proof steel coated or not coated with materials provided by our collaborators (TUT and Millidyne Oy).

Prior to biofilm tests, the steel coupons were autoclaved or disinfected with 70% ethanol and mounted in the wells of a sterile 6-well polystyrene plate. The coupons were immersed in R2 broth seeded with the bacterial strains, each to a density of 10 µg (wet weight) ml^{-1} (~10^6 cells ml^{-1}). The plate was covered with a lid and incubated under shaking (160 rpm) for 1 d at +45°C. The biofilms formed on the test coupons were rinsed with municipal tap water and stained for 5 min with the nucleic acid specific fluorochrome SYTO9 (5 µmol l^{-1}, Molecular Probes, Leiden, The Netherlands). The unbound stain was removed by rinsing with water and the emitted fluorescence measured with a scanning fluorometer (Wallac 1420 multilabel counter, Victor, Perkin Elmer, Wellesley, MA, USA) with an excitation wavelength of 485 nm and emission wavelength of 530 ± 10 nm. Because SYTO9 stains nucleic acids (DNA; RNA) the fluorescence intensity reflects the number of attached bacteria. The background fluorescence from non-seeded coupons of each coating material was subtracted. After measuring the fluorescence the coupons were stained with crystal violet (J.T.Baker, Phillipsburg, NJ, USA, 4 g l^{-1} in 20% v/v methanol), washed to remove non-adhering biomass and photographed.

Table 8. The bacterial strains used for growing biofilms

Strain	Origin of the strain
Deinococcus geothermalis E50051 (Hambi 2411)	Biofilm in the splash area of a paper machine,
Meiothermus silvanus B-R2A5-50-4 (Hambi 2477)	Biofilm in the wire section of a paper machine
Pseudoxanthomonas taiwanensis JN11306 (Hambi 2750)	Paper machine wet end, biofilm on a submerged surface
Bacillus pumilus TSP66	Paper product
Brevibacillus agri PMW-17	Biofilm, paper machine

The strains were from the collection of M. S. Salkinoja-Salonen, Department of Food and Environmental sciences, University of Helsinki.

Table 9. The studied coatings on steel. The steel used as a base was of the acid proof quality, AISI316L 2B for all coatings. The coated steels were novel designed materials provided by the collaborators (TUT and Millidyne Oy).

	L	L-FAS	FAS	MD1	MD2	MD3	Non-coated
Preparing method	Sol-gel	Sol-gel, spin coating	Spin coating	Sol-gel	Sol-gel	Sol-gel	-
Activity	Superhydrophilic, nano-topography (Boehmite)	Superhydrophobic, nano-topography (Boehmite, fluorosilane)	Hydrophobic (Fluorosilane)	Antimicrobial (biocide)			-
Water contact angle	<5°	150°	105°				70°

4 Results and Discussion

4.1 Means for preventing biofouling

4.1.1 Developing a method for quantification of biofilm on steel

A new method based on optical reading of the fluorescence was developed in this study for the quantification of biofilm on steel. This work was based on earlier developments reported by Kolari *et al.* (2001) and by Mattila *et al.* (2002) on quantitation of biofilms formed *in situ* at the paper machine. Bacteria attached on a surface are stained with a nucleic acid specific fluorochrome, such as SYTO9, and the emitted fluorescence is measured using a scanning fluorometer with excitation and emission wavelengths specific to the dye. We showed that the fluorescence output under these conditions was proportional to number of attached bacteria. The fluorescence values were translated to bacterial numbers using a calibration curve obtained by manual counting of the cells with a microscope. The autofluorescence from non-seeded coupons was measured for each of the coating materials and was subtracted from the biofilm value.

Before the evaluating a surface or a surface coating for its ability to repel or remove biofilm, methods to quantitate the amount of biofilm on the non-transparent abiotic surface is needed. Examples of methods used in biofilm research are listed in Table 6. Microscopic counting of bacterial cells is laborious, time consuming and not applicable on microcolonies or thick biofilms where it is impossible to distinguish each individual cell. Quantification techniques based on detaching the bacterial cells from the surface followed by counting by culture based methods are not suitable for bacteria forming tenacious biofilms, like *Deinococcus geothermalis. D. geothermalis* biofilm was shown to detach inefficiently by each of the methods applied, sonication, vigorous shaking and by chemical cleaning agents such as aquous solution of sodium dodecyl sulphate (Kolari *et al.*, 2002) or sodium hydroxide (Kolari *et al.*, 2002, Paper I).

Quantification methods based on crystal violet staining or determination of biomass dry-weight require thick biofilm, because of the low sensitivity. In our studies the experiments with biofilms were conducted after a maximum of two days growth in oligotrophic media at 45°C. This did not result to thick biofilms with the bacteria from paper machines. Longer cultivation would require usage of rich medium which no longer reflects conditions at paper machine. Moreover, Kolari (2003) reported that ample nitrogen in growth media prevented and low ionic strength favoured biofilm formation of the paper machine isolate *D. geothermalis* E50051.

The method developed in this study was fast to conduct for large number of surface samples, and is suitable also for relatively thin biofilms. Disadvantage of the fluorescence based method was that it did not suit to all materials. Some substrata or coating materials exhibited strong autofluorescence and some materials bound fluorochromes unspecifically resulting to background fluorescence values that were too high compared to the fluorescence emitted by the attached bacteria. For these reasons a value for background fluorescence was always measured from a stained, non-seeded surface.

4.1.2 How does coating of steel affect its tendency towards biofouling?

4.1.2.1 Adherence of microbes on steels coated with diamond like carbon or with fluoropolymers

Four different, phylogenetically distant biofilm forming bacteria were used to explore the bacterial adherence on to stainless steels with or without diamond like carbon (DLC) or fluoropolymer coatings (paper II). The bacterial species chosen for this study originated from widely different branches of the evolutionary tree: *Staphylococcus epidermidis* belongs to the phylum *Firmicutes.* This Gram-positive bacterium is known to form biofilms on medical implants (reviewed by Götz, 2002). Three other selected species were known formers of biofouling on paper machines. *Meiothermus silvanus* forms pink slime in paper machine wet end (Ekman *et al.*, 2007, Kolari *et al.*, 2003) and *Deinococcus geothermalis* forms pink deposits in splash areas of paper machines (Väisänen *et al.*, 1998, Peltola *et al.*, 2008b, Prince *et al.*, 2009) and pulp dryers. These species belong to the ancient phylum *Deinococcus-Thermus.* Bacteria of this phylum possess complex cell envelopes with many layers including an outer membrane. *Pseudoxanthomonas taiwanensis* is known for forming brown biofilms on submerged surfaces in the paper machine wet end (Kolari *et al.*, 2003, Suihko and Skyttä, 2009, Prince *et al.*, 2009, Suihko *et al.*, 2004). This species belongs to *Gammaproteobacteria, i.e.* the same subphylum as the extensively studied *Escherichia coli* and *Pseudomonas aeruginosa* (Table 2).

The coatings on stainless steel were done with two different diamond like carbon (DLC-A and DLC-B) and two fluoropolymers (AR-115 and AR-221, paper II). These coatings reduced the adhesion of all four biofilm forming bacteria (Fig. 8).

As described in paper II, we detected a new surface property parameter, skewness (S_{sk}) measured with AFM, that affected the adherence of bacteria to surfaces (Table in paper II). Slightly positive value (0.76 to 0.88) of S_{sk} indicated low tendency of adherence for each of the four different biofilm forming test bacteria used in this study. Furthermore, all test bacteria adhered densely to non-coated steels, which had the lowest Ss_k values (-0.43 and -

0.56). A positive S_{sk} value indicates lack of surface porosity *i.e.* a non-porous surface. In addition, we found that a high value (~7) of kurtosis (S_{ku}) predicted low tendency of adhesion by all bacteria tested. High value of S_{ku} indicates that the surface had sharp protrusions.

Very recently, Ivanova *et al.* (2010) reported that skewness and kurtosis were useful parameters for evaluating the tendency of surfaces to biofouling. They measured these parameters to evaluate titanium thin films and studied adherence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* onto such surfaces. Their results indicated that thickest Ti film (150 nm) with the higher values of S_{sk} (2.3 ± 0.1) and of S_{ku} (36.7 ± 1.1) attracted more *P. aeruginosa* and *Staph. aureus* than thinner Ti films (3 nm and 12 nm) with lower values of S_{sk} (1.2 ±0.2 and 1.0 ±0.1) and S_{ku} (9.2 ± 1.3 and 5.6 ± 0.1). Interestingly, they generalised their results opposite to those in our paper II where we concluded that a positive value of skewness and high value of kurtosis indicated decreased, rather than increased, tendency of bacterial adhesion. Closer inspection of their results reveals, however, that the skewness and kurtosis values reported by Ivanova *et al.* (2010) for the thick titanium film were far higher than those measured by us for the DLC and fluoropolymer coated steels in paper II (S_{sk} -0.56 to 0.88, and S_{ku} 3.17-7.54, paper II, table 4 and Fig. 8 in this thesis). So, Ivanova *et al.* compared high values to even higher values and did not study any material with truly low S_{sk} and S_{ku}. The thinnest (3 nm) Ti film of Ivanova *et al.*, which attracted least *P. aeruginosa* and *Staph. aureus*, had S_{sk} and S_{ku} values (1.2 ± 0.2 and 9.2 ± 1.3), *i.e.* close to the values of our DLC-B coating (0.88 and 7.54). The DLC-B coating attracted least amounts of *Staph. epidermidis* (the same genus as *Staph. aureus* of Ivanova *et al.* 2010). Moreover, the water contact angle values of these two surfaces were close to each other (Ti 76.3 ± 0.9 and DLC-B 79).

Our evidence shows that the bacterial repellence was not determined by surface hydrophobicity alone but other surface properties had a great impact. However, the measured water contact angles (Table 3, paper II and Fig. 8 in this thesis) indicated that *D. geothermalis* and *M. silvanus* preferred a hydrophilic surface ($\theta < 90°$) for adhesion whereas *Psx. taiwanensis* and *Staph. epidermidis* were more attracted to hydrophobic surfaces ($\theta > 90°$). Our findings could explain the observation of Cerca *et al.* (2005) that clinical isolates of *Staph. epidermidis* (n=11) adhered better on acrylic than on glass substrata. Moreover Katsikogianni *et al.* (2006) found that fluorinated PVC attracted more *Staph. epidermidis* than non-fluorinated or DLC coated PVC.

The results from paper II, in which biofouling of steel surfaces coated with DLC or with fluoropolymer coatings were compared, showed that the surface parameters skewness and kurtosis were more important in predicting attractiveness of the surfaces for biofilm formation than hydrophobicity/hydrophilicity (θ). Slightly positive values for skewness and values around 7 for kurtosis indicated that the surface was less prone to biofouling by the bacteria used in this study. Our findings showed that there are great differences between bacterial species and their preferences for adherence, is also important and new.

Paper II was the first paper describing adherence and biofilm formation of several essential bacterial species biofouling paper machines. Our study also is the first biofilm study done on adherence by *Meiothermus* sp. and by *Pseudoxanthomonas* sp.

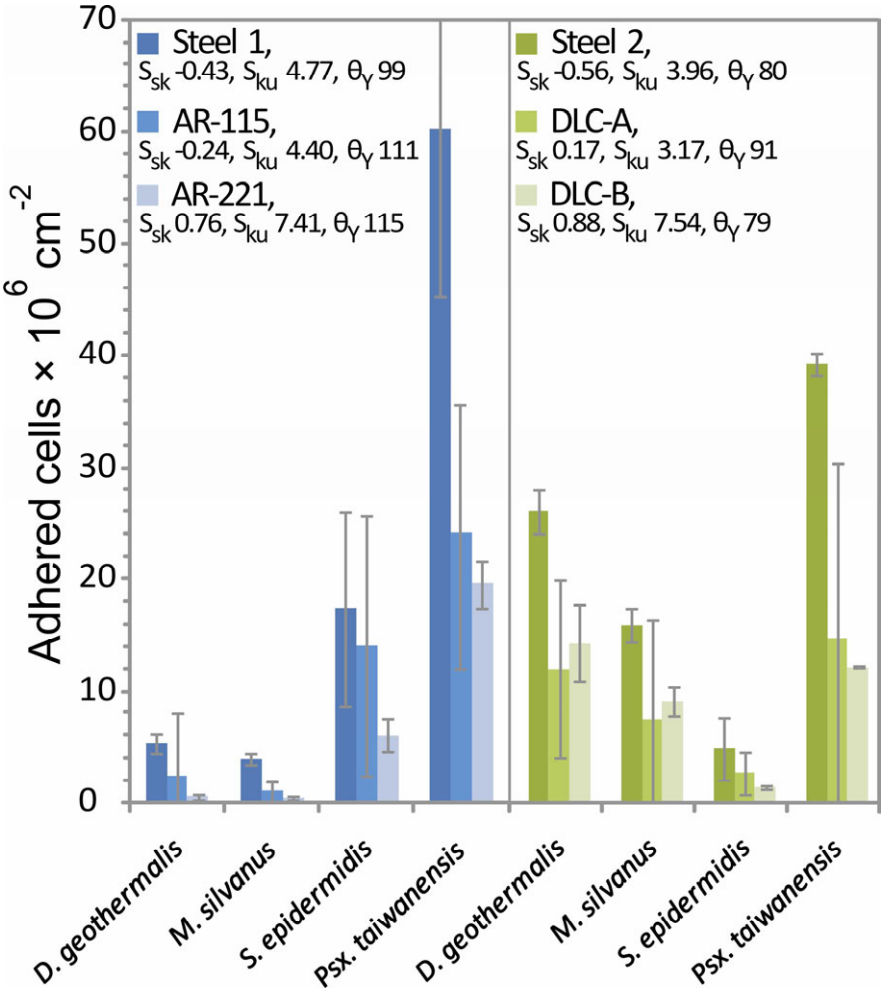

Fig. 8. Adherence of cultures of *Deinococcus geothermalis, Meiothermus silvanus, Pseudoxanthomonas taiwanensis* and *Staphylococcus epidermidis* (Table 8) onto coated or non-coated stainless steels (Table 2 in paper II). The steel coupons were immersed for 2 d at 45 37°C or at 37°C (*Staph. epidermidis)* in media seeded with the test bacteria. Non-adhered or loosely adhered cells were removed by washing with water. The coupons were stained with SYTO9 and the cell numbers were calculated from the fluorescence readings using a standard curve calibrated by microscopic counting. Error bars indicate standard deviations. Steel 1 = acid proof steel, Steel 2 = AISI316L/2B. The coatings: AR = fluoropolymer, DLC = diamond like carbon. S_{sk}= skewness, S_{ku} = kurtosis, θ_y = roughness corrected contact angle.

4.1.2.2 Coating of steel with or without antimicrobial agents

This study was done using mixed species cultures, of which three bacterial species were known to be capable of forming biofilms in paper machine environment, and two spore forming bacteria had been isolated from biofilms of a paper machine and from paper products (Table 8). Biofouling was measured by staining the bacteria adhered to the coated or non-coated coupons (Table 9). The degree of fouling was assessed by fluorescence emitted from the steel coupons by the method described under chapter 4.1.

The results summarised in Fig. 9 show that the surfaces coated with L-FAS, FAS and MD2 films emitted less fluorescence after exposure to the biofilm forming bacteria than the coupons of non-coated AISI316 steel or those with the L, MD1 or MD3 coatings. In addition, the results showed that the L-FAS coating on steel adsorbed the dye in such a high quantity that fluorescent emission, even in absence of bacteria, was so high that it compromised the measuring of bacterial fluorescence. Photographs of the coupons after staining the biomass with crystal violet (Fig. 11) show that this stain bound onto the L-FAS coating also without any biofilm. Thus, also this dye was unsuitable for analysing biofouling of the L-FAS coating. Furthermore, Fig. 11 shows that the biofilms formed ornamental patterns on several surfaces. These patterns reflect the clockwise flow of the liquid as the exposures were done on a clockwise rotating shaker. On L-FAS, on FAS and on MD2 coated surfaces this pattern was absent or less marked compared to the non-coated steel AISI316 or the L, MD1 or MD3 coatings, indicate lower adherence of bacteria. This supports the results from the measurements where fluorescence emission was used as measure for biomass (Fig. 9). Fig. 10 shows clearance of turbidity in the microplate wells holding coupons coated with MD2. This indicates that the coating emitted substance that killed the suspended bacteria rather than prevented them from adhering on the coupon.

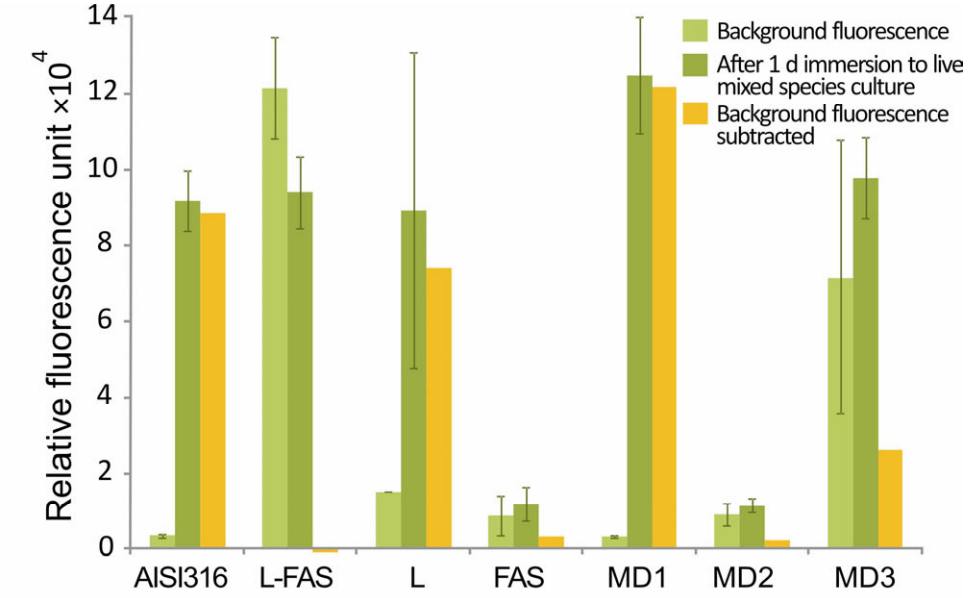

Fig. 9. Adherence of mixed inocula containing *Deinococcus geothermalis*, *Meiothermus silvanus*, *Pseudoxanthomonas taiwanensis*, *Bacillus pumilus* and *Brevibacillus agri* (Table 8) onto coated or non-coated coupons of steel. The coupons are described in Table 9. The medium R2 broth was seeded with the test strains and grown at 45°C, 160 rpm for 1 d. Non-adhered cells were removed by washing with water. The coupons were stained with SYTO9 and the resulting fluorescence was measured with a scanning fluorometer as described in chapter 4.1. Error bars indicate the standard deviations.

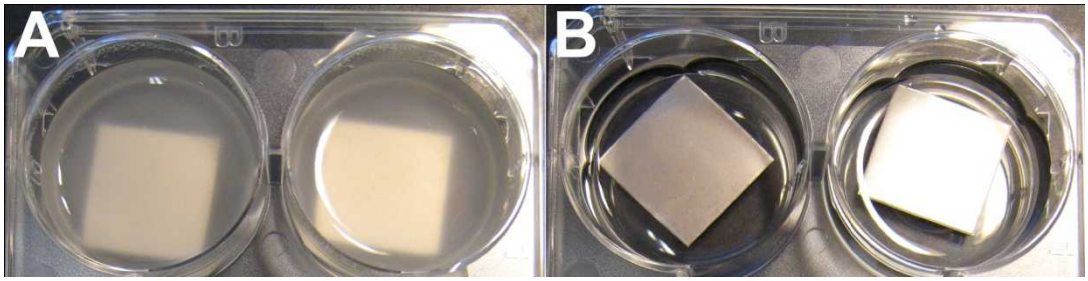

Fig. 10. Photographs of 6-well plates used for measuring biofouling of coated and non-coated acid proof steel (AISI316). Panel A shows the non-coated steel and panel B shows the steel coupons with biocide containing coating (MD2). The figure shows that the coating MD2 emitted substance that killed the bacteria not only on the coupon surface but also in the immersion medium (*i.e.* no turbidity in the liquid). The strains were listed in Table 8.

The coupons immersed into the bacterically seeded broth were inspected with FESEM. Examples of these results are shown in the 42 FESEM micrographs displayed in Figs. 12.1 to 12.7. Each micrograph shows only a small surface area (~75 µm^2 to 3050 µm^2) of the heterogeneous biofilms, selected by the author of this thesis out of a collection of 483 microscopic fields prepared by the author. Although the selection always is a subjective choice, the author has pursued to approach the physical reality as close by as possible.

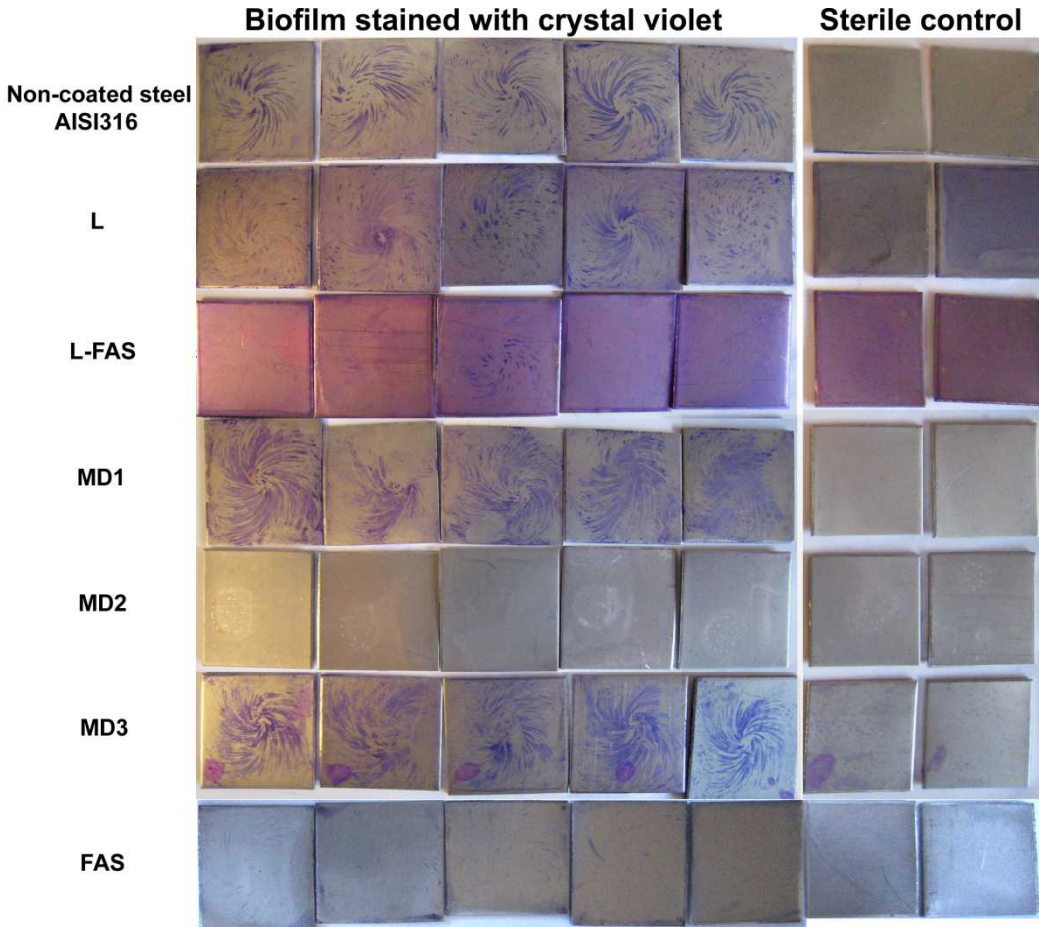

Fig. 11. Photograph of crystal violet stainings of coated and non-coated stainless steel coupons immersed in a broth seeded or not seeded (sterile control) with a mixed inoculum containing *Deinococcus geothermalis*, *Meiothermus silvanus*, *Pseudoxanthomonas taiwanensis*, *Bacillus pumilus* and *Brevibacillus agri*. The coupons are described in Table 9 and the strains and their origins are shown in Table 8. Crystal violet stains proteins blue, thus the blue colour indicates the presence of bacterial biomass, except for the L-FAS coating where the sterile control adsorbed colour and therefore stained blue. The ornamented blue patterns on the surfaces of other coupons reflected the concentric clockwise liquid flow due to the rotating shaker.

The bacterial species used as test bacteria in this study differed in cell sizes and shapes. Based on these morphological differences, it was possible to evaluate the contribution of each species in the biomass accumulated on the steel coupons. The cells of *Psx. taiwanensis* are the small, 1-2 µm × 0.3 µm, wrinkled, rod shaped bacteria. *D. geothermalis* are the spherical, smooth surfaced cells of 1 µm in diameter. *M. silvanus* cells are the short rods, 1 µm × 0.5 µm, frequently occurring in chains. *Bacillus* and *Brevibacillus* were visible as heavily ornamented spores, size 0.8-1 µm × 1.3-1.8 µm.

The images document that the species that most efficiently adhered onto the test surfaces under the conditions tested was *Psx. taiwanensis*. This species was shown to be efficient biofouler of various coatings in paper II as well. *D. geothermalis* adhered efficiently onto the most of the surfaces as well. Only superhydrophilic L coating was repelling *D. geothermalis*

cells, while *Psx. taiwanensis* and *M. silvanus* adhered efficiently onto this surface. In paper II it was shown, that *D. geothermalis* adhered more on hydrophilic than on hydrophobic coatings, but the water contact angle of these coatings was much lower (~80°) than that of superhydrophilic coating in this study (105°). Furthermore, it can be seen from Figs. 12.1 to 12.7, that when occurring as thin biofilms, these bacteria often formed single species microcolonies rather that scattered randomly.

The spores of *B. pumilus* and *Br. agri* were often seen stacked on layers of cells of the other species, indicating they adhered better on bacterial biomass than directly onto the abiotic surface. An exception to this was the superhydrophobic L-FAS coating, where several spores were seen attached directly onto the abiotic surface (Fig. 12.3 F). It has been reported previously that many *Bacillus* spp., including *B. pumilus,* have spores with hydrophobic character, (Doyle *et al.*, 1984, Faille *et al.*, 2010). Vegetative cells of these species were seen to adhere on the steels infrequently. It was earlier shown that *Bacillus* spp. did not form biofilm independently under conditions simulating the paper machine environment. Kolari *et al.* (2001) studied seven *Bacillus* species from paper machines and found that none adhered on stainless steel or polystyrene, including the *B. pumilus* TSP66 used in the present study. However, when the same strains were co-cultured with *D. geothermalis* E50051, mixed species biofilms were formed (Kolari *et al.*, 2001). In this thesis work, we saw that *B. pumilus* and *Br. agri* spores mostly adhered onto a biotic surface, *i.e.* biofilms formed by *Psx. taiwanensis,* a recently recognised primary coloniser from paper machine.

Combining the results from fluorescence measurements, photographs and FESEM micrographs, we conclude that the coating most repellent against biofouling for these bacterial species was the FAS coated stainless steel and that the antimicrobial substance emitting coating MD2 effectively killed the suspended bacteria.

Figs. 12.1 to 12.7. FESEM micrographs of coated and non-coated coupons of AISI316 steel biofouled with a mixture of paper machine bacteria: *Deinococcus geothermalis*, *Meiothermus silvanus*, *Pseudoxanthomonas taiwanensis*, *Bacillus pumilus* and *Brevibacillus agri*. The coupons (table 9) were exposed to a planktic mixture of test bacteria suspended in R2 broth, for 1 d at 45°C on a rotary shaker (160 rpm). The cell sizes and morphologies can be used to distinguish the species from the micrographs: *Psx. taiwanensis:* small 1-2 µm × 0.3 µm, wrinkled, rods; *D. geothermalis:* spherical Ø 1 µm, smooth; *M. silvanus:* short rods, 1 µm × 0.5 µm, occurring in chains; *B. pumilus*: rod, 0.6 µm × 2-3 µm; *Br. agri*: rod, 0.5-1 µm × 2-5 µm; spores: ornamented, egg shaped, 2 µm × 1 µm. The strains of the test bacteria and their origins are shown in Table 8. The test bacteria colonised the surfaces as heterogenous patches. Typical examples of these images were selected for the figure to show how the different bacteria assembled to biofilms alone or in mixed biofilms with other species.

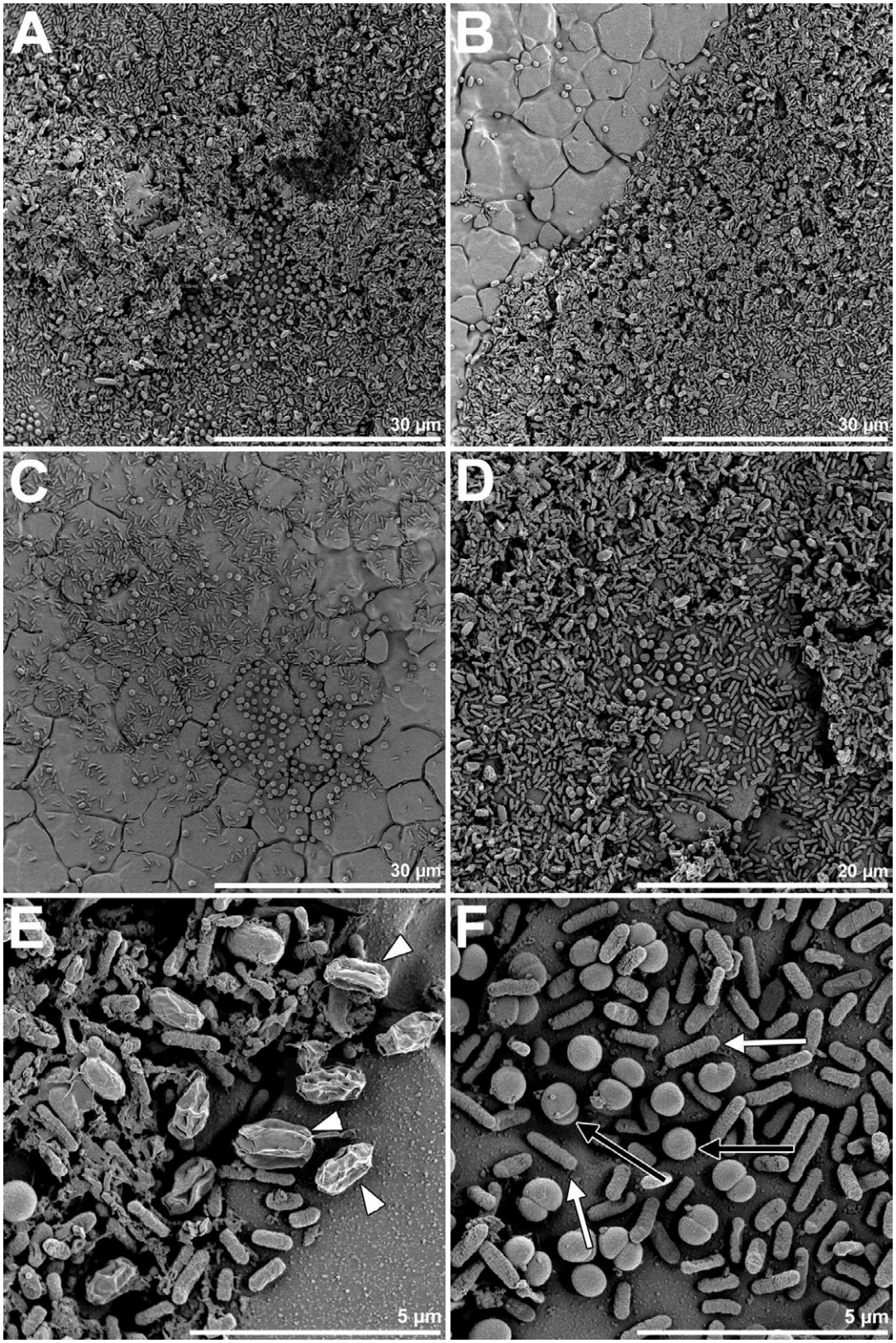

Fig. 12.1. Non-coated steel AISI316 biofouled with the five paper machine bacteria. Panels A, B, low magnification micrographs of the thick mixed species biofilm on steel. In the left upper corner of panel B the steel grain boundaries are visible. In panels C to F, cells of *Psx. taiwanensis* (white arrows) and *D. geothermalis* (black arrows) are adhered on steel. Panel E shows spores (white arrow heads) attached on a biofilm of *Psx. taiwanensis* on the steel.

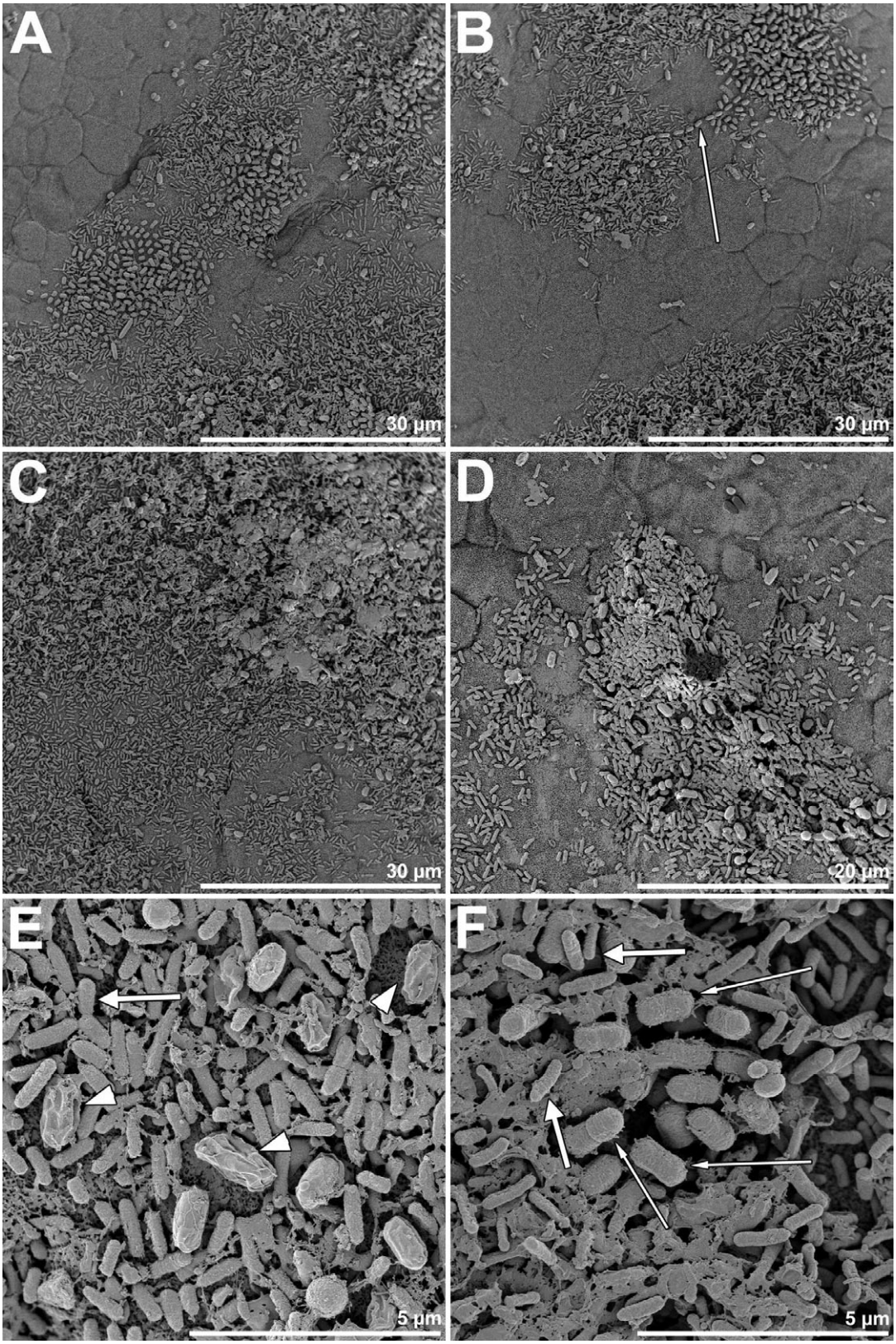

Fig. 12.2. L coating on steel AISI316 biofouled with the five paper machine bacteria. Panels A, B, low magnification micrographs of this mixed species biofilm on L coated steel. Microcolonies and a chain of cells formed by *M. silvanus* (thin, white arrow) are visible. A thick biofilm is visible in the upper right corner of panel C as well as in the middle of Panel D. Panels E, F, show high magnification views of spores (E, white arrow heads) and putative *M. silvanus* cells (F, thin, white arrows) adhered on a biofilm of *Psx. taiwanensis* (white arrows).

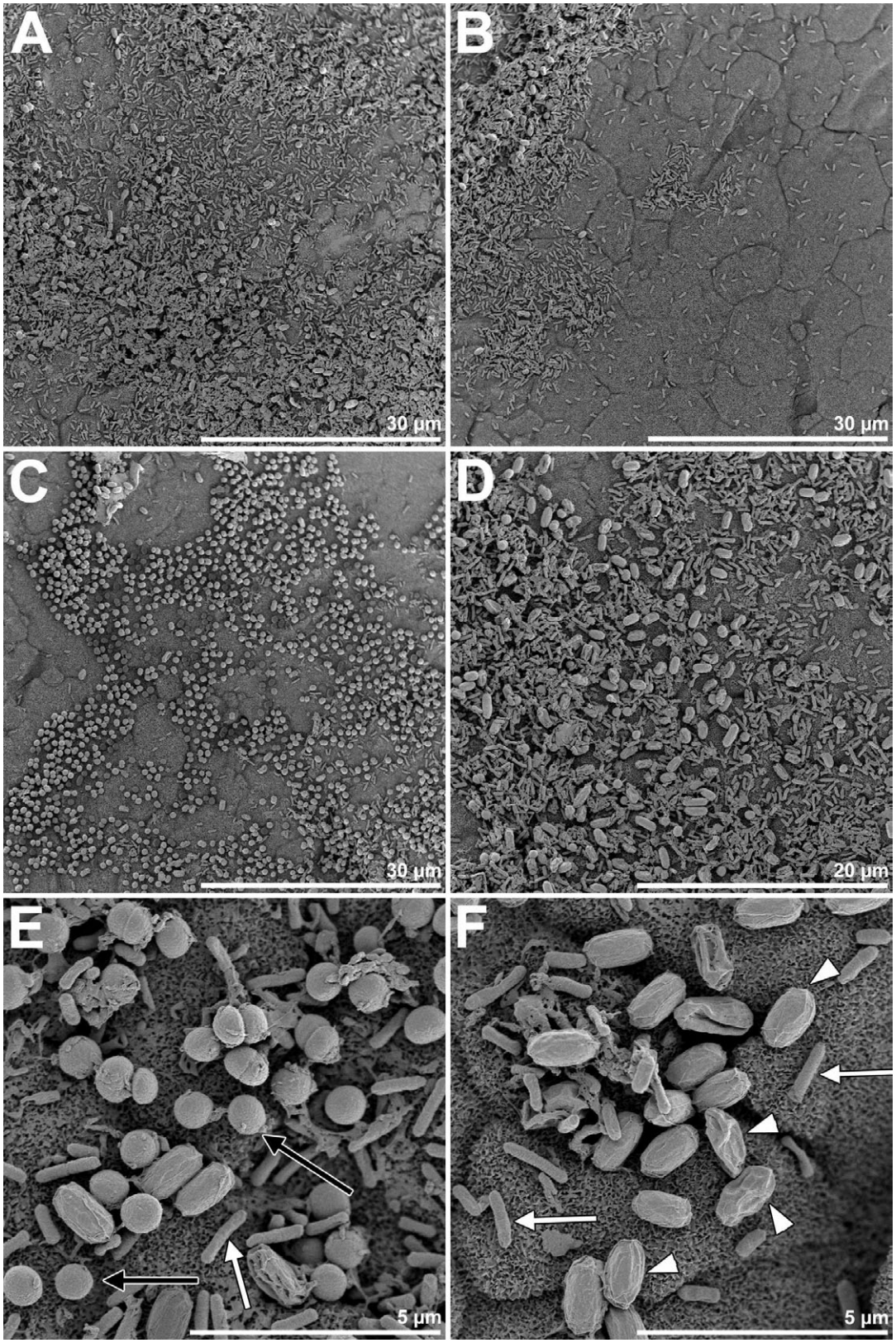

Fig. 12.3. L-FAS coating on steel AISI316 biofouled with the five paper machine bacteria. Panels A, B, low magnification micrographs of the mixed species biofilm on the L-FAS coated steel. Thick biofilm in the upper left corner and scattered cells of *Psx. taiwanensis* (white arrows) in the lower right corner of Panel B. Monolayer of adhered *D. geothermalis* (black arrows) cells is visible in Panel C. Panels D and F show numerous spores (white arrow heads) attached on the substratum. Topography of the L-FAS coating is visible between the adhered cells and spores in Panels E and F.

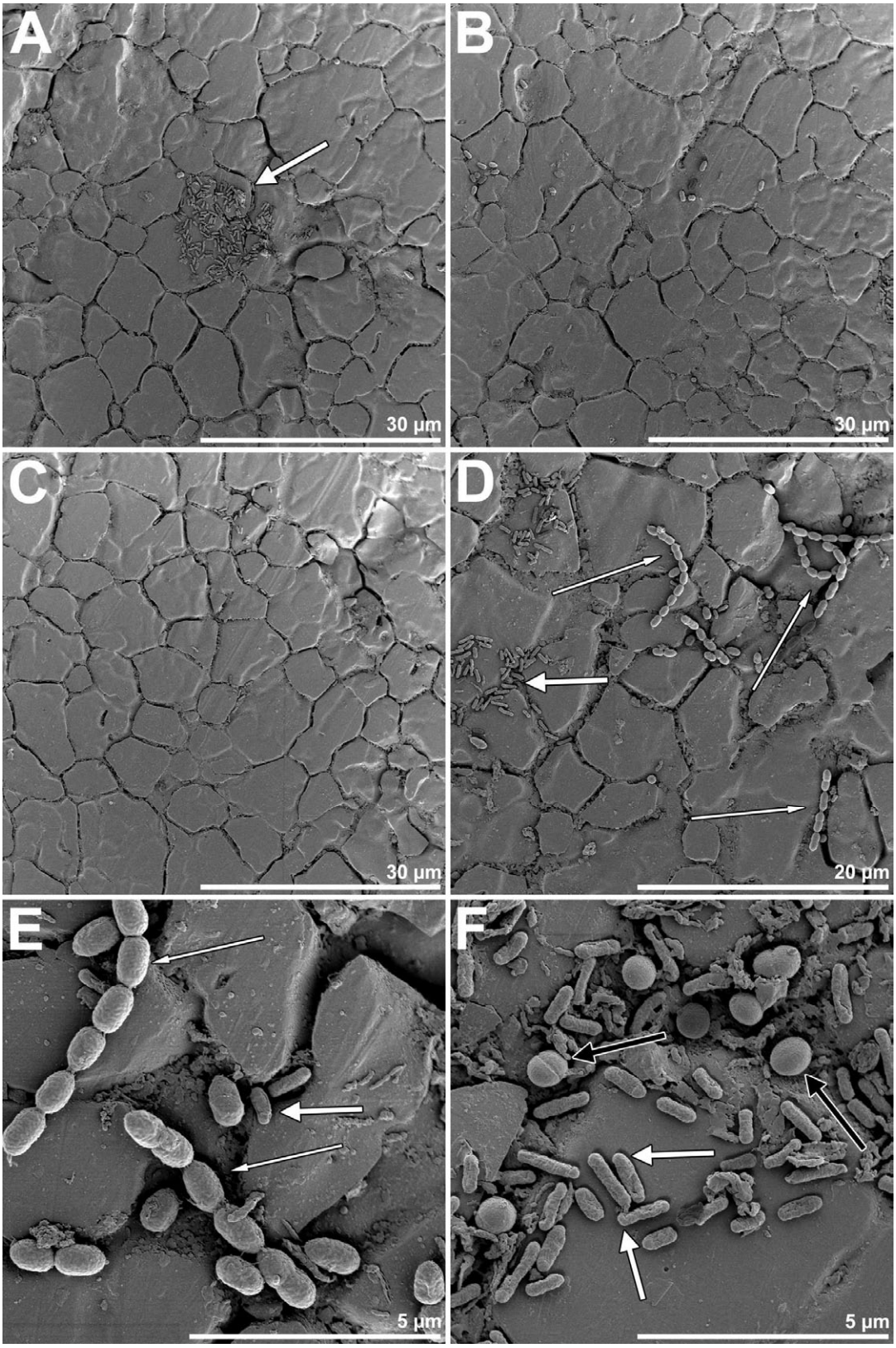

Fig. 12.4. FAS coating on steel AISI316 biofouled with the five paper machine bacteria. Panels A, B, only few adhered cells on the FAS coated steel are seen, compared to non-coated steel surface (Fig. 12.1). Large areas were vacant from any adhered bacteria as seen in panel C. In panels D and E, few *Psx. taiwanensis* cells (white arrows) and chains of *M. silvanus* cells (thin, white arrows) and in panel F, *Psx. taiwanensis* (white arrows) and *D. geothermalis* (black arrows) cells are adhered on the coated steel.

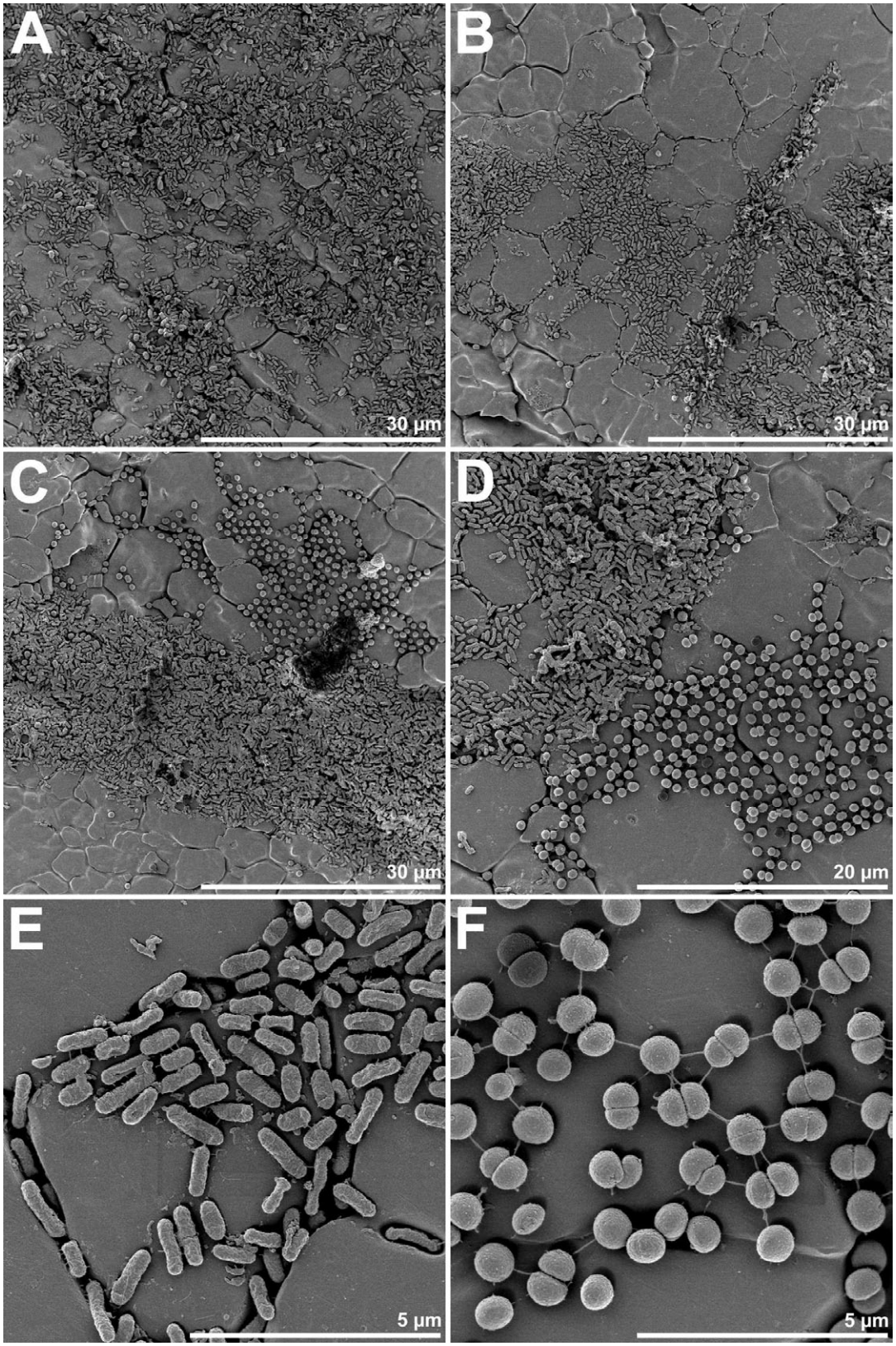

Fig. 12.5. MD1 coating on steel AISI316 biofouled with the five paper machine bacteria. Panels A, B, are low magnification micrographs of a thin mixed species biofilm on the MD1 coated steel. Large areas of vacant surface are also seen in Panel B. Panels C and D show *Psx. taiwanensis* and *D. geothermalis* adhered on the steel. A thick biofilm formed by *Psx. taiwanensis* is visible in Panel C. Panel E shows *Psx. taiwanensis* cells attached on grain boundaries of the steel substratum. Panel F, *D. geothermalis* cells attached to the abiotic surface and to each other by attachment threads.

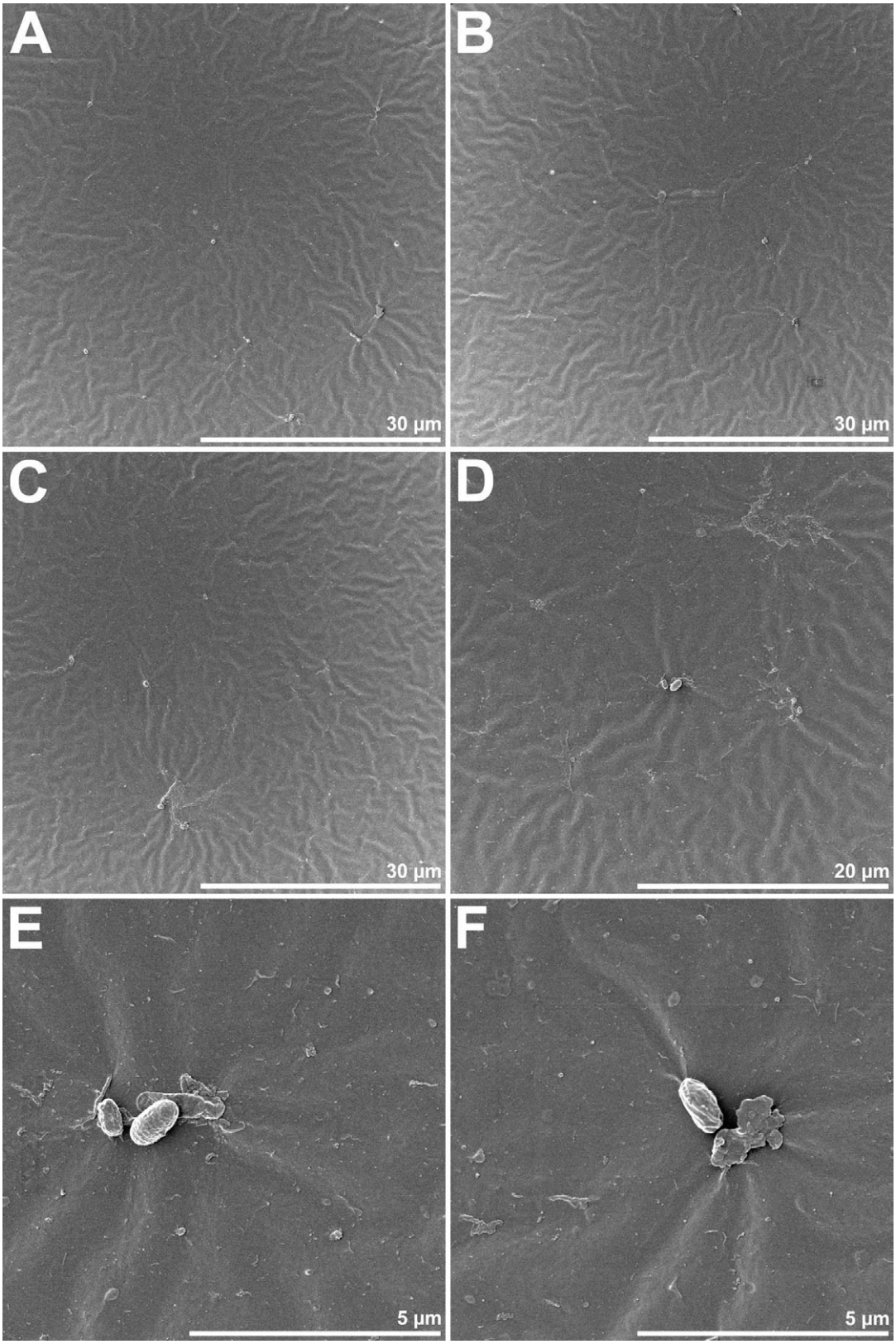

Fig. 12.6. MD2 coating on steel AISI316 biofouled with the five paper machine bacteria. Panels A to F, significantly fewer bacteria are seen adhered on surface compared to the non-coated steel (Fig. 12.1). Only a few individual bacterial cells or spores were seen on this coated steel surface.

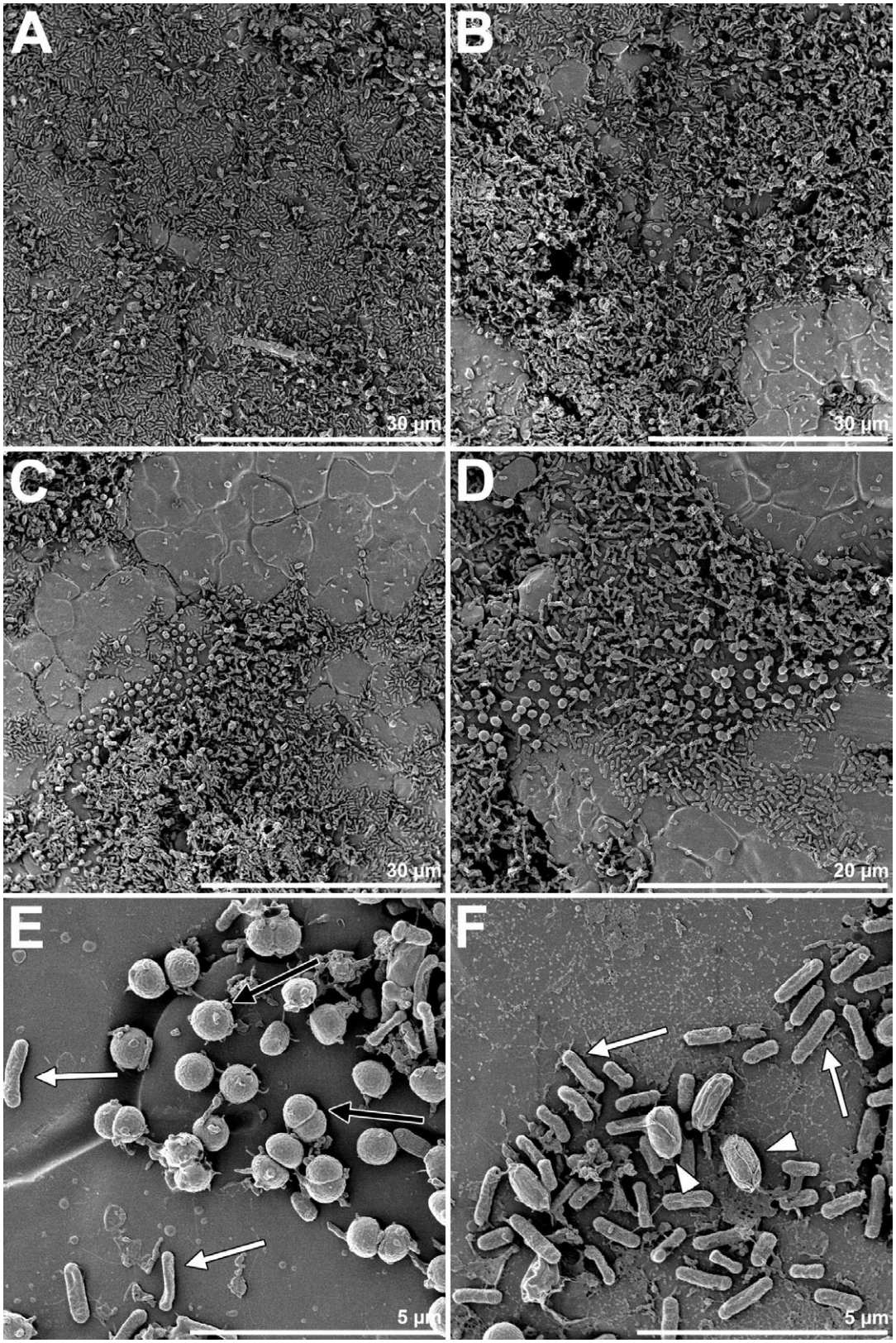

Fig. 12.7. MD3 coating on steel AISI316 biofouled with the five paper machine bacteria. Panels A, B, show low magnification micrographs of a thick mixed species biofilm on the coated steel. Higher magnification of these biofilms is shown in panels C and D. In Panel E *D. geothermalis* cells (black arrows) appear adhered on the coated steel surface. In panel F, cells of *Psx. taiwanensis* (white arrows) cells and *Bacillus* or *Brevibacillus* spores (white arrow heads) appear adhered on the coated steel surface.

We used the FESEM technique to study the ultrastructures of mixed species biofilms formed by five paper machine bacteria on surfaces of steel with different coatings or no coating. The bacterial strains *D. geothermalis*, *M. silvanus* and *Psx. taiwanensis* represented primary biofilm formers from paper machines, with shown capability to adhere onto plain steel as well as most of the coated steel surfaces. The species *B. pumilus* and *Br. agri* were also paper machine biofilm isolates, but only able to adhere as spores onto superhydrophobic L-FAS coating and onto biofilms formed by the other species. The results showed that most effective coating in repelling biofouling caused by these species was hydrophobic fluorosilane coating.

We have showed evidence that *Psx. taiwanensis* is an effective biofouler of abiotic surfaces in conditions simulating paper machine environment (paper II and this thesis). *Psx. taiwanensis* adhered onto a majority of the surfaces tested in these two studies: steel (Figs. 2 and 6 in paper II, Figs. 12.1 and 18 in this thesis), glass (Fig. 18 in this thesis), fluoropolymers (Figs. 2 and 6 in paper II), DLC (Figs. 2 and 6 in paper II), L-FAS, L as well as two different antimicrobial coatings (Figs. 12.2, 12.3, 12.5 and 12.7 in this thesis, respectively). Only the FAS coated steel (Fig. 12.4 in this thesis) which was mildly hydrophobic (θ 105°) but possessed no nanotopography, and the coating that emitted biocide (MD2, Fig. 12.6 in this thesis) to the immersing medium were relatively clean after the exposure to a seeded medium.

In addition to being able to adhere on different abiotic surfaces, *Psx. taiwanensis* was found to be capable of adhering to resin acid and wood extractive *i.e.* pitch emulsion droplets under conditions simulating those in paper machine wet end. Moreover, *Psx. taiwanensis* coagulated the pitch emulsion so that large rafts (up to 15 µm) were formed (Leino *et al.*, 201×b, Leino *et al.*, 201×a). Such lipid containing rafts are low in gravity and may float, depositing on the surface of the paper web when it is formed on the wire. Surface exposed bacterial-pitch deposits may connect to the fouling of hot calender surfaces observed at the dry end of the paper machine during on-line calendering.

4.1.3 Biofilms can be destroyed by photocatalysis

There are plenty of studies describing the potential of photocatalysis for disinfection or to improve hygiene by destroying microbes attached on surfaces. There is less information on the potential of photocatalysis to destroy actual biofilms on nonliving surface. We investigated this aspect using photocatalytic TiO_2 films, prepared by the ALD or the sol-gel

method, as a substratum for *D. geothermalis* –biofilm (paper I). Biofilms pre-grown on the TiO_2 coated coupons were illuminated (λ 360 nm) for 1 d. The coupons were then stained with a fluorogenic nucleic acid stain to measure the quantity of bacteria that remained on the surface. The fluorescence emission was recorded by a scanning fluorescence reader. The coupons were also examined with epifluorescence microscope and with scanning electron microscope.

The results (Figs. 2 to 7 in paper I and Figs. 13 to 15 in this thesis) showed that surfaces with photocatalytic coating carried a lesser amount of bacteria when illuminated (λ 360 nm) than when kept in the dark.

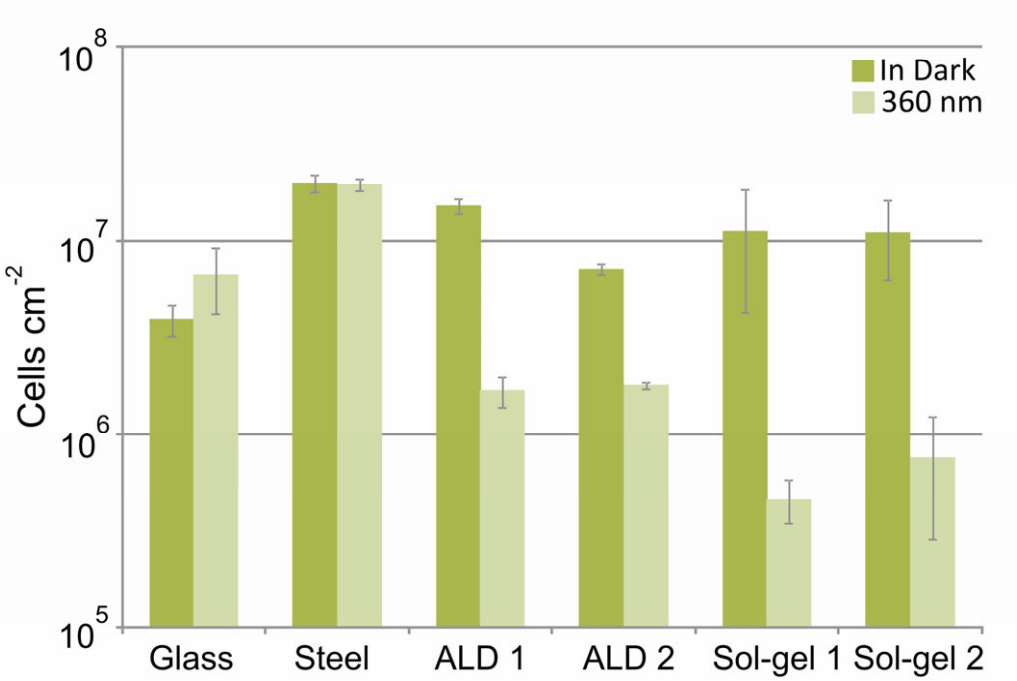

Fig. 13. Removal of *D. geothermalis* biofilms from various abiotic substrata, coated and non-coated with TiO_2, by exposure to illumination at 360 nm. The biofilms had been grown on the coupons for 2 d in the dark and were then illuminated for 1 d, 1 W m^{-2} at 360 nm or not. The biofilms were quantitated by dyeing with the nucleic acid responsive fluorochrome SYTO9. Fluorescence emission values were measured with a scanning fluorometer. Glass = borosilicate glass, Steel = AISI316, ALD 1 = borosilicate glass substratum coated with anatase TiO_2 by ALD method, ALD 2 = borosilicate glass substratum coated with rutile TiO_2:S by ALD method, Sol-gel 1 = titanium substratum coated with macroporous anatase TiO_2 with sol-gel method, Sol-gel 2 = titanium substratum coated with anatase TiO_2 with sol-gel method.

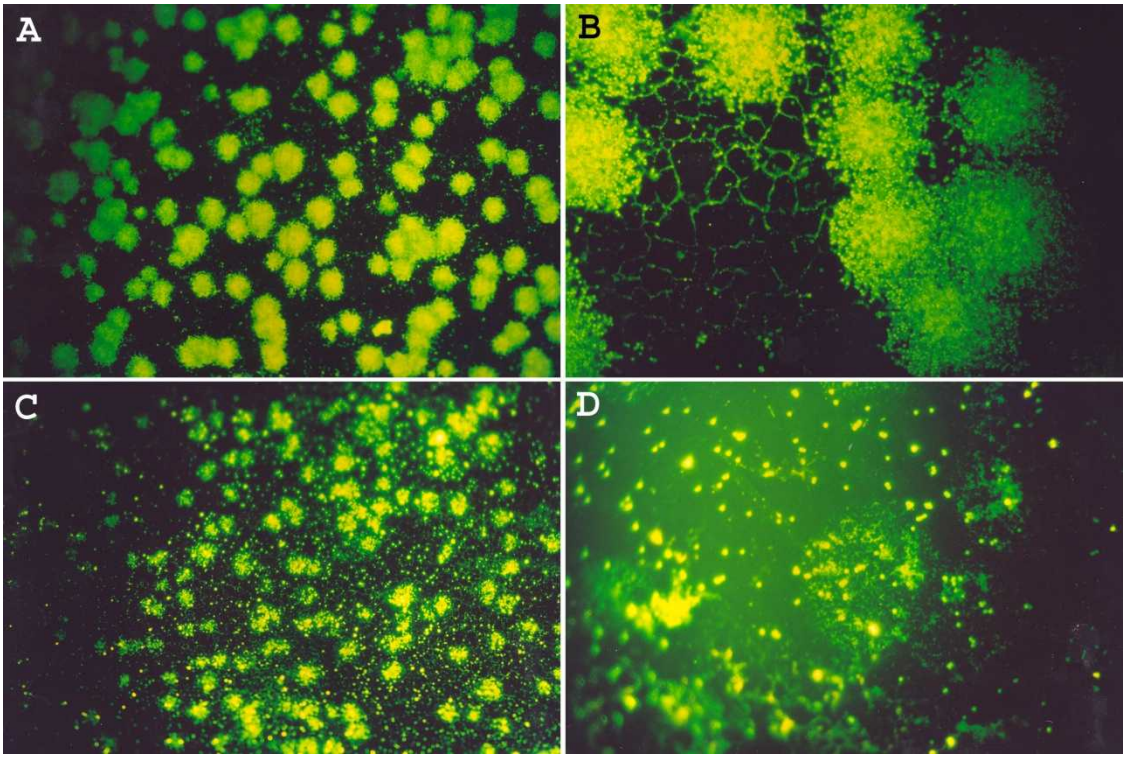

Fig. 14. Epifluorescence images of *D. geothermalis* biofilm on coupons of stainless steel stained with the nucleic acid responsive fluorochrome SYTO9. Panels A and B show biofilm grown for 2 d. Plenty of green fluorescing cells. Panels C and D show similarly prepared coupons fluorescently dyed after irradiated for 1 d, 1 W m^{-2} at 360 nm. Most of the biomass fluorescently stainable by SYTO9 persisted the irradiation.

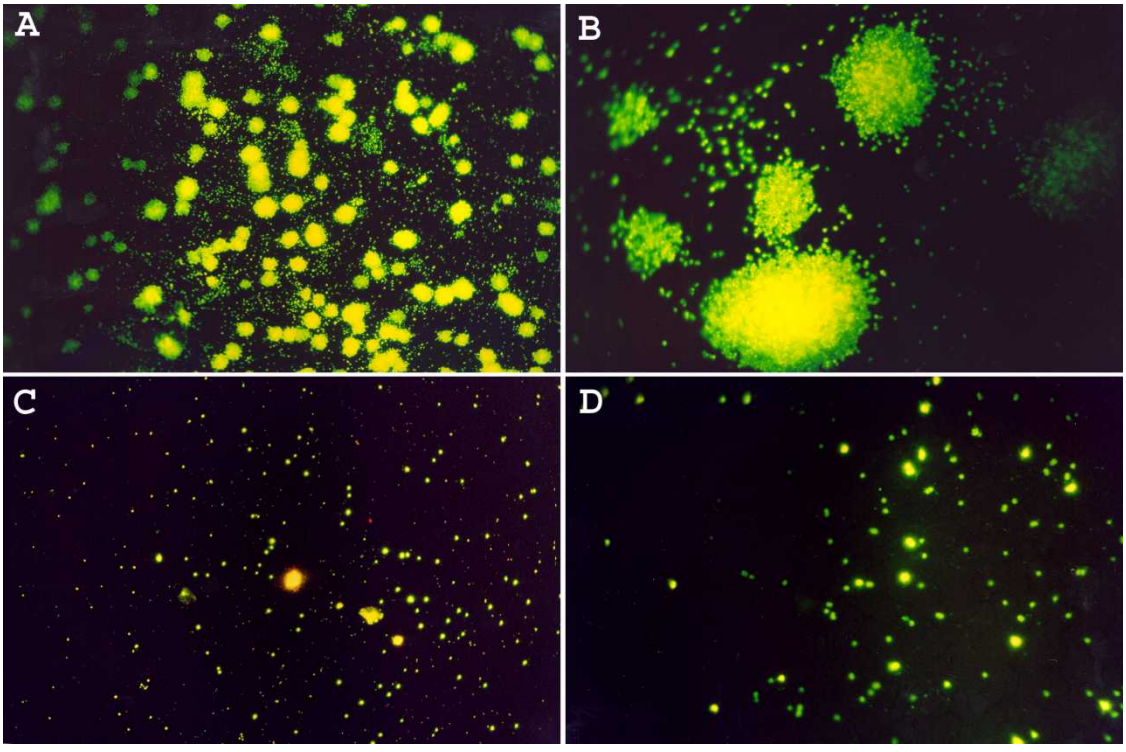

Fig. 15. Epifluorescence images of *D. geothermalis* biofilm on a stainless steel coupon coated with TiO_2 (ALD 3). The images were taken after the coupons had been dyed with the nucleic acid responsive fluorochrome SYTO9. Panels A and B show biofilm grown for 2 d. Plenty of green fluorescing cells. Panels C and D show similarly prepared coupons irradiated for 1 d, 1 W m^{-2} at 360 nm and then fluorescently dyed. Almost all fluorescently stainable material is absent.

There are plenty of studies describing the potential of photocatalysis for disinfection or for improving hygiene by destroying microbes attached on surfaces. However, little is known on the potential of photocatalysis to destroy biofilms on abiotic surfaces. It is well known that bacteria in biofilms tolerate well many stressors, such as antimicrobial agents, desiccation and radiation (Costerton *et al.*, 1987 and 1999, Mah and O'Toole, 2001, Stewart and William Costerton, 2001, Lewis, 2008). Interestingly, *D. geothermalis* is radiation and desiccation resistant (Ferreira *et al.*, 1997) and was observed to produce more biofilm when exposed to certain biocides such as, methylene bisthiosyanate (MBT) or 2,2-dibromo-3-nitrilopropionamide (DBNPA) at pH 5 than when not exposed (Kolari *et al.*, 2003).

In paper I we showed that *D. geothermalis* biofilms on TiO_2 coated coupons were effectively (reduction by 1 to 2 log units) removed by photocatalytic action of the TiO_2 when illuminated at 360 nm. My scanning electron micrographs revealed that the *D. geothermalis* biofilm was extensively damaged by the photocatalytic action (Figs. 2 and 3 in paper I): the adhesion threads normally connecting the adjacent cells to one another and the cells to the substratum practically disappeared from the *D. geothermalis* cells on TiO_2 coated coupons, whereas the same bacteria on plain steel appeared to possess undamaged threads after similar irradiation (Figs. 4 and 5 in paper I).

My data published elsewhere (Keskinen *et al.*, 2006) showed that irradiation catalysed cell density reduction by > 1 log in *D. geothermalis* biofilms on steel coated with TiO_2 by the flame spray method. This photocatalytic killing was less effective when silver nanoparticles were applied together with the TiO_2 on the steel substratum. However, in absence of illumination the silver containing coatings attracted > 1 log less of *D. geothermalis* cells than the steel substratum coated with TiO_2 only or with none. Storgårds *et al.* (2007) presented preliminary results from experiment in brewery filling department. They exposed steel coupons with no coating or coated with plain TiO_2 or TiO_2 together with silver to production environment. Results showed >1 log reduction in the counts for aerobic bacteria on TiO_2/Ag coated steel compared to the non-coated steel and 3 log reduction in the counts of pseudomonads on both TiO_2 and TiO_2/Ag coated steel after 200 d exposure. The experiment was conducted without added illumination. The results from these two studies showed that applying silver on photocatalytic surface reduced biofilm load more efficiently that plain TiO_2 in conditions with no sufficien illumination for photocatalysis to occur. However, the use of silver coatings to suppress biofilm growth is questionable, since silver induces resistance (Silver *et al.*, 2006).

Summarising all these data, (the results in paper I, and elsewhere) showed that photocatalysis is a potential tool for removing biofilms from abiotic surfaces in transparent media, such as application in water handling industries and facilities where high level of hygiene is required.

Stainless steel is the material of choice in many industrial process equipments and surfaces. Examples of industrial settings reported to have biofilm problems were compiled in Table 3. The results from my studies (papers I and II and this thesis) showed that biofilm forming bacteria from three phylogenetically distant phyla are capable of colonising and forming a biofilm on steel. Coating the steel surfaces with material that reduces biofouling would be beneficial in a broad range of industrial applications. However, the requirements of the coating material are demanding. In addition to the repellency towards microbial colonisation, demonstrated in this study, the material should be easy to apply, affordable and durable against cleaning procedures and wearing. No material has yet fullfilled all these requirements.

4.1.4 Use of starter cultures to modify biofilm structure in barley kernel tissues

Paper III describes ultrastructural details of the natural microbiota of barley kernels before the steeping step of malting (Fig. 2 in paper III and Fig. 16 A to C) and after (Fig. 3 in paper III and Fig. 16 D to F) as revealed by field emission scanning electronmicroscope. Amending the steeping water with a culture of *Lactobacillus plantarum* E76 reduced the accumulation of EPS during the subsequent steeping. The strain of *L. plantarum* used in this study as a starter culture was successful in colonising the tissues of barley kernels (Fig. 4 in paper III and Fig. 16 G to I in this thesis). This colonisation resulted into reduced growth of slime producing bacteria. A positive effect on filterability of the mash was also observed (Fig. 5 in paper III). Lactic acid starter bacteria have elsewhere been shown effective in controlling undesirable microbial contaminants, particularly pseudomonads and the *Fusarium* fungi in malting (Laitila *et al.*, 2006, reviewed by Lowe and Arendt, 2004).

Adding the yeast *Wickerhamomyces anomalus* C565 (synonym *Pichia anomala*) to steeping water was shown to suppress the growth of contaminating yeasts and filamentous fungi, but did not noticeably affect bacterial growth in grain (Laitila *et al.* 2007b). The final malts were of good quality, but the separation of wort slowed down. Based on this knowledge we decided to use two starters simultaneously, *Lactobacillus plantarum* E76 and *Wickerhamomyces anomalus* C565 in the steeping water. When we examined biofilms on

the barley kernel surfaces with FESEM we found that both the *L. plantarum* and the *W. anomalus* strains had entered the husk layer of the barley grain and adhered on the surfaces of the kernel suppressing the growth of the EPS producing bacteria (Fig. 1 in Laitila *et al.*, 201× and Fig. 16 G to I in this thesis).

Microbial communities are an integral part of all cereals including barley as is visible in Fig. 16 A to F. It is shown that microbial community in barley kernels consists of diverse bacteria, yeasts and fungi, located within the outer layers of kernel. The natural microbiota of grains has great impact on the technical performance and the quality of cereal products (Laitila *et al.*, 2007a, Noots *et al.*, 1999). Steeping in water stimulates the microbiota in the barley kernel to grow and to produce extracellular substance. In the micrographs in Fig. 3 in paper II and Fig. 16 D to F and Fig. 19 D to F in this thesis it is shown that the tissues of steeped barley kernel became covered by slime which embedded microbes. Kernel associated biofilms and EPS may deteriorate germination (Laitila *et al.*, 2007a, Doran and Briggs, 1993, Kelly and Briggs, 1992) and be harmful in brewing, by reducing the separation of wort and filterability of the mash (Laitila *et al.*, 1999). The results in this thesis show that starter cultures can be used in steeping water to modify the microbial community and the structure of the biofilms inside barley kernels.

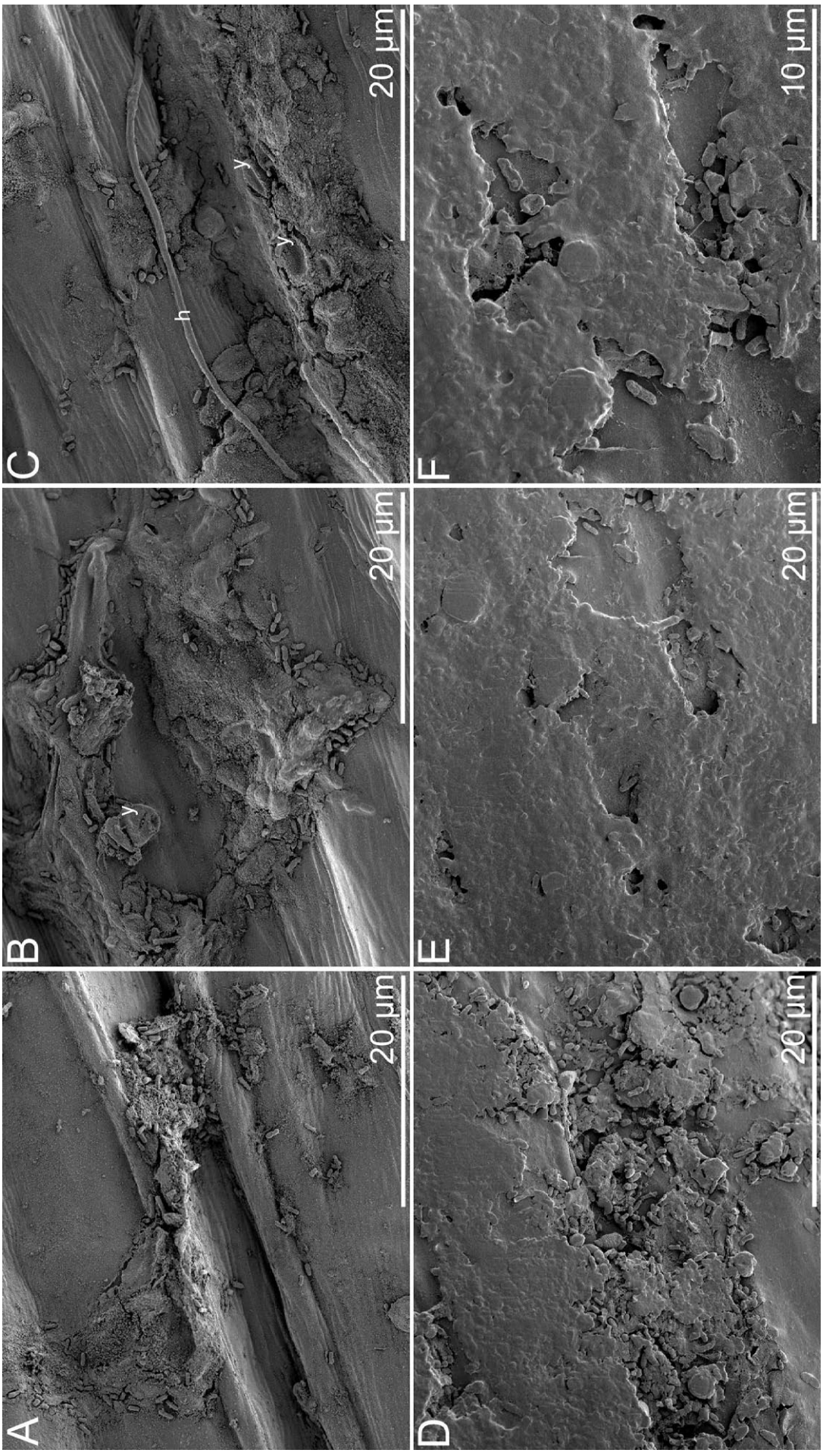
A
20 µm
B
20 µm
y
C
20 µm
h
y
y
D
20 µm
E
20 µm
F
10 µm

Fig. 16. FESEM micrographs of surfaces of the epidermis layers of barley kernels. Panels A to C show clusters of bacteria on a dry kernel of barley, collapsed fungal hyphae (h) and yeast cells (y). Panels D to F show 2 d old biofilms on a steeped barley kernel. Most of the kernel surface and the attached bacteria are embedded in amorphous slime. Panels G to I show 2 d old biofilms on a barley kernel steeped in the presence of the starter *Lactobacillus plantarum* E76 with *Wickerhamomyces anomalus* C565 (synonym *Pichia anomala*). Cells looking like *L. plantarum* (short rods) have colonized the kernel surface. In panel G yeast cells (y), putative *W. anomalus*, are in close proximity to putative *L. plantarun* cells. In panel I, the belt-like structures of *L. plantarum* cells are visible (white arrows).

4.2 Ultrastructural studies of bacteria adhered on abiotic surfaces using FESEM

4.2.1 Mechanisms of adhesion to biotic and abiotic surfaces by phylogenetically remote bacteria

We found by field emission scanning electron microscopy (FESEM) that several biofilm forming bacterial species utilise thread like appendages to anchor their cells onto a surface and also to bridge neighbouring cells to one another (Papers I-III and Fig. 17 in this thesis). As model organisms we used two members of the phylum *Deinococcus-Thermus* (*Deinococcus geothermalis* and *Meiothermus silvanus*), one *Firmicute* (*Staphylococcus epidermidis*) and one *Gammaproteobacterium* (*Pseudoxanthomonas taiwanensis*) (Table 2).

We observed by FESEM (paper I) that although the adhesion organelles of *D. geothermalis* were resistant towards washing with alkali (0.1% w/v NaOH), they were destroyed by photocatalysis. Irradiation with 360 nm light of *D. geothermalis* cells adhered onto TiO_2 coated coupons destroyed the adhesion organelles completely (Figs. 2 and 3 in paper I), whereas same irradiation did not visibly affect the cells adhered onto coupons of plain steel (Fig. 4 in paper I). The TiO_2 coating itself was not toxic to *D. geothermalis*, as shown by biofilms on TiO_2 coated coupons incubated in the dark (Figs. 2 and 3 in paper I). The cells adhered on TiO_2 coated steel displayed adhesion organelles similar to those seen on cells adhered on plain steel. *D. geothermalis* may possess a second mean of attachment besides these adhesion threads, since some of the cells persistently adhered on the TiO_2 coated coupon even after irradiation at 360 nm. Kolari *et al.* (2002) measured with AFM the adhesive forces between the surface of *D. geothermalis* cell adhered on glass and silicon tip of the cantilever. They found that the cell surface was heterogeneous in adhesiveness. The surface topography of adhered *D. geothermalis* cells looked patchy also in FESEM micrographs of high magnification (Fig. 5 in paper I). The local adhesive areas on the surface of *D. geothermalis* possibly mediated adhesion to certain abiotic surfaces. *D. geothermalis* type strain (Ferreira *et al.*, 1997) as well as paper machine biofilm isolate E50051 (Fig. 1 D in paper IV), are known to produce capsule-like layer surrounding and connecting adjacent cells. Capsular layer has been shown to act as adhesin (reviewed by An and Friedman, 1998).

In paper II (Fig. 4) we showed that in *D. geothermalis* biofilms on non-coated and DLC coated steels most cells displayed a large number of appendages. These thread shaped organelles connected neighbouring cells to one another or to the abiotic surface. Only few, thin appendages were present on the cells grown on steel coated with fluoropolymer and

planktic cells grown in the same culture medium were void of any thread-like structures. *D. geothermalis* grown on glass surface also expressed the appendages (Fig. 17 in this thesis).

Kolari *et al.* (2002) found , using FESEM and AFM, the first evidence on the presence of peritrichous appendages, in *D. geothermalis* biofilms and suggested that the bacterium uses these for cell-to-cell attachment and adhesion onto abiotic surfaces. Later Saarimaa *et al.* (2006) found glycosylated proteins, reminiscent on type IV pili in washing supernatants of *D. geothermalis* E50051 cells grown on agar plates. In the same study Minna Peltola and I showed that the appendages only appear during surface attached growth and were absent in planktic cells. We did this by imaging with FESEM and CLSM combined with lectin staining (Figs. 1 and 3 Fig. in Saarimaa *et al.* 2006 and Fig. 17 in this thesis). However, very recently Tian *et al.* (2010) reported fimbrial pilin and type IV pilus assembly proteins from planktic cells of *D. geothermalis* DSM 11300^{T} by proteomic analysis of membrane proteins.

The pili and the numerous cell surface -exposed glycoconjugates (Peltola *et al.*, 2008a), and unique cell envelope proteins and repair enzymes (Liedert *et al.*, 2010) expressed by *D. geothermalis* E50051, could explain the firm attachment and formation of tenacious biofilms by this bacterium, which is persistent against desiccation, radiation, aggressive washings and antimicrobial agents.

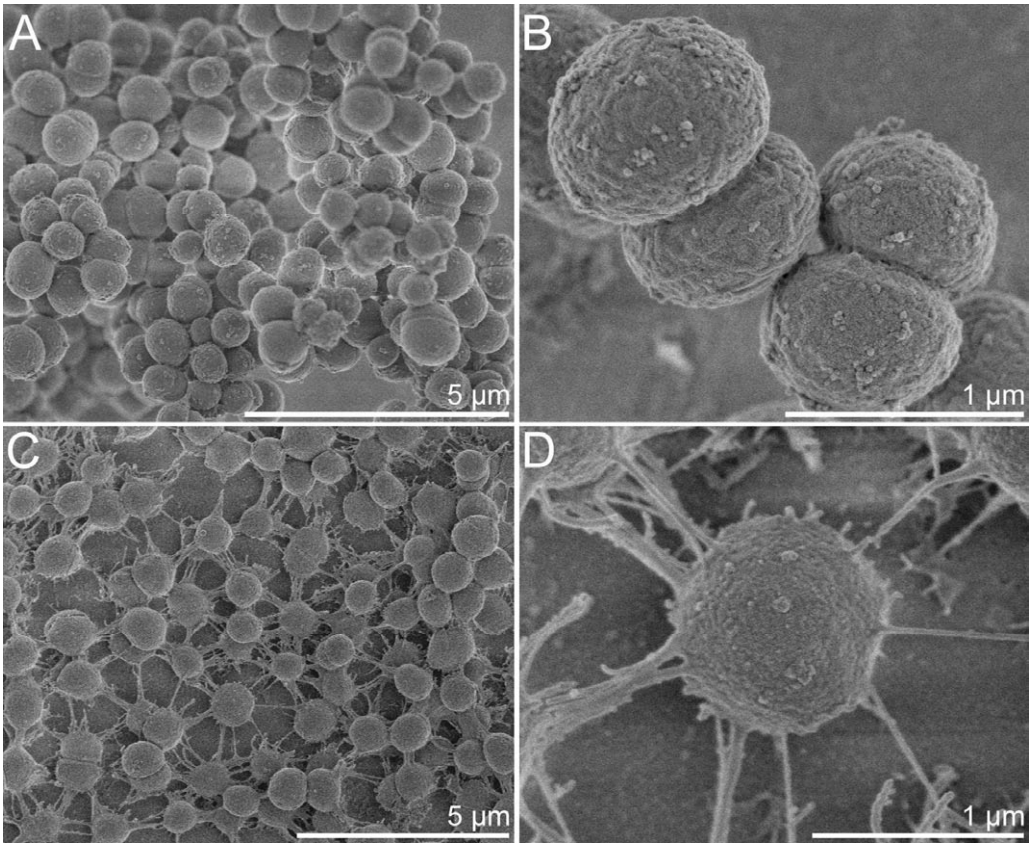

Fig. 17. FESEM micrographs of planktic (A and B) and sessile (C and D) cells of *Deinococcus geothermalis* E50051 isolated from a pink biofilm in a paper machine. Sessile cells (on glass surface) expressed adhesion threads, absent in the planktic cells.

We found that in biofilms of *Psx. taiwanensis* large amounts of cell ghosts were distributed on the abiotic surface among and underneath the adhered intact cells (Fig. 2 in paper II, Fig. 18 in this thesis). We hypothesise that this bacterium uses a suicidal strategy to form a biofilm: part of the population is sacrificed to mediate the adhesion of the remaining part of the community onto the abiotic substratum. No such ghost cells were observed in liquid culture or among *Psx. taiwanensis* cells adhered onto coupons of steel coated with fluoropolymers. This phenomenon is yet to be studied in detail to understand the actual events taking place. Is the suicidal biofilm generation a fact or an artifact? Having viewed hundreds of micrographs of *Pxt. taiwanensis* and other species attached and not attached on surfaces, after the same procedures of fixation and dehydration, we conclude that the suicidal biofilm formation likely reflects the real life situation. Nevertheless, more study is required by independent methods (*e.g.* light microscopy with live cells) to confirm this hypothesis.

The hypothesis of an altruistic suicide among bacteria gets support from reports where release of extracellular DNA through an autolysin-mediated lysis of a subpopulation of bacteria was shown to advance biofilm formation by *Staph. epidermidis*, by *Enterococcus faecalis* and by *Streptococcus mutans* (Qin *et al.*, 2007, Perry *et al.*, 2009, Thomas *et al.*, 2008). Cell lysis also provides nutrients for the surviving part of the population. It has been suggested that autolysis of bacteria is functionally similar to the programmed cell death (apoptosis) in eukaryotes, promoting the development of biofilm architecture (Lewis, 2000) and is linked to the resistance of biofilm bacteria against bactericidal antimicrobials (reviewed by Lewis, 2001 and Bayles, 2007).

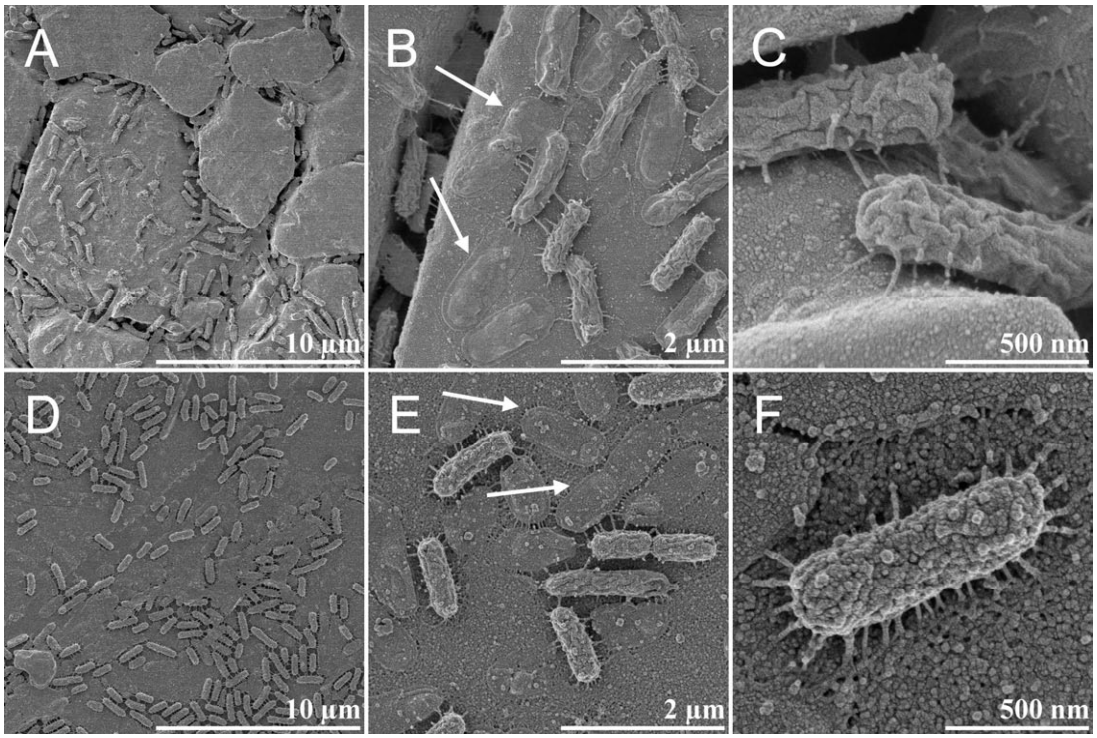

Fig. 18. FESEM micrographs of the paper machine biofilm isolate *Pseudoxanthomonas taiwanensis* JN 11306 adhered to abiotic surfaces. Panels A to C, acid proof steel (Steel #1, Table 2 in paper II). Panels D to F, glass. Arrows in Panels B and E point at cell ghosts.

When grown on non-coated steel *Staph. epidermidis* (Fig. 5 in paper II and Fig. 6 in this thesis) displayed slimy appearance and expressed thick threads bridging the neighbouring cells. In contrast, on DLC or fluoropolymer coated steel adhered cells of the same strain consisted of non-slimy cells interconnected by thin appendages only. The strain (O-47 PIA^+) used in this study is known as a producer of the polysaccharide intercellular adhesin, PIA (Heilmann,C., 1996). PIA is a component of the EPS of *Staph. epidermidis* and contributes to its adherence (Izano *et al.*, 2008, Mack *et al.*, 1994). Fig. 5 A in paper II shows that the biofilm of *Staph. epidermidis* O-47 PIA^+ had a slimy appearance, possibly due to the production of PIA.

Adhesion threads similar to those displayed in paper II and in Figs. 17 and 18 in this thesis were also seen in bacteria adhered onto barley kernels studied in paper III. The appendages shown in Fig. 19 B and C of this thesis and Figs. 2 and 3 in paper III resemble those observed by Pawar *et al.* (2005) in electron micrographs of curli-expressing *Escherichia coli* O157:H7 5-11 adhered on an abiotic surface. Inside the steeped barley kernel the threads connected bacteria to each other and to the seed-coat tissues to dense networks. Some of the microbial cells seemed to make contact with the barley kernel surface by means of a slime layer (Fig. 19 D to F in this thesis). The slime covered up to 95% of the accessible surface in the husk and on the outer epidermis of the barley kernels after steeping.

We noticed the presence of a belt-like structure on the surface of *L. plantarum* cells and hypothesised that this belt connected the bacterium to tissues of the barley kernel (Fig. 4 in paper III and Fig. 16 I in this thesis). Those could represent some of the 12 putative adhesion factors reported by Boekhorst *et al.* (2006) from the proteome of *L. plantarum*.

Literature offers examples of appendages visualised on bacterial cells with scanning electron microscopy; *e.g.* type IV bundle-forming pili of enteropathogenic *E. coli* (Bieber *et al.*, 1998), lateral flagellar filaments in *Vibrio parahaemolyticus* (Belas and Colwell, 1982) and flagelli-like filaments and the thin fibrillar structures of *Stenotrophomonas maltophilia* (de Oliveira-Garcia *et al.*, 2002). Ishii *et al.* (2004) reported two morphologically different cell organelles from *Acinetobacter* sp. and showed FESEM images as well as variable pressure FESEM (VP-FESEM) images of the same structures. The specimens for VP-FESEM were fixed but not dehydrated for imaging. Later Ishii *et al.* (2008) showed that these structures were only expressed in adhered cells. Also, the electrically conductive bacterial nanowires produced by *Shewanella oneidensis* were visualised by Gorby *et al.*

(2006) by scanning tunneling microscopy, SEM as well as epifluorescence microscopy. Later Ray *et al.* (2010) imaged the nanowires by SEM from adhered and planktic *Shewanella oneidensis* grown with and without oxygen. Type IV pili of *Haemophilus influenza* were visualized with SEM and with immunofluorescence confocal microscopy by Jurcisek and Bakaletz (2007).

As a conclusion, our results from the studies described in papers I, II, and III showed that phylogenetically remote bacteria utilised similar tools, adhesion threads, to attach on biotic and abiotic surfaces. From electron micrographs alone, it is not possible to decide if threads were genuine appendages or if they consisted of dehydrates slime produced by the bacteria. However, the adhesion threads of *Deinococcus geothermalis* were seen also in hydrated samples by CLSM and by atomic force microscopy (AFM) (Saarimaa *et al.*, 2006, Peltola *et al.*, 2008a, Kolari *et al.*, 2002). For the other species where we also saw adhesion threads, *e.g. Psx. taiwanesis, M. silvanus, Staph. epidermidis* and the native colonisers of barley kernels, the true nature of adhesion organelles remains to be elucidated.

Flagellar motility has been reported to an important factor for adherence and for biofilm formation for many human pathogens (Table 1). Results in papers I and II and in this thesis showed, that also non-flagellated biofilm forming bacterial species are capable of adhering to abiotic surfaces under hydraulic flow. Such bacteria were exemplified by the species *D. geothermalis, M. silvanus, Psx. taiwanensis* and *Staph. epidermidis,* none of which is flagellated (compiled in Table 2). *Deinobacterium chartae* is further example of a non-flagellated, biofilm forming bacterium (paper IV, Table 7 in this thesis). Convective transport with the liquid flow may provide the energy needed for the non-motile bacteria to penetrate the diffusion layer and to reach the abiotic surface.

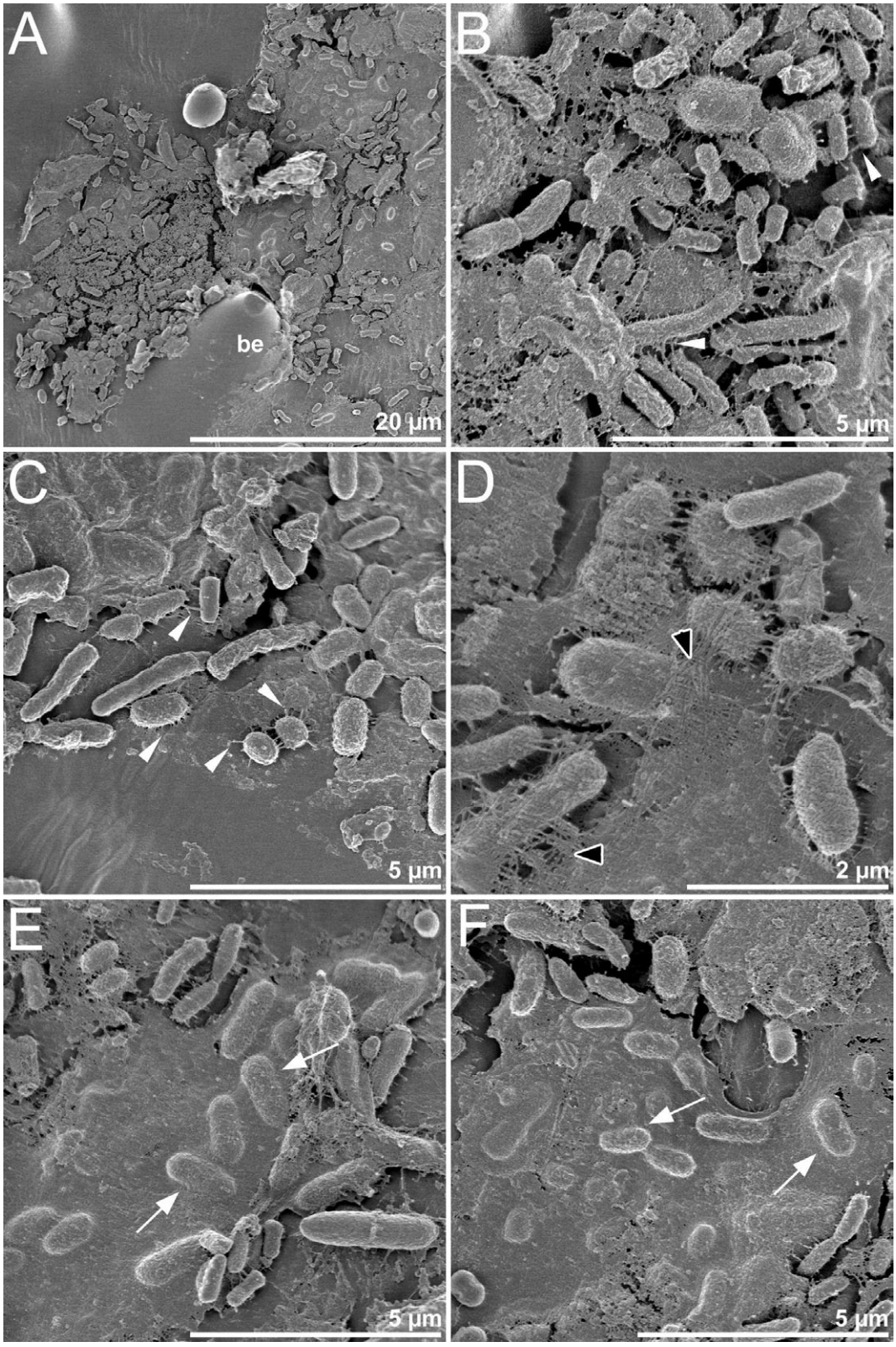

Fig. 19. FESEM micrographs of 2 days old native biofilms on the epidermis of a steeped barley kernel. Panel A shows the biofilm located in between the testa and the outer epidermis of the kernel. It shows numerous microbes, exopolymeric matrix and the kernel epidermis (be). Panels B and C show biofilm bacteria on the barley biofilm adhering to one another and to the kernel surface (white arrow heads). Panel D shows bacteria embedded in fibrous slime (black arrow heads). Panels E and F show bacteria embedded in amorphous slime (white arrows).

4.2.2 Bacteria respond to the quality of an abiotic surface

Ultrastructures of the single species biofilms formed by four unrelated species of bacteria, representing three different phyla, were investigated by field emission scanning electron microscope (FESEM) in paper II. Cultures of the bacteria were grown under high liquid flow for 2 days at 45°C on coupons of coated and non-coated stainless steels. In Figs. 1 to 5 in Paper II it was already shown that each of the four biofilm forming bacteria possessed a different cell surface ultrastructure depending on whether it grew in planktic form or as a monoculture biofilm. Expanding on this, we describe below that these bacteria adapted the types of their adhesion organelles, cell morphology and the strategy for forming biofilm, to the surface they were colonising.

D. geothermalis showed numerous adhesion threads of various lengths and thicknesses connecting cells to one another and to steel surface. The cells that adhered onto fluoropolymer coating produced threads thinner and fewer in number than those observed on steel (Fig. 4 in paper II). This was also observed in paper I. The threads mediating adhesion of *D. geothermalis* to steel and to TiO_2 coated steel coupons were fewer in number but longer and thicker than those on the cells adhered to glass (Figs. 1 to 5 in paper I). Later Peltola *et al.* (2008a) showed by CLSM, that the adhesion of *D. geothermalis* to acid proof steel was partly mediated by molecules with lectin specificities different from those adhering to glass. *Staph. epidermidis* (PIA^+) produced both thick and thin appendages on steel, but used only the thin adhesion threads on coated steels. Furthermore, the colonies appeared less slimy on fluoropolymer coated steel compared to those on plain steel. The fluoropolymer coating on steel appeared to induce downregulation of the formation of the adhesion threads as compared to plain steel.

M. silvanus formed giant cells (> 20 µm long) when adhered onto DLC coated steels or to non-coated steels, but no such cells were seen among those that adhered to fluoropolymer coated steels (Fig. 3 in paper II and Fig. 20 in this thesis). Biofilms on hydrophilic surfaces (DLC, θ = 79° to 91°) contained a large number of elongated cells but the biofilms on hydrophobic surfaces (fluoropolymer, θ = 111° to 115°) contained none. Thus, the number of giant cells responded to the contact angle of the substratum (Table 3 in paper II). Giant cells were also observed among *M. silvanus* cells that adhered to glass (not shown), which is a hydrophilic surface. The change of the cell shape is impossible to explain based on any artifacts from sample preparation.

There was large amounts of cell ghosts (lysed cells) among the intact looking *Psx. taiwanensis* cells adhered on non-coated steel, DLC coated steel and glass (Fig. 2 in paper II and Fig. 18 in this thesis).

None of these morphological features of those four phylogenetically remote bacterial species were present on cells of the same strains if grown in the same medium and temperature in liquid culture (Fig. 1 in paper II and Fig. 17 in this thesis).

As a summary, when investigating the ultrastructure of four totally unrelated bacterial species adhered on surfaces we came across the surprising finding that each of these four bacteria used a different mode of adhesion onto plain steel compared to that used by the same species on the same steel coated with diamond like carbon (DLC) coatings or fluoropolymers. The adhesion organelles of *D. geothermalis* and *Staph. epidermidis* were shorter and thinner when growing on steels coated with fluoropolymers or with DLC coatings than those of the same bacteria on the same steel with no coating, immersed in the same medium at the same temperature. In addition, giant cells or cell ghosts were present frequent in the biofilms formed by *M. silvanus* and *Psx. taiwanensis* on stainless steel coated or not coated with DLC, but absent on steels coated with fluoropolymers.

We conclude from these observations that the four studied bacteria were capable of sensing the quality of the surface they are adhering to and adapted to it.

After the confocal scanning laser microscopy (CLSM) was applied to biofilm research in 1990s, the use of electron microscopy has diminished substantially. The reason for this is understandable: Electron microscopy of biological samples requires pretreatments, fixation and dehydration. Additionally, transmission electron microscopy (TEM) requires staining, embedding and sectioning. Scanning electron microscopy (SEM) requires coating of the specimens with metallic vapours. Extensive sample preparation can cause severe artifacts, distort the structures and change interactions between the components, for instance, by removing the lipids soluble in solvents used for the dehydration. Interpretation of the images thus requires caution. For CLSM the samples can remain fully hydrated which reduces the risk for artifacts. Yet, electron microscopy has a major advantage compared to light microscopy: its ultrahigh resolution. Highest possible magnification in light microscopy is 1000 ×, in electron microscopy 200000 × can be reached. Nevertheless, the results acquired using electron microscopy should be confirmed by other techniques to rule out artifacts.

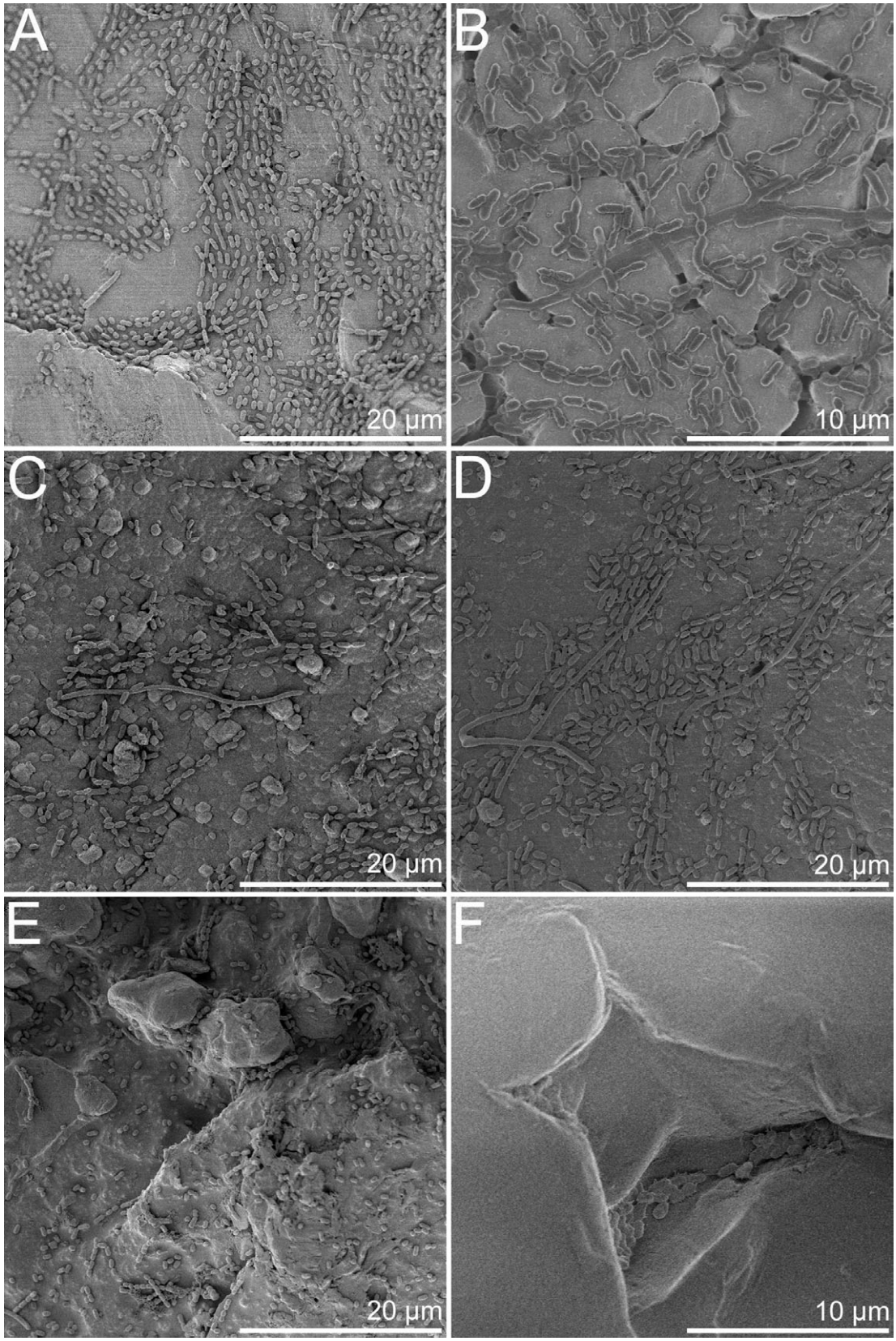

Fig. 20. FESEM micrographs of the paper machine biofilm isolate *Meiothermus silvanus* B-R2A5-50.4 adhered on coated and non-coted steels. Panel A shows *M. silvanus* cells adhered on acid proof steel AISI 316L/2B (steel #2 in Table 2 in paper II). Plenty of cells occurred in chains and few giant cells are visible. Panel B shows chains of *M. silvanus* cells adhered on another brand of acid proof steel (steel #1). Also here a giant cell is seen. Panels C, D show *M. silvanus* giant cells adhered on diamond like carbon coatings on steel#2 (DLC-A and DLC-B,

respectively). Panels E, F show *M. silvanus* adhered on fluoropolymer coatings on steel #1 (Ar-115 and AR-221, respectively). No giant cells were found. Panel F shows that *M. silvanus* cells only adhered into the crevices of the AR-221 fluoropolymer coated steel.

4.3 Oxalate and silicate in deposits formed in a paper mill (a case study)

Sometimes petrified deposits accumulate in paper mill water circuits. Petrified means that the deposit is insoluble in water or cleaning liquids used to clean the machines (warm aqueous 1% NaOH or surfactants). We addressed the question whether microbes play a role in the formation of such deposits?

Petrified deposits (Fig. 21) were obtained from a paper mill. They originated from the pulper (75°C, pH 10, "pulper deposit") and from the vacuum pump (45°C, pH 7.8, "pump deposit") of a paper machine using recycled fibre as raw material.

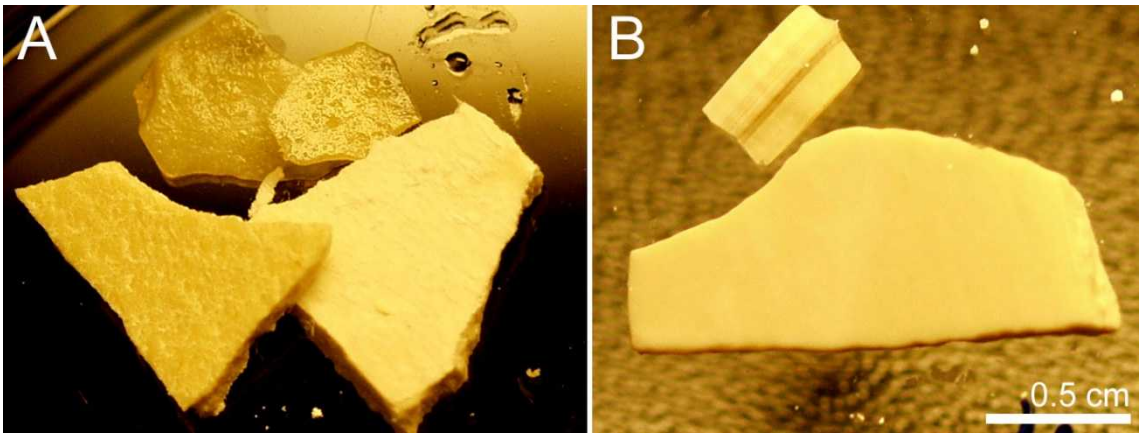

Fig. 21. Petrified deposits from a paper mill. Panel A, deposit from a pulper ("pulper deposit"); Panel B, deposit from a vacuum pump ("pump deposit") of the mill.

The deposits were insoluble in 1 M HCl, indicating that carbonates were not the main constituent. The "pump deposit" contained 0.3 mg oxalic acid g^{-1} while oxalic acid could not be detected in the "pulper deposit" by assay conducted with commercial enzymatic kit. Enzymatic assay may have been insufficient method for measuring oxalic acid from the deposits, since Ca-oxalate dissolves poorly.

The deposits were air dried and inspected with SEM and CLSM (Figs. 22 and 23). SEM micrographs displayed a crystal of 10 µm × 5 µm on the surface of the "pulper deposit". On the "pump deposit" fungal hyphae and spores were visible.

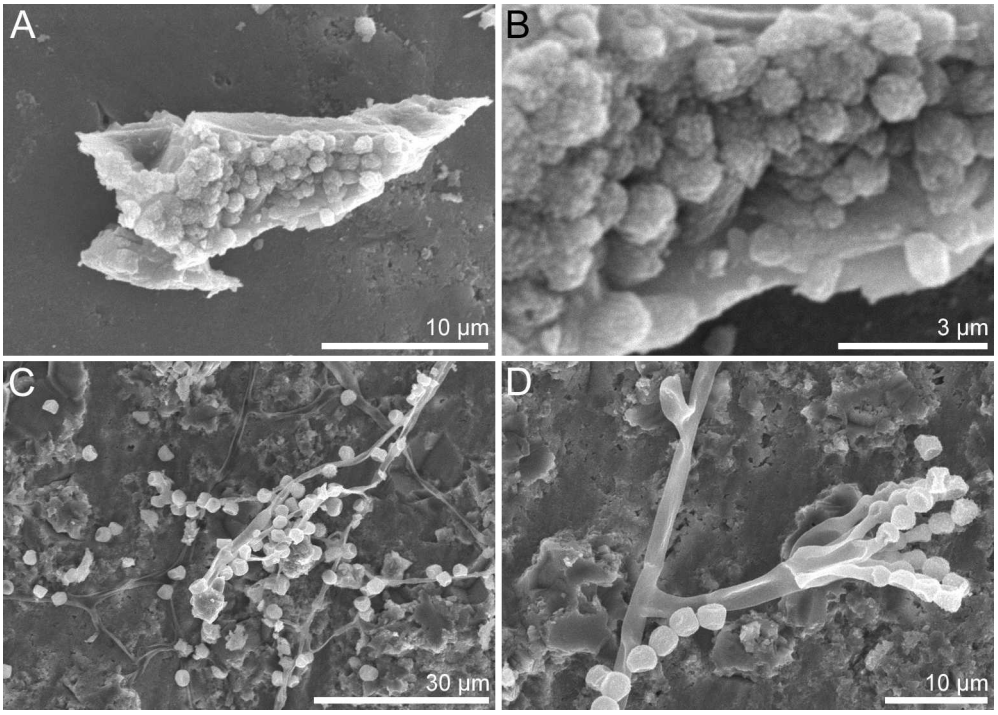

Fig. 22. Scanning electron micrographs (SEM) of deposits from a paper machine using recycled fiber; Panels A and B show a crystal on the surface of a deposit from a pulper of the machine. Panels C and D show fungal hyphae and spores on surface of the deposit from a vacuum pump of the machine.

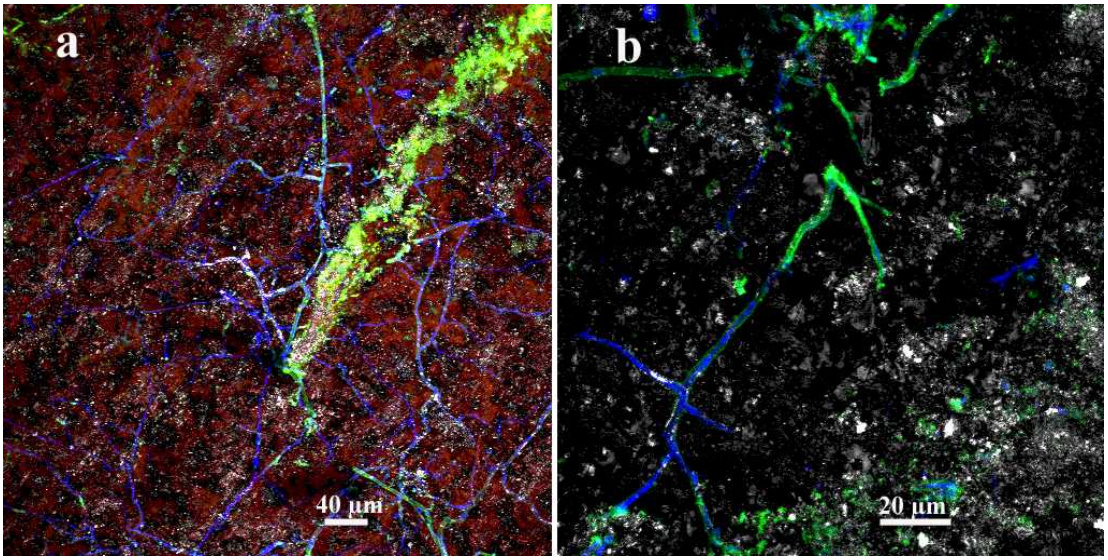

Fig. 23 A deposit from a vacuum pump of a paper machine was stained with fluorescent labelled *Triticum vulgaris* lectin (green) and nucleic acid stain, SYTO60 (blue) and inspected by CLSM. The information was collected in reflection (=grey), in green (=green), red (=red), far red (=blue) channels. The figure shows fungal hyphae –like filaments. The lectin bound to some junctions of the filaments. SYTO60 (blue) indicates the locations where DNA or RNA is present. Thickness of the optical section in panel a was 24 µm and in panel b 16 µm. The samples were prepared as described by Peltola *et al.*, (2008a). The images are courtesy of Minna Peltola.

CLSM micrograph of "pulper deposit" (Fig. 23), stained with the *Triticum vulgaris* (wheat germ) lectin and a nucleic acid reactive fluorochrome, shows the network of fungal hyphae on surface of the deposit. Some of the filaments (Fig. 23) appear to penetrate the

surface indicating that the organism may have grown inside the deposit. The Fig. 23 also shows that the filaments were living material since they were positive for nucleic acid specific fluorochrome SYTO60. This indicates that the filaments contained DNA and /or RNA. Fig. 23 B shows lectin binding to certain parts of the filaments, indicating growing points of the organism. The *Triticum vulgaris* lectin is specific for β-GlcNac, sialic acid and GlcNAc(β1,4)GlcNAc. The binding of the lecting thus indicates the presence of bacterial (murein) or fungal (chitin) cell wall material. The lectin showed also unspecific binding, maybe to pulp material of the paper machine (Fig. 23 A).

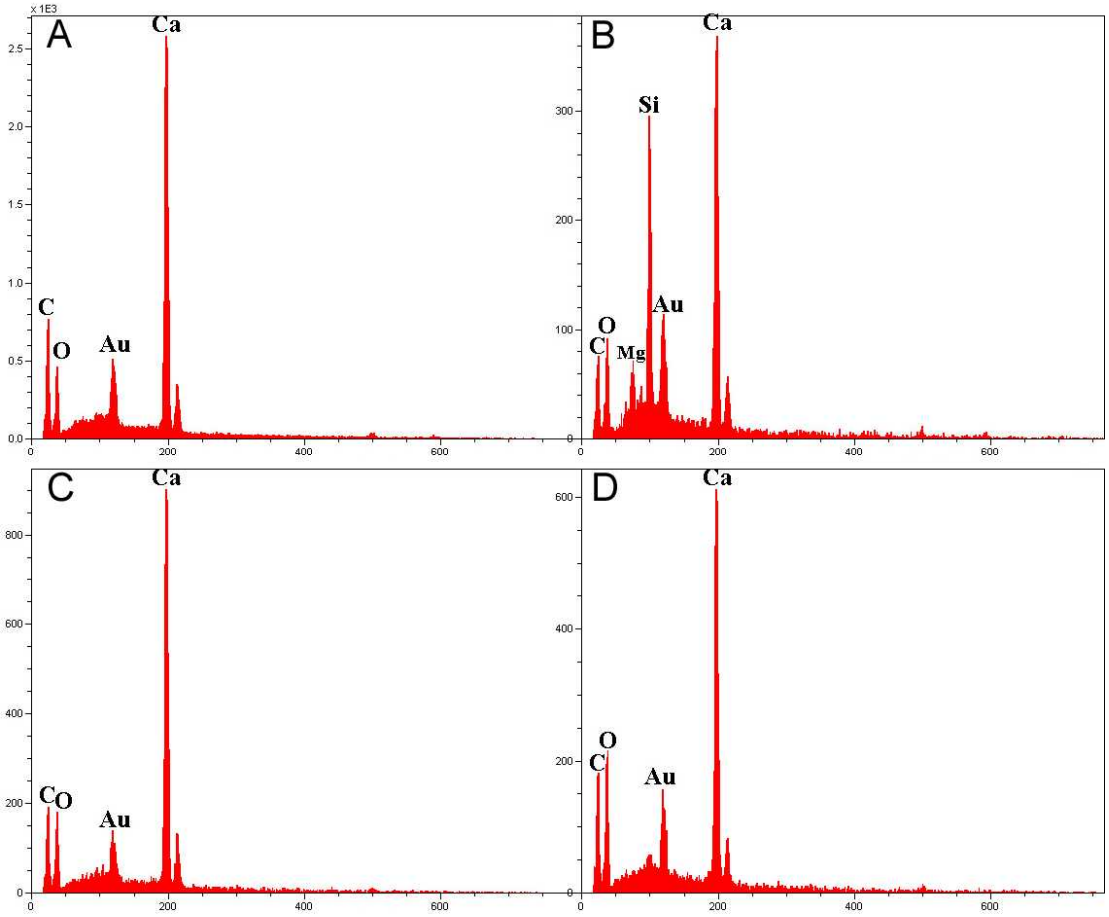

Fig. 24. EDS spectra of two petrified deposits from a paper mill. The deposits were gilded (Au) before the EDS-analysis. Panel A, "pulper deposit" (overall spectrum); Panel B, a crystal from the "pulper deposit"; Panel C, "pump deposit"; Panel D, Model substance Ca-oxalate.

The deposits were air dried and analysed for chemical elements using EDS (Energy Dispersive Spectrometry, Fig. 24). Both deposits contained calcium as the main metal. The EDS spectrum of the "pump deposit" (Fig. 24 C), prepared of gilded sample (explaining the Au) resembles that of calcium oxalate (reference material, shown in panel D). The EDS spectrum of the "pulper deposit" (Fig. 24 A) also resembles that of calcium oxalate. EDS analysis (Fig. 24 B) of the crystal (Fig. 22 A and B) found in the "pulper deposit" gave signals of silicon (Si) and carbon (C). In other areas of the "pulper deposit" there was less or

no Si (Fig. 24 A). Silicon and oxalate have been associated with microbially influenced corrosion (MIC) of steel in conditions prevailing in paper machine (Uutela *et al.*, 2003). Uutela *et al.* (2003) reported that the corrosion pits formed on steel accumulated large amounts of silicon containing ovoid particles of the shape and the size similar to those seen in Fig 22 B.

We cultured the deposits (Fig. 21) on an agar to which a blue stain (Victoria Blue B), $CaCl_2$ and pulp (pine wood cellulose and CTMP) were added. The blue agar allows detection of the light coloured calcium oxalate deposits surrounding colonies of oxalic acid producing bacteria. Colonies surrounded by light zones were thus searched for. Seventeen such isolates were subcultured (7 d at 45°C 160 rpm) in R2 broth amended with softwood pulp and sodium pyruvate. Concentrations of soluble oxalates were determined in the growth medium. Seven isolates produced oxalic acid 15 to 22 mg l^{-1} after the 7 d cultivation. None of the oxalic acid producing isolates was from the "pump deposit", suggesting that the putative oxalic acid was formed by the fungi which were seen in the micrographs (Figs. 22 and 23). Our attempts to subculture the oxalate producer(s) from this deposit failed.

These results show that petrified deposits from paper mill most likely contained calcium oxalate. Other possible constituents, insoluble in dilute acids, such as calcium phosphate, calsium sulphate and polythionates were excluded bacause these should have given a phosphorus or sulphur signal in EDS, respectively. In EDS only carbon, calcium and oxygen signals were seen for the "pump deposit" and additionally silicon (Si) for the "pulper deposit", indicating the presence of silicates. The deposits also contained microbiota. The organisms may have been involved in the synthesis or in the utilization of the petrified substance (possibly calcium oxalate) and of the silicon containing crystal.

4.4 Do microbes penetrate ceramic coating materials?

We measured the penetration of bacteria into and through ceramic with materials prepared by our collaborating laboratory (Ceramic materials laboratory, Technical University of Tampere, TUT). The ceramic sheets of five different compositions, were silicon glued on coupons of sterilised stainless steel. These coupons were immersed in sterilized white water from a paper machine amended with yeast extract, starch, sodium pyruvate, sodium thiosulphate and calcium chloride, and inoculated with the oxalic acid producing *M. silvanus* strain ox-13 and the biofilm former *D. geothermalis* strain E50051. After 17 d of shaking at 45°C the ceramic sheets were removed carefully from the steel coupons and both the ceramic sheet and the steel coupon surfaces were fluorescently stained

and examined with epifluorescence microscope. The micrographs (Fig. 25) show that a thick biofilm had formed on the surface of ceramic coating (Fig. 25 A) and that a number of bacteria had penetrated the ceramic and adhered on the underlying steel surface (Fig. 25 B). The results obtained with different ceramic coatings were largely similar. The steel coupons were boiled in HCl and the oxalic acid concentrations were measured using an enzyme assay. No soluble oxalates were detected from the steel surfaces.

The results demonstrated that penetration of bacteria through the ceramic coating material does occur. Thus, such penetration could take place also on the ceramically coated steel rolls of the paper machine press section.

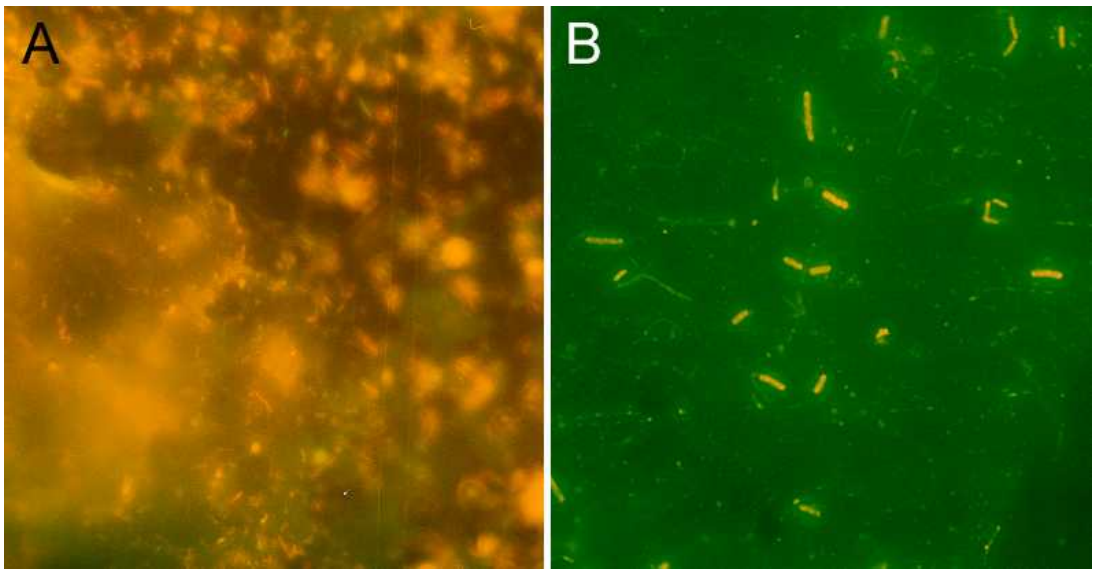

Fig. 25. Epifluorescence micrographs of the ceramic sheet and the steel surface after 17 d immersion in nutrient supplemented white water. The coupons were stained with acridine orange. Panel A, thick biofilm had formed on a ceramic sheet (75 % CrO_3, 25 % TiO_2); panel B, bacterial cells had penetrated the ceramic coating and adhered on the steel surface.

4.5 A novel bacterial taxon, *Deinobacterium chartae* gen. nov. sp. nov., isolated from a paper machine

We characterized and described the strain K4.1 isolated from a paper machine biofilm. It represented a novel genus, named by us *Deinobacterium,* and a novel species, *Deinobacterium chartae* (paper IV). The strain K4.1^T originated from a biofilm growing in the wire section of a Finnish paper mill producing folding boxboard. Temperature at the site of isolation was 45 – 50°C. 16S rRNA gene sequence analysis showed that the strain K4.1^T was closest to the type strains of *Deinococcus pimensis* (90.0 %) and *D. pereridilitoris* (89.6 %) and that the strain K4.1^T formed a separate branch inside the phylum *Deinococcus-Thermus,* vicinal to the genus *Deinococcus.* The maximum likelihood tree, constructed for

the 16S rRNA gene sequences of the strain K4.1^T and 50 validly described species of the phylum *Deinococcus-Thermus* is shown as a supplementary Fig. S4 in paper IV.

Figs. 26 to 27 and Table 7 display some features of this novel genus and its novel species. Fig. 26 A shows malachite green and Gram–stainings of the strain K4.1^T, showing that this strain produced no endospores. The strain K4.1^T stained Gram-negative, was a rod shaped bacterium and changing the length of its cells with growth temperature from 0.8-1 µm at 37°C to 1-2 µm at 45°C (Fig. 26 C and D) when grown on TSA plates. Paper IV showed that cells of the type strain K4.1^T of the novel species were longer when grown in a nutrient rich liquid broth (TSB) than on TSA plates. The cell length was from 3 µm to 8 µm at 45°C when grown for 1 d and filamentous (up to 40 µm) after 2 d (Supplementary Fig. S1 in paper IV and Fig. 26 E and F in this thesis).

Table 7. Characteristics of *Deinobacterium chartae* type strain K4.1^T

	Deinobacterium chartae
Phylum	*Deinococcus-Thermus*
Metabolism	Aerobic, chemoorganotrophic
Cell wall	The cell envelope had a less complex multilayered appearance than that of many *Deinococcus* species. The strain K4.1^T did not have capsule-like structure surrounding the cell like *D. geothermalis.*
Flagelli	0
Fimbriae	Not detected
G + C mol%	66.8
Cell morphology	Stains Gram-negative but has features of Gram-positive bacteria. Rod 0.8-1.3 µm × 1.4-2.5 µm, non-motile, nonsporing
Pigment	Pale pink colonies on R2A at 45°C
Peptidoglycan type	A3β (L-Orn - Gly – Gly)
Respiratory quinone	Menaquinone 8
Optimum T°C	37 to 45
Isolation site	Biofilm collected from a Finnish paper machine
Other	Radiation resistant
Reference	Paper IV

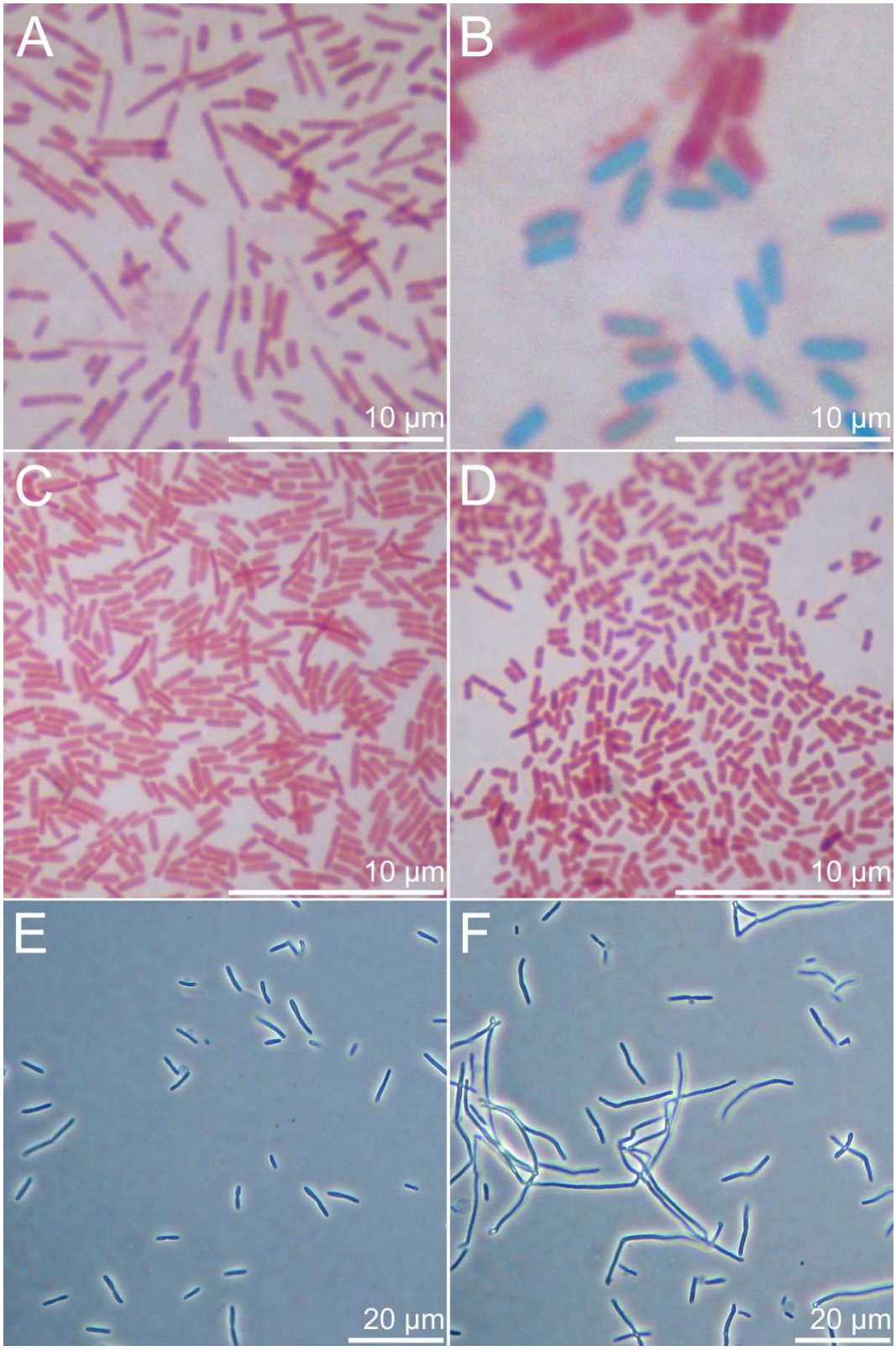

Fig. 26. Light microscopic images showing morphology of *Deinobacterium chartae* K4.1^T. Panels A and B show malachite green -stainings of *Deinobacterium chartae,* gen. nov, sp. nov. strain K4.1^T grown on tryptic soy agar (TSA) plates for 5 d (panel A) and reference spore former *Bacillus cereus* F4810/72 (panel B) grown similarly. The spores in *B. cereus* culture stained turquoise. No endospores were present in the culture of *Db. chartae* K1.4^T. Panel C, Gram-stained *Db. chartae* K4.1^T grown on TSA, 37°C for 1 d and Panel D, TSA at 45°C for 1 d. Panels E and F, phase-contrast images of cells grown in tryptic soy broth for 1 d and 2 d at +45 °C, respectively.

Cell surface ultrastructures of the strain K4.1^T were examined with scanning electron microscope (Fig. 1 in paper IV, Fig. 27 in thesis). Cell surface of *Db. chartae* K4.1^T, gen. nov. sp. nov. was smooth (Fig. 27), and no shrinkage due to sample preparation, characteristic to the appearance of the outer membrane of Gram-negative cells was seen. The transmission electron micrographs (Fig. 28) revealed a typical Gram-positive cell wall morphology, with no outer membrane (Fig. 1 C in paper IV, Fig. 28 in this thesis), even though the strain K4.1^T stained Gram-negative.

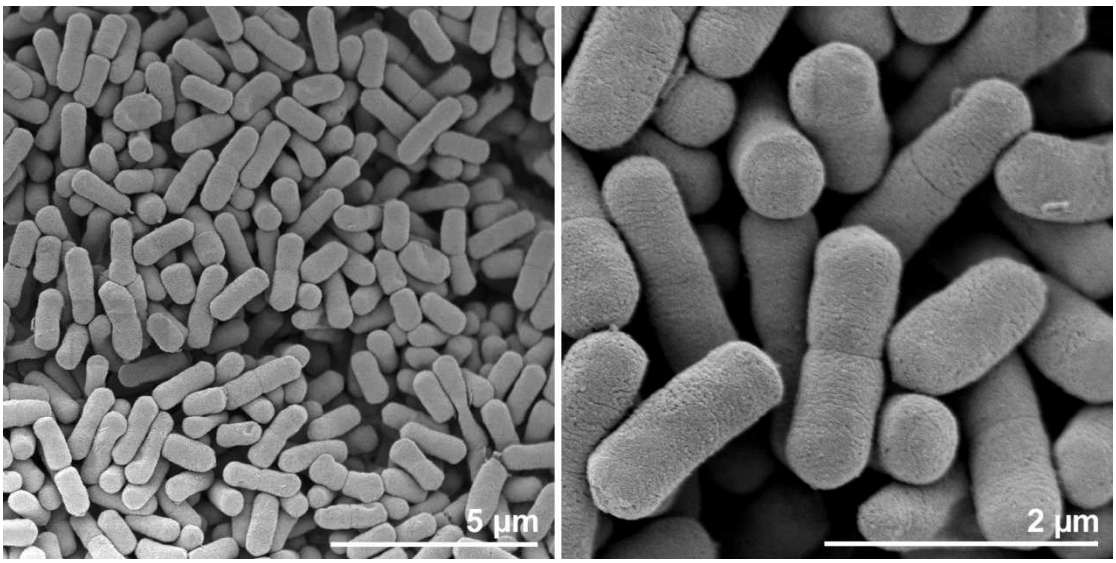

Fig. 27. Field emission scanning electron micrographs of the type strain K4.1^T of *Deinobacterium chartae,* gen. nov, sp. nov. The figure shows smooth ultrastructure of the cell surface. For FESEM *Db. chartae* was grown in R2-broth for 3 d at 37 °C.

According to TEM image (Fig. 1 C in paper IV) the cell envelope of *Db. chartae* K4.1^T was multilayered but less complex than that of *Deinococcus* species (Battista and Rainey, 2001, Ferreira *et al.*, 1997). *Db. chartae* K4.1^T cell envelope was very different from that of *D. geothermalis* E50051, also isolated from paper machine (Fig. 1 D in paper IV). The thinsection of the *Db. chartae* K4.1^T resembled that of *D. radiopugnans* (Battista and Rainey, 2001). Several kinds of inclusion bodies were detected in *Db. chartae* K4.1^T with TEM (Fig. S2 in paper IV, Fig. 28 in thesis). Part of the inclusion bodies fluoresced yellow when stained with DAPI, indicating polyphosphate (Tijssen *et al.*, 1982). According to a review by Shively (1974) polyphosphate granules (volutine) are widely distributed in prokaryotes and occur at a fairly constant number during the exponential phase of growth and decrease in number under limiting phosphate conditions. Polyphosphates are known to function as energy storage or as a phosphate reserve (Shively, 1974). The *D. radiodurans* strains SARK and R1 are also known to contain round inclusion bodies (Battista and Rainey, 2001).

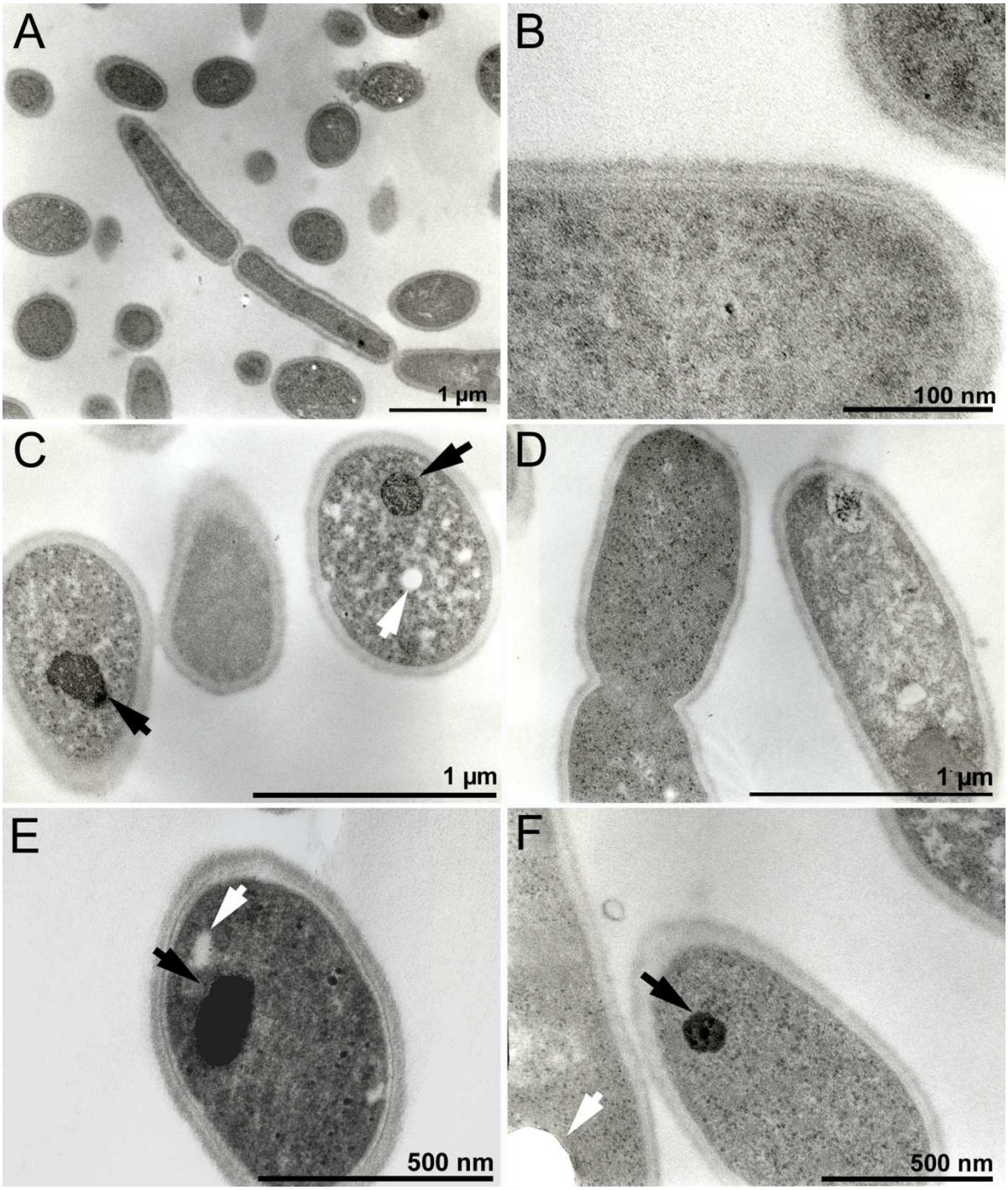

Fig. 28. Transmission electron micrographs of thin sections of *Deinobacterium chartae,* gen. nov, sp. nov. strain K4.1^T. Panel A shows cell size and morphology, Panel B the cell wall structure, Panels C to F show inclusion bodies, presumably polyphosphate granules (black arrow heads) and lipid granules (white arrow heads). For ultra thin sections the strain K4.1T was grown on TSA for 3 d at 37°C or 45°C.

The characteristics of *Db. chartae,* gen. nov, sp. nov. strain K4.1^T, *i.e.* growth at 45°C, utilisation of starch, ability to form biofilms and plausible resistance to desiccation (connected to radiation resistance, Supplementary Fig. S3 in paper IV), show that this bacterium is adapted for conditions prevailing at paper machines.

5 Conclusions

1. Electron microscopic analysis of adhered and not adhered cells of *Staphylococcus epidermidis, Meiothermus silvanus, Pseudoxanthomonas taiwanensis* and *Deinococcus geothermalis* showed that these strains adhered on abiotic surfaces by using adhesion threads.

2. We found remarkable differences in the ultrastructural details of adhered cells of the *Staphylococcus epidermidis, Meiothermus silvanus, Pseudoxanthomonas taiwanensis* and *Deinococcus geothermalis* under same conditions on different abiotic surface materials. These four bacterial species sensed the quality of the abiotic surface and responded accordingly.

3. Results in this thesis showed that biofilms of *D. geothermalis* on titanium dioxide coated coupons of steel or glass were destroyed (1 to 2 log units) by photocatalysis generated by titanium dioxide irradiated at 360 nm. The reduction in biomass was not detected on non-coated steel or glass. We showed that photocatalysis is a powerful technique for biofilm removal and applicable in water industries as well as for processes and facilities where high level of hygiene is required.

4. This study showed that coating reduced biofouling of steel. Moreover, it showed great differences between the preferences of different bacterial species to adhere the various surfaces. In contrast to most biofouling studies, usually executed with a single strain of a single species, we used four biofilm forming species of widely distant phylogenetic origins. We found that fluoropolymer and diamond like carbon coatings repelled all four biofilm formers, *Staphylococcus epidermidis, Meiothermus silvanus, Pseudoxanthomonas taiwanensis* and *Deinococcus geothermalis* on steel.

5. We found new criteria to predict bacterial repellence by abiotic surfaces. The new parameters skewness (S_{sk}) and kurtosis (S_{ku}), measured with AFM, were found important in predicting attractiveness of surfaces for bacteria forming biofilms. Surfaces with skewness values mildly positive, and kurtosis values around 7, were less prone to biofouling than surfaces with negative skewness value and kurtosis value around 4. Hydrophobicity/hydrophilicity, expressed by the water contact angle (θ), is traditionally believed to be an important parameter to predict biofouling. Our results indicated that

impact of this parameter for adherence greatly differed between bacterial species. *D. geothermalis* and *M. silvanus* preferred a hydrophilic surface ($\theta < 90°$) for adhesion whereas *Psx. taiwanensis* and *Staph. epidermidis* were more attracted to hydrophobic surfaces ($\theta > 90°$).

6. This is the first study describing the factors determining adherence and formation of biofilm for the bacterial species shown as essential biofoulers of paper machines. For the first time the conditions determining adherence by species of *Meiothermus* and of *Pseudoxanthomonas* were described.

7. We developed a method for quantifying biofilm by means of optical reading of the emitted fluorescence. This method can be applied for *in situ* quantification of biofilms directly on a surface.

8. It was shown that starter cultures of *Lactobacillus plantarum* and of *Wickerhamomyces anomalus* entered the barley kernel and colonised the kernel tissues in concerted action when simultaneously dispensed in steeping water during the malting process. Use of these starter cultures decreased the formation of detrimental extracellular polymeric substances (EPS) during the steeping and positively influenced the mash properties.

9. The family of biofilm formers in paper machines was expanded by a genus novel to science, *Deinobacterium*. Its species *Db. chartae* is validly described in this thesis.

10. It was shown in this thesis that even petrified deposits from paper mills contained microbiota, in addition to calcium oxalate and silicates.

11. It was demonstrated that bacterial penetration through a ceramic coating materials does occur under conditions (45°C, oligotrophic medium) prevailing at the paper machine wet end. Penetration thus could take place also on the ceramically coated steel rolls of the paper machine press section.

6 Acknowledgements

This thesis work was carried out in the Department of Food and Environmental Sciences, Division of Microbiology, University of Helsinki. The work was funded as a part of the technology programme Clean Surfaces 2002-2006 (PINTA) and applied research project "Surface modifications and active processes for cleaner paper machine" (PIMU), both supported by the Finnish Funding Agency for Technology and Innovation (Tekes). The support by the Academy of Finland Center of Excellence grants Nr. 53305 (Microbial Resources) and 118637 (Photobiomics) and the Finnish industrial partners are gratefully acknowledged.

I want to express my gratitude to my supervisor prof. Mirja Salkinoja-Salonen. I thank you for sharing your knowledge and valuable time for the benefit of my thesis. Your enthusiasm for science is admirable.

I wish to acknowledge the reviewers Doc. Marja Tiirola and Doc. Leif Kronberg for their valuable comments on my thesis.

I thank Dr. Arja Laitila for introducing the fascinating microbial life on barley kernels to me. I thank her also for the constructive comments on the thesis manuscript. They improved the thesis considerably.

The papers in my thesis have been a result from a co-operation of several partners in science and in industry. I own my gratitude to the co-authors for sharing their data and expertise: Jaakko Ekman, Dr. Hans-Jürgen Busse, Dr. David Fewer, Dr. Viljami Pore, Dr. Sami Areva, Prof. Mikko Ritala, Prof. Markku Leskelä, Doc. Mika Lindén, Prof. Jarl B. Rosenholm, Doc. Kari Lounatmaa, Dr. Annika Wilhelmson, Dr. Arja Laitila, Dr. Mikael Järn, Juhana Ahola, Prof. Jouko Peltonen, Sanna Tervakangas, Jukka Kolehmainen, Timo Ruokolainen and Pekka Narko.

I thank the stuff of the Division of Microbiology for kind assictance during my PhD work. I wish to thank Helsinki University Viikki Science Library, Faculty of Agriculture and Forestry Instrument Centre and Electron Microscopy Unit. Leena Steininger, Hannele Tukiainen and Tuula Suortti are acknowledged for many kinds of help.

I thank all of my present and past colleagues in the MSS group for the extraordinary group spirit. Especially: Camelia, Douwe, Elina×2, Hanna×2, Irina, Jaakko×2, Juhana, Pikku-Mari, Maria, Marko, Minna, Mirva, Raimo, Ranad, Stiina and Teemu, just to mention some names. The coffee breaks and Friday cakes have made my PhD work so much more fun and bar evenings, barbecue parties, sports events and karonkkas have done the same thing to my life.

I thank my friends and family for all the support and good things not related to the science at all. Thanks mum, dad, Suski, Johanna, Erkka, Aku, Anja, Matti, Arttu and Heta. Thank you my team mates: Anis, Heidi, Inka, Linda, Senni & Tessa, for all the great get-togethers!

Special thanks go to my dear Antti, personal chef and bartender, thank you for making me laugh ♥ and Riimi and Santtu for taking me out for a walk every day,

7 References

Allesen-Holm, M., Barken, K. B., Yang, L., Klausen, M., Webb, J. S., Kjelleberg, S., Molin, S., Givskov, M. and Tolker-Nielsen, T. 2006. A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms. Mol. Microbiol. 59, 1114-1128.

Allion, A., Baron, J. P. and Boulange-Petermann, L. 2006. Impact of surface energy and roughness on cell distribution and viability. Biofouling 22, 269-278.

Allison, D. G. 2003. The biofilm matrix. Review. Biofouling 19, 139-150.

Amézaga-Madrid, P., Nevárez-Moorillón, G. V., Orrantia-Borunda, E. and Miki-Yoshida, M. 2002. Photoinduced bactericidal activity against *Pseudomonas aeruginosa* by TiO_2 based thin films. FEMS Microbiol. Lett. 211, 183-188.

An, Y. H. and Friedman, R. J. 1998. Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. Review. J. Biomed. Mater. Res. 43, 338-348.

Anderson, G. G. and O'Toole, G. A. 2008. Innate and induced resistance mechanisms of bacterial biofilms. Review. Curr. Top. Microbiol. Immunol. 322, 85-105.

Apetroaie-Constantin, C., Mikkola, R., Andersson, M. A., Teplova, V., Suominen, I., Johansson, T. and Salkinoja-Salonen, M. 2009. *Bacillus subtilis* and *B. mojavensis* strains connected to food poisoning produce the heat stable toxin amylosin. J. Appl. Microbiol. 106, 1976-1985.

Arnold, J. W. and Bailey, G. W. 2000. Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: scanning electron and atomic force microscopy study. Poult. Sci. 79, 1839-1845.

Austin, J. W., Sanders, G., Kay, W. W. and Collinson, S. K. 1998. Thin aggregative fimbriae enhance *Salmonella enteritidis* biofilm formation. FEMS Microbiol. Lett. 162, 295-301.

Aykent, F., Yondem, I., Ozyesil, A. G., Gunal, S. K., Avunduk, M. C. and Ozkan, S. 2010. Effect of different finishing techniques for restorative materials on surface roughness and bacterial adhesion. J. Prosthet. Dent. 103, 221-227.

Barken, K. B., Pamp, S., Yang, L., Gjermansen, M., Bertrand, J. J., Klausen, M., Givskov, M., Whitchurch, C. B., Engel, J. N. and Tolker-Nielsen, T. 2008. Roles of type IV pili, flagellum-mediated motility and extracellular DNA in the formation of mature multicellular structures in *Pseudomonas aeruginosa* biofilms. Environ. Microbiol. 10, 2331-2343.

Barthlott, W. and Neinhuis, C. 1997. Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta 202, 1-8.

Battin, T. J., Kaplan, L. A., Newbold, J. D., Cheng, X. and Hansen, C. 2003. Effects of current velocity on the nascent architecture of stream microbial biofilms. Appl. Environ. Microbiol. 69, 5443-5452.

Battista, J. R. and Rainey, F. A. 2001. Genus *I. Deinococcus* Brooks and Murray 1981. In: Garrity, G. M., Staley, J. T. and Boone, D. R. (Eds.), Bergey's Manual of Systematic Bacteriology, The *Archaea* and the Deeply Branching and Phototrophic Bacteria. Springer, New York, USA, pp. 396-403.

Baveja, J. K., Willcox, M. D., Hume, E. B., Kumar, N., Odell, R. and Poole-Warren, L. A. 2004. Furanones as potential anti-bacterial coatings on biomaterials. Biomaterials 25, 5003-5012.

Bayles, K. W. 2007. The biological role of death and lysis in biofilm development. Nat. Rev. Microbiol. 5, 721-726.

Belas, M. R. and Colwell, R. R. 1982. Scanning electron microscope observation of the swarming phenomenon of *Vibrio parahaemolyticus*. J. Bacteriol. 150, 956-959.

Benjamin, A. M. and Tamhane, D. V. 1966. Observations on the formation of a pigment by strains of *Escherichia coli*. Archiv fur Mikrobiologie 53, 242-247.

Berry, D., Xi, C. and Raskin, L. 2006. Microbial ecology of drinking water distribution systems. Review. Curr. Opin. Biotechnol. 17, 297-302.

Beyenal, H., Lewandowski, Z. and Harkin, G. 2004. Quantifying biofilm structure: facts and fiction. Biofouling 20, 1-23.

Bieber, D., Ramer, S. W., Wu, C. Y., Murray, W. J., Tobe, T., Fernandez, R. and Schoolnik, G. K. 1998. Type IV pili, transient bacterial aggregates, and virulence of enteropathogenic *Escherichia coli*. Science 280, 2114-2118.

Blanco, M. A., Negro, C., Gaspar, I. and Tijero, J. 1996. Slime problems in the paper and board industry. Review. Appl Microbiol Biot 46, 203-208.

Boekhorst, J., Wels, M., Kleerebezem, M. and Siezen, R. J. 2006. The predicted secretome of *Lactobacillus plantarum* WCFS1 sheds light on interactions with its environment. Microbiology 152, 3175-3183.

Borenstein, S. W. 1994. Microbiologically influenced corrosion handbook. Industrial Press Inc., New York, USA.

Brooijmans, R. J., de Vos, W. M. and Hugenholtz, J. 2009. *Lactobacillus plantarum* WCFS1 electron transport chains. Appl. Environ. Microbiol. 75, 3580-3585.

Cao, B., Nagarajan, K. and Loh, K. C. 2009. Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches. Review. Appl. Microbiol. Biotechnol. 85, 207-228.

Casutt, M. S., Huber, T., Brunisholz, R., Tao, M., Fritz, G. and Steuber, J. 2010. Localization and Function of the Membrane-bound Riboflavin in the Na+-translocating NADH:Quinone Oxidoreductase (Na^+-NQR) from *Vibrio cholerae*. J. Biol. Chem. 285, 27088-27099.

Cerca, N., Pier, G. B., Vilanova, M., Oliveira, R. and Azeredo, J. 2005. Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of *Staphylococcus epidermidis*. Res. Microbiol. 156, 506-514.

Charman, K. M., Fernandez, P., Loewy, Z. and Middleton, A. M. 2009. Attachment of *Streptococcus oralis* on acrylic substrates of varying roughness. Lett. Appl. Microbiol. 48, 472-477.

Chen, M. Y., Tsay, S. S., Chen, K. Y., Shi, Y. C., Lin, Y. T. and Lin, G. H. 2002. *Pseudoxanthomonas taiwanensis* sp. nov., a novel thermophilic, N_2O-producing species isolated from hot springs. Int. J. Syst. Evol. Microbiol. 52, 2155-2161.

Chen, T. L., Chou, Y. J., Chen, W. M., Arun, B. and Young, C. C. 2006. *Tepidimonas taiwanensis* sp. nov., a novel alkaline-protease-producing bacterium isolated from a hot spring. Extremophiles 10, 35-40.

Chen, W., Liu, Y., Courtney, H. S., Bettenga, M., Agrawal, C. M., Bumgardner, J. D. and Ong, J. L. 2006. In vitro anti-bacterial and biological properties of magnetron co-sputtered silver-containing hydroxyapatite coating. Biomaterials 27, 5512-5517.

Chiang, W. C., Schroll, C., Hilbert, L. R., Moller, P. and Tolker-Nielsen, T. 2009. Silver-palladium surfaces inhibit biofilm formation. Appl. Environ. Microbiol. 75, 1674-1678.

Cho, M., Chung, H., Choi, W. and Yoon, J. 2005. Different inactivation behaviors of MS-2 phage and Escherichia coli in TiO_2 photocatalytic disinfection. Appl. Environ. Microbiol. 71, 270-275.

Chua, P. H., Neoh, K. G., Kang, E. T. and Wang, W. 2008. Surface functionalization of titanium with hyaluronic acid/chitosan polyelectrolyte multilayers and RGD for promoting osteoblast functions and inhibiting bacterial adhesion. Biomaterials 29, 1412-1421.

Chuang, H. F., Smith, R. C. and Hammond, P. T. 2008. Polyelectrolyte multilayers for tunable release of antibiotics. Biomacromolecules 9, 1660-1668.

Claus, D. and Berkely, R. C. W. 1986. Genus *Bacillus* Cohn 1872. In: Holt, J. G., Sneath, P. H. A., Mair, N. S. and Sharpe, M. E. (Eds.), Bergey's Manual of Systematic Bacteriology. Springer, New York, USA, pp. 1105-1139.

Coetser, S. E. and Cloete, T. E. 2005. Biofouling and Biocorrosion in Industrial Water Systems. Crit. Rev. Microbiol. 31, 213-232.

Cooper, T. F., Rozen, D. E. and Lenski, R. E. 2003. Parallel changes in gene expression after 20,000 generations of evolution in *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A. 100, 1072-1077.

Costerton, J. W., Irvin, R. T. and Cheng, K. J. 1981. The bacterial glycocalyx in nature and disease. Review. Annu. Rev. Microbiol. 35, 299-324.

Costerton, J. W., Cheng, K. J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M. and Marrie, T. J. 1987. Bacterial biofilms in nature and disease. Review. Annu. Rev. Microbiol. 41, 435-464.

Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R. and Lappin-Scott, H. 1995. Microbial Biofilms. Review. Annu. Rev. Microbiol. 49, 711-745.

Costerton, J. W., Stewart, P. S. and Greenberg, E. P. 1999. Bacterial biofilms: a common cause of persistent infections. Review. Science 284, 1318-1322.

Costerton, J. W., Montanaro, L. and Arciola, C. R. 2005. Biofilm in implant infections: its production and regulation. Review. Int. J. Artif. Organs 28, 1062-1068.

Costerton, J. W. 2007. The Biofilm Primer. Springer-Verlag, Berlin.

Cucarella, C., Solano, C., Valle, J., Amorena, B., Lasa, I. and Penades, J. R. 2001. Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. J. Bacteriol. 183, 2888-2896.

Danese, P. N., Pratt, L. A. and Kolter, R. 2000. Exopolysaccharide production is required for development of *Escherichia coli* K-12 biofilm architecture. J. Bacteriol. 182, 3593-3596.

Danhorn, T. and Fuqua, C. 2007. Biofilm formation by plant-associated bacteria. Review. Annu. Rev. Microbiol. 61, 401-422.

de Oliveira-Garcia, D., Dall'Agnol, M., Rosales, M., Azzuz, A. C., Martinez, M. B. and Giron, J. A. 2002. Characterization of flagella produced by clinical strains of Stenotrophomonas maltophilia. Emerg. Infect. Dis. 8, 918-923.

de Oliveira-Garcia, D., Dall'Agnol, M., Rosales, M., Azzuz, A. C., Alcantara, N., Martinez, M. B. and Giron, J. A. 2003. Fimbriae and adherence of *Stenotrophomonas maltophilia* to epithelial cells and to abiotic surfaces. Cell. Microbiol. 5, 625-636.

De Weger, L. A., van der Vlugt, C. I., Wijfjes, A. H., Bakker, P. A., Schippers, B. and Lugtenberg, B. 1987. Flagella of a plant-growth-stimulating *Pseudomonas fluorescens* strain are required for colonization of potato roots. J. Bacteriol. 169, 2769-2773.

Denner, E. B., Kolari, M., Hoornstra, D., Tsitko, I., Kampfer, P., Busse, H. J. and Salkinoja-Salonen, M. 2006. *Rubellimicrobium thermophilum* gen. nov., sp. nov., a red-pigmented, moderately thermophilic bacterium isolated from coloured slime deposits in paper machines. Int. J. Syst. Evol. Microbiol. 56, 1355-1362.

Desjardins, E. and Beaulieu, C. 2003. Identification of bacteria contaminating pulp and a paper machine in a Canadian paper mill. J. Ind. Microbiol. Biotechnol. 30, 141-145.

Di Martino, P., Cafferini, N., Joly, B. and Darfeuille-Michaud, A. 2003. *Klebsiella pneumoniae* type 3 pili facilitate adherence and biofilm formation on abiotic surfaces. Res. Microbiol. 154, 9-16.

Dobretsov, S., Teplitski, M. and Paul, V. 2009. Mini-review: quorum sensing in the marine environment and its relationship to biofouling. Biofouling 25, 413-427.

Doran, P. J. and Briggs, D. E. 1993. Microbes and grain germination. J. Inst. Brew. 99, 165-170.

Doyle, R. J., Nedjat-Haiem, F. and Singh, J. S. 1984. Hydrophobic characteristics of *Bacillus* spores. Curr. microbiol. 10, 329-332.

Dunne, W. M.,Jr. 2002. Bacterial adhesion: seen any good biofilms lately? Review. Clin. Microbiol. Rev. 15, 155-166.

Ekman, J., Kosonen, M., Jokela, S., Kolari, M., Korhonen, P. and Salkinoja-Salonen, M. 2007. Detection and quantitation of colored deposit-forming *Meiothermus* spp. in paper industry processes and end products. J. Ind. Microbiol. Biotechnol. 34, 203-211.

Faille, C., Lequette, Y., Ronse, A., Slomianny, C., Garénaux, E. and Guerardel, Y. 2010. Morphology and physico-chemical properties of Bacillus spores surrounded or not with an exosporium: Consequences on their ability to adhere to stainless steel. Int. J. Food Microbiol. 143, 125-135

Falkinham, J. O.,3rd. 1996. Epidemiology of infection by nontuberculous mycobacteria. Review. Clin. Microbiol. Rev. 9, 177-215.

Falkinham, J. O.,3rd, Norton, C. D. and LeChevallier, M. W. 2001. Factors influencing numbers of *Mycobacterium avium*, *Mycobacterium intracellulare*, and other Mycobacteria in drinking water distribution systems. Appl. Environ. Microbiol. 67, 1225-1231.

Farmer III, J. J., Janda, M. J., Brenner, F. W., Cameron, D. N. and Birkhead, K. M. 2005. Genus I *Vibrio* Pacini 1854. In: Garrity, G. M., Brenner, D. J., Krieg, N. R. and Staley, J. T. (Eds.), Bergey's Manual of Systematic Bacteriology, *The proteobacteria*, Part B *The Gammaproteobacteria*. Springer, New York, USA, pp. 494-546.

Ferreira, A. C., Nobre, M. F., Rainey, F. A., Silva, M. T., Wait, R., Burghardt, J., Chung, A. P. and da Costa, M. S. 1997. *Deinococcus geothermalis* sp. nov. and *Deinococcus murrayi* sp. nov., two extremely radiation-resistant and slightly thermophilic species from hot springs. Int. J. Syst. Bacteriol. 47, 939-947.

Finkmann, W., Altendorf, K., Stackebrandt, E. and Lipski, A. 2000. Characterization of N_2O-producing *Xanthomonas*-like isolates from biofilters as *Stenotrophomonas nitritireducens* sp. nov., *Luteimonas mephitis* gen. nov., sp. nov. and *Pseudoxanthomonas broegbernensis* gen. nov., sp. nov. Int. J. Syst. Evol. Microbiol. 50 Pt 1, 273-282.

Flemming, H. C. 2002. Biofouling in water systems-cases, causes and countermeasures. Review. Appl. Microbiol. Biotechnol. 59, 629-640.

Flemming, H. C., Neu, T. R. and Wozniak, D. J. 2007. The EPS matrix: the "house of biofilm cells". Review. J. Bacteriol. 189, 7945-7947.

Flemming, H. C. and Wingender, J. 2010. The biofilm matrix. Review. Nat. Rev. Microbiol. 8, 623-633.

Fletcher, M. and Loeb, G. I. 1979. Influence of Substratum Characteristics on the Attachment of a Marine Pseudomonad to Solid Surfaces. Appl. Environ. Microbiol. 37, 67-72.

Fujishima, A., Rao, T. N. and Tryk, D. A. 2000. Titanium dioxide photocatalysis. J Photochem. Photobio. C. 1, 1-21.

Fux, C. A., Shirtliff, M., Stoodley, P. and Costerton, J. W. 2005. Can laboratory reference strains mirror 'real-world' pathogenesis? Trends Microbiol. 13, 58-63.

Gamble, R. and Muriana, P. M. 2007. Microplate fluorescence assay for measurement of the ability of strains of *Listeria monocytogenes* from meat and meat-processing plants to adhere to abiotic surfaces. Appl. Environ. Microbiol. 73, 5235-5244.

Geesey, G. G., Richardson, W. T., Yeomans, H. G., Irvin, R. T. and Costerton, J. W. 1977. Microscopic examination of natural sessile bacterial populations from an alpine stream. Can. J. Microbiol. 23, 1733-1736.

Geesey, G. G., Mutch, R., Costerton, J. W. and Green, R. B. 1978. Sessile Bacteria: An Important Component of the Microbial Population in Small Mountain Streams. Limnol. Oceanogr. 23, 1214-1223.

Ghigo, J. M. 2001. Natural conjugative plasmids induce bacterial biofilm development. Nature 412, 442-445.

Giaouris, E. D. and Nychas, G. E. 2006. The adherence of *Salmonella* Enteritidis PT4 to stainless steel: The importance of the air–liquid interface and nutrient availability. Food Microbiol. 23, 747-752.

Gogniat, G., Thyssen, M., Denis, M., Pulgarin, C. and Dukan, S. 2006. The bactericidal effect of TiO_2 photocatalysis involves adsorption onto catalyst and the loss of membrane integrity. FEMS Microbiol. Lett. 258, 18-24.

Gohl, O., Friedrich, A., Hoppert, M. and Averhoff, B. 2006. The thin pili of *Acinetobacter* sp. strain BD413 mediate adhesion to biotic and abiotic surfaces. Appl. Environ. Microbiol. 72, 1394-1401.

Goller, C. C. and Romeo, T. 2008. Environmental influences on biofilm development. Review. Curr. Top. Microbiol. Immunol. 322, 37-66.

Gorby, Y. A., Yanina, S., McLean, J. S., Rosso, K. M., Moyles, D., Dohnalkova, A., Beveridge, T. J., Chang, I. S., Kim, B. H., Kim, K. S., Culley, D. E., Reed, S. B., Romine, M. F., Saffarini, D. A., Hill, E. A., Shi, L., Elias, D. A., Kennedy, D. W., Pinchuk, G., Watanabe, K., Ishii, S., Logan, B., Nealson, K. H. and Fredrickson, J. K. 2006. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. Proc. Natl. Acad. Sci. U. S. A. 103, 11358-11363.

Gottenbos, B., Grijpma, D. W., van der Mei, H. C., Feijen, J. and Busscher, H. J. 2001. Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria. J. Antimicrob. Chemother. 48, 7-13.

Gross, M., Cramton, S. E., Götz, F. and Peschel, A. 2001. Key role of teichoic acid net charge in *Staphylococcus aureus* colonization of artificial surfaces. Infect. Immun. 69, 3423-3426.

Guilbaud, M., de Coppet, P., Bourion, F., Rachman, C., Prevost, H. and Dousset, X. 2005. Quantitative detection of *Listeria monocytogenes* in biofilms by real-time PCR. Appl. Environ. Microbiol. 71, 2190-2194.

Götz, F. 2002. *Staphylococcus* and biofilms. Review. Mol. Microbiol. 43, 1367-1378.

Haapala, A., Liimatainen, H., Körkkö, M., Ekman, J., Salkinoja-Salonen, M. and Niinimäki, J. 2010. Web defects in newsprint - a mill case study. Appita Journal 63, 358-363.

Hall-Stoodley, L., Costerton, J. W. and Stoodley, P. 2004. Bacterial biofilms: from the natural environment to infectious diseases. Review. Nat. Rev. Microbiol. 2, 95-108.

Harmsen, M., Lappann, M., Knochel, S. and Molin, S. 2010. Role of extracellular DNA during biofilm formation by *Listeria monocytogenes*. Appl. Environ. Microbiol. 76, 2271-2279.

Harris, L. G., Meredith, D. O., Eschbach, L. and Richards, R. G. 2007. *Staphylococcus aureus* adhesion to standard micro-rough and electropolished implant materials. J. Mater. Sci. Mater. Med. 18, 1151-1156.

Hartmann, I., Carranza, P., Lehner, A., Stephan, R., Eberl, L. and Riedel, K. 2010. Genes involved in *Cronobacter sakazakii* biofilm formation. Appl. Environ. Microbiol. 76, 2251-2261.

Heilmann, H-D. 1974. On the Peptidoglycan of the Cell Walls of *Pseudomonas aeruginosa*. European Journal of Biochemistry 43, 35-38.

Heilmann, C., Gerke, C., Perdreau-Remington, F. and Götz, F. 1996. Characterization of Tn917 insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. Infect. Immun. 64, 277-282.

Henrici, A. T. 1933. Studies of Freshwater Bacteria: I. A Direct Microscopic Technique. J. Bacteriol. 25, 277-287.

Hentzer, M., Teitzel, G. M., Balzer, G. J., Heydorn, A., Molin, S., Givskov, M. and Parsek, M. R. 2001. Alginate overproduction affects *Pseudomonas aeruginosa* biofilm structure and function. J. Bacteriol. 183, 5395-5401.

Hentzer, M., Riedel, K., Rasmussen, T. B., Heydorn, A., Andersen, J. B., Parsek, M. R., Rice, S. A., Eberl, L., Molin, S., Hoiby, N., Kjelleberg, S. and Givskov, M. 2002. Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. Microbiology 148, 87-102.

Hentzer, M., Wu, H., Andersen, J. B., Riedel, K., Rasmussen, T. B., Bagge, N., Kumar, N., Schembri, M. A., Song, Z., Kristoffersen, P., Manefield, M., Costerton, J. W., Molin, S., Eberl, L., Steinberg, P., Kjelleberg, S., Hoiby, N. and Givskov, M. 2003. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J. 22, 3803-3815.

Houry, A., Briandet, R., Aymerich, S. and Gohar, M. 2010. Involvement of motility and flagella in *Bacillus cereus* biofilm formation. Microbiology 156, 1009-1018.

Huang, Z., Maness, P., Blake, D. M., Wolfrum, E. J., Smolinski, S. L. and Jacoby, W. A. 2000. Bactericidal mode of titanium dioxide photocatalysis. J. Photoch. Photobio. A. 130, 163-170.

Huber, B., Riedel, K., Kothe, M., Givskov, M., Molin, S. and Eberl, L. 2002. Genetic analysis of functions involved in the late stages of biofilm development in *Burkholderia cepacia* H111. Mol. Microbiol. 46, 411-426.

Hugh, R. and Leifson, E. 1964. The proposed Neotype Strains of *Pseudomonas Aeruginosa* (Schroeter 1872) Migula 1900. Int. Bull. Bacteriol. Nomencl. Taxon 14, 69-84.

Hume, E. B., Baveja, J., Muir, B., Schubert, T. L., Kumar, N., Kjelleberg, S., Griesser, H. J., Thissen, H., Read, R., Poole-Warren, L. A., Schindhelm, K. and Willcox, M. D. 2004. The control of *Staphylococcus epidermidis* biofilm formation and in vivo infection rates by covalently bound furanones. Biomaterials 25, 5023-5030.

Ireland, J. C., Klostermann, P., Rice, E. W. and Clark, R. M. 1993. Inactivation of *Escherichia coli* by titanium dioxide photocatalytic oxidation. Appl. Environ. Microbiol. 59, 1668-1670.

Irie, Y. and Parsek, M. R. 2008. Quorum sensing and microbial biofilms. Review. Curr. Top. Microbiol. Immunol. 322, 67-84.

Ishii, S., Koki, J., Unno, H. and Hori, K. 2004. Two morphological types of cell appendages on a strongly adhesive bacterium, *Acinetobacter* sp. strain Tol 5. Appl. Environ. Microbiol. 70, 5026-5029.

Ishii, S., Miyata, S., Hotta, Y., Yamamoto, K., Unno, H. and Hori, K. 2008. Formation of filamentous appendages by *Acinetobacter* sp. Tol 5 for adhering to solid surfaces. J. Biosci. Bioeng. 105, 20-25.

Ivanova, E. P., Truong, V. K., Wang, J. Y., Berndt, C. C., Jones, R. T., Yusuf, I. I., Peake, I., Schmidt, H. W., Fluke, C., Barnes, D. and Crawford, R. J. 2010. Impact of nanoscale roughness of titanium thin film surfaces on bacterial retention. Langmuir 26, 1973-1982.

Izano, E. A., Amarante, M. A., Kher, W. B. and Kaplan, J. B. 2008. Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. Appl. Environ. Microbiol. 74, 470-476.

Jacoby, W. A., Maness, P. C., Wolfrum, E. J., Blake, D. M. and Fennell, J. A. 1998. Mineralization of Bacterial Cell Mass on a Photocatalytic Surface in Air. Environ. Sci. Technol. 32, 2650-2653.

Jaglic, Z., Michu, E., Holasova, M., Vlkova, H., Babak, V., Kolar, M., Bardon, J. and Schlegelova, J. 2010. Epidemiology and characterization of *Staphylococcus epidermidis* isolates from humans, raw bovine milk and a dairy plant. Epidemiol. Infect. 138, 772-782.

Jeon, H., Yi, S. and Oh, S. 2003. Preparation and antibacterial effects of Ag–SiO_2 thin films by sol–gel method. Biomaterials 24, 4921-4928.

Josset, S., Keller, N., Lett, M. C., Ledoux, M. J. and Keller, V. 2008. Numeration methods for targeting photoactive materials in the UV-A photocatalytic removal of microorganisms. Chem. Soc. Rev. 37, 744-755.

Jurcisek, J. A. and Bakaletz, L. O. 2007. Biofilms formed by nontypeable *Haemophilus influenzae* in vivo contain both double-stranded DNA and type IV pilin protein. J. Bacteriol. 189, 3868-3875.

Kalyon, B. D. and Olgun, U. 2001. Antibacterial efficacy of triclosan-incorporated polymers. Am. J. Infect. Control 29, 124-125.

Kandler, O. and Weiss, N. 1986. Genus *Lactobacillus* Beijerinck 1901. In: Holt, J. G., Sneath, P. H. A., Mair, N. S. and Sharpe, M. E. (Eds.), Bergey's Manual of Systematic Bacteriology. Springer, New York, USA, pp. 1209-1234.

Kang, Y., Liu, H., Genin, S., Schell, M. A. and Denny, T. P. 2002. *Ralstonia solanacearum* requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. Mol. Microbiol. 46, 427-437.

Kanto Öqvist, C. 2008. Microbial life and deposits in paper machine circuits. Dissertationes Biocentri Viikki Universitatis Helsingiensis 20/2008, PhD Thesis.

Kathariou, S. 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J. Food Prot. 65, 1811-1829.

Katsikogianni, M., Spiliopoulou, I., Dowling, D. P. and Missirlis, Y. F. 2006. Adhesion of slime producing *Staphylococcus epidermidis* strains to PVC and diamond-like carbon/silver/fluorinated coatings. J. Mater. Sci. Mater. Med. 17, 679-689.

Kelly, L. and Briggs, D. 1992. The influence of the grain microflora on the germinative physiology of barley. J. Inst. Brew. 98, 395-400.

Keskinen, H., Mäkelä, J. M., Aromaa, M., Keskinen, J., Areva, S., Teixeira, C. V., Rosenholm, J. B., Pore, V., Ritala, M., Leskelä, M., Raulio, M., Salkinoja-Salonen, M., Levänen, E. and Mäntylä, T. 2006. Titania and titania-silver nanoparticle deposits made by Liquid Flame Spray and their functionality as photocatalyst for organic- and biofilm removal. Catalysis Letters 111, 127-132.

Kikuchi, Y., Sunada, K., Iyoda, T., Hashimoto, K. and Fujishima, A. 1997. Photocatalytic bactericidal effect of TiO_2 thin films: dynamic view of the active oxygen species responsible for the effect. J. Photochem. Photobiol. A. 106, 51-56.

Kim, S. H. and Wei, C. I. 2009. Molecular characterization of biofilm formation and attachment of *Salmonella enterica* serovar Typhimurium DT104 on food contact surfaces. J. Food Prot. 72, 1841-1847.

Kinnari, T. J., Esteban, J., Gomez-Barrena, E., Zamora, N., Fernandez-Roblas, R., Nieto, A., Doadrio, J. C., Lopez-Noriega, A., Ruiz-Hernandez, E., Arcos, D. and Vallet-Regi, M. 2009. Bacterial adherence to SiO_2-based multifunctional bioceramics. J. Biomed. Mater. Res. A. 89, 215-223.

Klausen, M., Heydorn, A., Ragas, P., Lambertsen, L., Aaes-Jørgensen, A., Molin, S. and Tolker-Nielsen, T. 2003a. Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants. Mol. Microbiol. 48, 1511-1524.

Klausen, M., Aaes-Jørgensen, A., Molin, S. and Tolker-Nielsen, T. 2003b. Involvement of bacterial migration in the development of complex multicellular structures in *Pseudomonas aeruginosa* biofilms. Mol. Microbiol. 50, 61-68.

Kloos, W. E. and Schleifer, K-H. 1986. Genus IV *Staphylococcus*. In: Holt, J. G., Sneath, P. H. A., Mair, N. S. and Sharpe, M. E. (Eds.), Bergey's Manual of Systematic Bacteriology. Springer, New York, USA, pp. 1013-1035.

Koch, K. and Ensikat, H. 2008. The hydrophobic coatings of plant surfaces: Epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. Micron 39, 759-772.

Kodjikian, L., Burillon, C., Lina, G., Roques, C., Pellon, G., Freney, J. and Renaud, F. N. 2003. Biofilm formation on intraocular lenses by a clinical strain encoding the ica locus: a scanning electron microscopy study. Invest. Ophthalmol. Vis. Sci. 44, 4382-4387.

Kohnen, W., Kolbenschlag, C., Teske-Keiser, S. and Jansen, B. 2003. Development of a long-lasting ventricular catheter impregnated with a combination of antibiotics. Biomaterials 24, 4865-4869.

Kolari, M., Nuutinen, J. and Salkinoja-Salonen, M. S. 2001. Mechanisms of biofilm formation in paper machine by *Bacillus* species: the role of *Deinococcus geothermalis* . J. Ind. Microbiol. Biotechnol. 27, 343-351.

Kolari, M., Schmidt, U., Kuismanen, E. and Salkinoja-Salonen, M. S. 2002. Firm but slippery attachment of *Deinococcus geothermalis*. J. Bacteriol. 184, 2473-2480.

Kolari, M., Nuutinen, J., Rainey, F. A. and Salkinoja-Salonen, M. S. 2003. Colored moderately thermophilic bacteria in paper-machine biofilms. J. Ind. Microbiol. Biotechnol. 30, 225-238.

Kolari, M. 2003. Attachment mechanisms and properties of bacterial biofilms on non-living surfaces. Dissertationes Biocentri Viikki Universitatis Helsingiensis 12/2003, PhD Thesis.

Kolter, R. and Greenberg, E. P. 2006. Microbial sciences: the superficial life of microbes. Review. Nature 441, 300-302.

Kongpol, A., Kato, J. and Vangnai, A. S. 2008. Isolation and characterization of *Deinococcus geothermalis* T27, a slightly thermophilic and organic solvent-tolerant bacterium able to survive in the presence of high concentrations of ethyl acetate. FEMS Microbiol. Lett. 286, 227-235.

Korber, D. R., Lawrence, J. R. and Caldwell, D. E. 1994. Effect of Motility on Surface Colonization and Reproductive Success of *Pseudomonas fluorescens* in Dual-Dilution Continuous Culture and Batch Culture Systems. Appl. Environ. Microbiol. 60, 1421-1429.

Kühn, K. P., Chaberny, I. F., Massholder, K., Stickler, M., Benz, V. W., Sonntag, H. and Erdinger, L. 2003. Disinfection of surfaces by photocatalytic oxidation with titanium dioxide and UVA light. Chemosphere 53, 71-77.

Kumar, C. G. and Anand, S. K. 1998. Significance of microbial biofilms in food industry: a review. Int. J. Food Microbiol. 42, 9-27.

Kuosmanen, T., Peltola, M., Raulio, M., Pulliainen, M., Laurila, T., Selin, J. -., Huopalainen, H. and Salkinoja-Salonen, M. S. 2006. Effect of polarization on manganese biofouling of heat exchanger surfaces. In: Müller-Steinhagen, H., Malayeri, M. R. and Watkinson, A. P. (Eds.), Proceedings of the 6th International Conference on Heat Exchangers Fouling and Cleaning - Challenges and Opportunities. Engineering Conferences International, ECI Symposium Series, pp. 283-288.

Kuula, H., Kononen, E., Lounatmaa, K., Konttinen, Y. T. and Kononen, M. 2004. Attachment of oral gram-negative anaerobic rods to a smooth titanium surface: an electron microscopy study. Int. J. Oral Maxillofac. Implants 19, 803-809.

Lafuma, A. and Quere, D. 2003. Superhydrophobic states. Nat. Mater. 2, 457-460.

Lahtinen, T., Kosonen, M., Tiirola, M., Vuento, M. and Oker-Blom, C. 2006. Diversity of bacteria contaminating paper machines. J. Ind. Microbiol. Biotechnol. 33, 734-740.

Laitila, A., Schmedding, D., van Gestel, M., Vlegels, P. and Haikara, A. 1999. Lactic acid starter cultures in malting- an application for prevention of wort filtration problems caused by bacteria in barley containing split barley kernels. Proceedings of the European Brewery Convention Congress, Caen, France, 559-566.

Laitila, A., Wilhelmson, A., Kotaviita, E., Olkku, J., Home, S. and Juvonen, R. 2006a. Yeasts in an industrial malting ecosystem. J. Ind. Microbiol. Biotechnol. 33, 953-966.

Laitila, A., Sweins, H., Vilpola, A., Kotaviita, E., Olkku, J., Home, S. and Haikara, A. 2006b. *Lactobacillus plantarum* and *Pediococcus pentosaceus* starter cultures as a tool for microflora management in malting and for enhancement of malt processability. J. Agric. Food Chem. 54, 3840-3851.

Laitila, A. 2007. Microbes in the tailoring of barley malt properties. Academic dissertation in Microbiology VTT publications 645, Helsinki University, Department of Applied Chemistry and Microbiology.

Laitila, A., Kotaviita, E., Peltola, P., Home, S. and Wilhelmson, A. 2007a. Indigenous microbial community of barley greatly influences grain germination and malt quality. J. Inst. Brew. 113, 9-20.

Laitila, A., Sarlin, T., Kotaviita, E., Huttunen, T., Home, S. and Wilhelmson, A. 2007b. Yeasts isolated from industrial maltings can suppress *Fusarium* growth and formation of gushing factors. J. Ind. Microbiol. Biotechnol. 34, 701-713.

Laitila, A., Sarlin, T., Raulio, M., Wilhelmson, A., Kotaviita, E., Huttunen, T. and Juvonen, R. 201×. Yeasts in malting with emphasis on *Wickerhamomyces anomalus* (synonym *Pichia anomala*). Review. A Van Leeuw. J. Microb. DOI 10.1007/s10482-010-9511-8

Lappann, M., Claus, H., van Alen, T., Harmsen, M., Elias, J., Molin, S. and Vogel, U. 2010. A dual role of extracellular DNA during biofilm formation of *Neisseria meningitidis*. Mol. Microbiol.

Latasa, C., Roux, A., Toledo-Arana, A., Ghigo, J. M., Gamazo, C., Penades, J. R. and Lasa, I. 2005. BapA, a large secreted protein required for biofilm formation and host colonization of *Salmonella enterica* serovar Enteritidis. Mol. Microbiol. 58, 1322-1339.

Lawrence, J. R., Korber, D. R., Hoyle, B. D., Costerton, J. W. and Caldwell, D. E. 1991. Optical sectioning of microbial biofilms. J. Bacteriol. 173, 6558-6567.

LeChevallier, M. W., Babcock, T. M. and Lee, R. G. 1987. Examination and characterization of distribution system biofilms. Appl. Environ. Microbiol. 53, 2714-2724.

Lee, D., Cohen, R. E. and Rubner, M. F. 2005. Antibacterial properties of Ag nanoparticle loaded multilayers and formation of magnetically directed antibacterial microparticles. Langmuir 21, 9651-9659.

Lee, D. S., Ryu, S. H., Hwang, H. W., Kim, Y. J., Park, M., Lee, J. R., Lee, S. S. and Jeon, C. O. 2008. *Pseudoxanthomonas sacheonensis* sp. nov., isolated from BTEX-contaminated soil in Korea, transfer of *Stenotrophomonas dokdonensis* Yoon *et al.* 2006 to the genus *Pseudoxanthomonas* as *Pseudoxanthomonas dokdonensis* comb. nov. and emended description of the genus *Pseudoxanthomonas*. Int. J. Syst. Evol. Microbiol. 58, 2235-2240.

Lehti, T. A., Bauchart, P., Heikkinen, J., Hacker, J., Korhonen, T. K., Dobrindt, U. and Westerlund-Wikström, B. 2010. Mat fimbriae promote biofilm formation by meningitis-associated *Escherichia coli*. Microbiology 156, 2408-2417.

Leino, T., Raulio, M., Stenius, P., Laine, J. and Salkinoja-Salonen, M. 201×a. Flocculation of white water bacterium with cationic polyelectrolytes and wood pitch. J. Ind. Microbiol. Submitted.

Leino, T., Raulio, M., Salkinoja-Salonen, M., Stenius, P. and Laine, J. 201×b. Adsorption of bacteria and polycations on model surfaces of cellulose, hemicellulose and wood extractives studied by QCM-D. Colloid Surface B. Submitted.

Lejeune, P. 2003. Contamination of abiotic surfaces: what a colonizing bacterium sees and how to blur it. Review. Trends Microbiol. 11, 179-184.

Leroy, S., Lebert, I., Chacornac, J. P., Chavant, P., Bernardi, T. and Talon, R. 2009. Genetic diversity and biofilm formation of *Staphylococcus equorum* isolated from naturally fermented sausages and their manufacturing environment. Int. J. Food Microbiol. 134, 46-51.

Lewis, K. 2008. Multidrug tolerance of biofilms and persister cells. Review. Curr. Top. Microbiol. Immunol. 322, 107-131.

Lewis, K. 2001. Riddle of biofilm resistance. Review. Antimicrob. Agents Chemother. 45, 999-1007.

Lewis, K. 2000. Programmed death in bacteria. Review. Microbiol. Mol. Biol. Rev. 64, 503-514.

Lewis, K. and Klibanov, A. M. 2005. Surpassing nature: rational design of sterile-surface materials. Review.Trends Biotechnol. 23, 343-348.

Li, B. and Logan, B. E. 2005. The impact of ultraviolet light on bacterial adhesion to glass and metal oxide-coated surface. Colloid Surface B 41, 153-161.

Li, C., Zhang, X. and Whitbourne, R. 1999. In vitro antimicrobial activity of a new antiseptic central venous catheter. J. Biomater. Appl. 13, 206-223.

Li, Y., Leung, W. K., Yeung, K. L., Lau, P. S. and Kwan, J. K. 2009. A multilevel antimicrobial coating based on polymer-encapsulated ClO_2. Langmuir 25, 13472-13480.

Lichter, J. A., Van Vliet, K. J. and Rubner, M. F. 2009. Design of Antibacterial Surfaces and Interfaces: Polyelectrolyte Multilayers as a Multifunctional Platform. Macromolecules 42, 8573-8586.

Lichter, J. A. and Rubner, M. F. 2009. Polyelectrolyte multilayers with intrinsic antimicrobial functionality: the importance of mobile polycations. Langmuir 25, 7686-7694.

Liedert, C., Bernhardt, J., Albrecht, D., Voigt, B., Hecker, M., Salkinoja-Salonen, M. and Neubauer, P. 2010. Two-dimensional proteome reference map for the radiation-resistant bacterium *Deinococcus geothermalis*. Proteomics 10, 555-563.

Lindberg, L. E., Holmbom, B. R., Väisänen, O. M., Weber, A. M. and Salkinoja-Salonen, M. S. 2001. Sugar composition of biofilms produced by paper mill bacteria. Appl. Microbiol. Biotechnol. 55, 638-643.

Linsebigler, A. L., Lu, G. and Yates, J. T.,Jr. 1995. Photocatalysis on TiO_2 Surfaces: Principles, Mechanisms, and Selected Results. Review. Chem. Rev. 95, 735-758.

Lipski, A. and Stackebrandt, E. S. 2005. Genus VI *Pseudoxanthomonas* Finkmann, Altendorf, Stackebrandt and Lipski 2000. In: Garrity, G. M., Brenner, D. J., Krieg, N. R. and Staley, J. T. (Eds.), Bergey's Manual of Systematic Bacteriology, *The proteobacteria*, Part B *The Gammaproteobacteria*. Springer, New York, USA, pp. 105-107.

Liu, Y. and Tay, J. -. 2001. Metabolic response of biofilm to shear stress in fixed-film culture. J. Appl. Microbiol. 90, 337-342.

Lowe, D. P. and Arendt, E. K. 2004. The use and effects of lactic acid bacteria in malting and brewing with their relationships to antifungal activity, mycotoxins and gushing: a review. J. Inst. Brew. 110, 163-180.

Mack, D., Nedelmann, M., Krokotsch, A., Schwarzkopf, A., Heesemann, J. and Laufs, R. 1994. Characterization of transposon mutants of biofilm-producing *Staphylococcus epidermidis* impaired in the accumulative phase of biofilm production: genetic identification of a hexosamine-containing polysaccharide intercellular adhesin. Infect. Immun. 62, 3244-3253.

Mack, D., Fischer, W., Krokotsch, A., Leopold, K., Hartmann, R., Egge, H. and Laufs, R. 1996. The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. J. Bacteriol. 178, 175-183.

Mah, T. F. and O'Toole, G. A. 2001. Mechanisms of biofilm resistance to antimicrobial agents. Review. Trends Microbiol. 9, 34-39.

Maness, P. C., Smolinski, S., Blake, D. M., Huang, Z., Wolfrum, E. J. and Jacoby, W. A. 1999. Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. Appl. Environ. Microbiol. 65, 4094-4098.

Marouani-Gadri, N., Augier, G. and Carpentier, B. 2009. Characterization of bacterial strains isolated from a beef-processing plant following cleaning and disinfection — Influence of isolated strains on biofilm formation by Sakaï and EDL 933 *E. coli* O157:H7. Int. J. Food Microbiol. 133, 62-67.

Matsunaga, T., Tomoda, R., Nakajima, T. and Wake, H. 1985. Photoelectrochemical sterilization of microbial cells by semiconductor powders. FEMS Microbiol. Lett. 29, 211-214.

Mattila, K., Weber, A. and Salkinoja-Salonen, M. S. 2002. Structure and on-site formation of biofilms in paper machine water flow. J. Ind. Microbiol. Biotechnol. 28, 268-279.

May, T. B., Shinabarger, D., Maharaj, R., Kato, J., Chu, L., DeVault, J. D., Roychoudhury, S., Zielinski, N. A., Berry, A. and Rothmel, R. K. 1991. Alginate synthesis by *Pseudomonas aeruginosa*: a key pathogenic factor in chronic pulmonary infections of cystic fibrosis patients. Review. Clin. Microbiol. Rev. 4, 191-206.

McCann, M. T., Gilmore, B. F. and Gorman, S. P. 2008. *Staphylococcus epidermidis* device-related infections: pathogenesis and clinical management. J. Pharm. Pharmacol. 60, 1551-1571.

Medilanski, E., Kaufmann, K., Wick, L.,Y., Wanner, O. and Harms, H. 2002. Influence of the Surface Topography of Stainless Steel on Bacterial Adhesion. Biofouling 18, 193-203.

Merritt, P. M., Danhorn, T. and Fuqua, C. 2007. Motility and chemotaxis in *Agrobacterium tumefaciens* surface attachment and biofilm formation. J. Bacteriol. 189, 8005-8014.

Mills, A. and Le Hunte, S. 1997. An overview of semiconductor photocatalysis. Review. J. Photochem. Photobiol. A. 108, 1-35.

Mitik-Dineva, N., Wang, J., Truong, V. K., Stoddart, P., Malherbe, F., Crawford, R. J. and Ivanova, E. P. 2009. *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* attachment patterns on glass surfaces with nanoscale roughness. Curr. Microbiol. 58, 268-273.

Moreira, C., Rainey, F. A., Nobre, M. F., da Silva, M. T. and da Costa, M. S. 2000. *Tepidimonas ignava* gen. nov., sp. nov., a new chemolithoheterotrophic and slightly thermophilic member of the *beta-Proteobacteria*. Int. J. Syst. Evol. Microbiol. 50, 735-742.

Morgan, T. D. and Wilson, M. 2001. The effects of surface roughness and type of denture acrylic on biofilm formation by *Streptococcus oralis* in a constant depth film fermentor. J. Appl. Microbiol. 91, 47-53.

Moscoso, M., Garcia, E. and Lopez, R. 2006. Biofilm formation by *Streptococcus pneumoniae*: role of choline, extracellular DNA, and capsular polysaccharide in microbial accretion. J. Bacteriol. 188, 7785-7795.

Murata, H., Koepsel, R. R., Matyjaszewski, K. and Russell, A. J. 2007. Permanent, non-leaching antibacterial surface-2: how high density cationic surfaces kill bacterial cells. Biomaterials 28, 4870-4879.

Nagorska, K., Ostrowski, A., Hinc, K., Holland, I. B. and Obuchowski, M. 2010. Importance of eps genes from *Bacillus subtilis* in biofilm formation and swarming. J. Appl. Genet. 51, 369-381.

Nicolella, C., van Loosdrecht, M. C. and Heijnen, J. J. 2000. Wastewater treatment with particulate biofilm reactors. J. Biotechnol. 80, 1-33.

Njoroge, J. and Sperandio, V. 2009. Jamming bacterial communication: new approaches for the treatment of infectious diseases. Review. EMBO Mol. Med. 1, 201-210.

Nobre, M. F. and da Costa, M. S. 2001. Genus II. *Meiothermus* Nobre, Trüper and Milton da Costa. In: Garrity, G. M., Staley, J. T. and Boone, D. R. (Eds.), Bergey's Manual of Systematic Bacteriology, The *Archaea* and the Deeply Branching and Phototrophic Bacteria. Springer, New York, USA, pp. 414-420.

Nobre, M. F., Truper, H. G. and Da Costa, M. S. 1996. Transfer of *Thermus ruber* (Loginova *et al.* 1984), *Thermus silvanus* (Tenreiro *et al.* 1995), and *Thermus chliarophilus* (Tenreiro *et al.* 1995) to *Meiothermus* gen. nov. as *Meiothermus ruber* comb. nov., *Meiothermus silvanus* comb. nov., and *Meioithermus chliarophilus* comb. nov., Respectively, and Emendation of the Genus *Thermus*. Int. J. Syst. Bacteriol. 46, 604-606.

Noots, I., Delcour, J. A. and Michiels, C. W. 1999. From field barley to malt: detection and specification of microbial activity for quality aspects. Review. Crit. Rev. Microbiol. 25, 121-153.

Ojeniyi, B., Christensen, J. and Bisgaard, M. 2000. Comparative investigations of *Listeria monocytogenes* isolated from a turkey processing plant, turkey products, and from human cases of listeriosis in Denmark. Epidemiol. Infect. 125, 303-308.

Oppong, D., King, V. M., Zhou, X. and Bowen, J. A. 2000. Cultural and biochemical diversity of pink-pigmented bacteria isolated from paper mill slimes. J. Ind. Microbiol. Biotechnol. 25, 74.

O'Sullivan, T., Walsh, Y., O'Mahony, A., Fitzgerald, G. and van Sinderen, D. 1999. A comparative study of malthouse and brewhouse microflora. J. Inst. Brew. 105, 55-61.

O'Toole, G. A. and Kolter, R. 1998a. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. 30, 295-304.

O'Toole, G. A. and Kolter, R. 1998b. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Mol. Microbiol. 28, 449-461.

Palleroni, N. J. 2005. Genus I *Pseudomonas* Migula 1894. In: Garrity, G. M., Brenner, D. J., Krieg, N. R. and Staley, J. T. (Eds.), Bergey's Manual of Systematic Bacteriology, *The Proteobacteria*, Part B *The Gammaproteobacteria*. Springer, New York, USA, pp. 323-379.

Parkar, S. G., Flint, S. H., Palmer, J. S. and Brooks, J. D. 2001. Factors influencing attachment of thermophilic bacilli to stainless steel. J. Appl. Microbiol. 90, 901-908.

Pawar, D. M., Rossman, M. L. and Chen, J. 2005. Role of curli fimbriae in mediating the cells of enterohaemorrhagic *Escherichia coli* to attach to abiotic surfaces. J. Appl. Microbiol. 99, 418-425.

Pederson, C. S. 1936. A Study of the Species *Lactobacillus plantarum* (Orla-Jensen) Bergey *et al.* J. Bacteriol. 31, 217-224.

Peltola, M., Neu, T. R., Raulio, M., Kolari, M. and Salkinoja-Salonen, M. S. 2008a. Architecture of *Deinococcus geothermalis* biofilms on glass and steel: a lectin study. Environ. Microbiol. 10, 1752-1757.

Peltola, M., Kanto Oqvist, C., Ekman, J., Kosonen, M., Jokela, S., Kolari, M., Korhonen, P. and Salkinoja-Salonen, M. 2008b. Quantitative contributions of bacteria and of *Deinococcus geothermalis* to deposits and slimes in paper industry. J. Ind. Microbiol. Biotechnol. 35, 1651-1657.

Pereni, C. I., Zhao, Q., Liu, Y. and Abel, E. 2006. Surface free energy effect on bacterial retention. Colloids Surf. B Biointerfaces 48, 143-147.

Perry, J. A., Cvitkovitch, D. G. and Levesque, C. M. 2009. Cell death in *Streptococcus mutans* biofilms: a link between CSP and extracellular DNA. FEMS Microbiol. Lett. 299, 261-266.

Petters, H., Flannigan, B. and Austin, B. 1988. Quantitative and qualitative studies of the microflora of barley malt production. J. Appl. Bacteriol. 65, 279-297.

Pirttijarvi, T. S., Graeffe, T. H. and Salkinoja-Salonen, M. S. 1996. Bacterial contaminants in liquid packaging boards: assessment of potential for food spoilage. J. Appl. Bacteriol. 81, 445-458.

Podsiadlo, P., Paternel, S., Rouillard, J. M., Zhang, Z., Lee, J., Lee, J. W., Gulari, E. and Kotov, N. A. 2005. Layer-by-layer assembly of nacre-like nanostructured composites with antimicrobial properties. Langmuir 21, 11915-11921.

Pompermayer, D. M. C. and Gaylarde, C. C. 2000. The influence of temperature on the adhesion of mixed cultures of *Staphylococcus aureus* and *Escherichia coli* to polypropylene. Food Microbiol. 17, 361-365.

Pratt, L. A. and Kolter, R. 1999. Genetic analyses of bacterial biofilm formation. Curr. Opin. Microbiol. 2, 598-603.

Pratt, L. A. and Kolter, R. 1998. Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol. Microbiol. 30, 285-293.

Prince, V., Simao-Beaunoir, A. M. and Beaulieu, C. 2009. Amplified ribosomal DNA restriction analysis of free-living bacteria present in the headbox of a Canadian paper machine. Can. J. Microbiol. 55, 810-817.

Puckett, S. D., Taylor, E., Raimondo, T. and Webster, T. J. 2010. The relationship between the nanostructure of titanium surfaces and bacterial attachment. Biomaterials 31, 706-713.

Qin, Z., Ou, Y., Yang, L., Zhu, Y., Tolker-Nielsen, T., Molin, S. and Qu, D. 2007. Role of autolysin-mediated DNA release in biofilm formation of *Staphylococcus epidermidis*. Microbiology 153, 2083-2092.

Raaska, L., Sillanpää, J., Sjöberg, A. M. and Suihko, M. L. 2002. Potential microbiological hazards in the production of refined paper products for food applications. J. Ind. Microbiol. Biotechnol. 28, 225-231.

Rajagopal, G., Maruthamuthu, S., Mohanan, S. and Palaniswamy, N. 2006. Biocidal effects of photocatalytic semiconductor TiO_2. Colloid Surface B 51, 107-111.

Rao, T. S., Kora, A. J., Chandramohan, P., Panigrahi, B. S. and Narasimhan, S. V. 2009. Biofouling and microbial corrosion problem in the thermo-fluid heat exchanger and cooling water system of a nuclear test reactor. Biofouling 25, 581-591.

Raulio, M., Pore, V., Areva, S., Ritala, M., Leskelä, M., Linden, M., Rosenholm, J. B., Lounatmaa, K. and Salkinoja-Salonen, M. 2006. Destruction of Deinococcus geothermalis biofilm by photocatalytic ALD and sol-gel TiO(2) surfaces. J. Ind. Microbiol. Biotechnol. 33, 261-268.

Raulio, M., Järn, M., Ahola, J., Peltonen, J., Rosenholm, J. B., Tervakangas, S., Kolehmainen, J., Ruokolainen, T., Narko, P. and Salkinoja-Salonen, M. 2008. Microbe repelling coated stainless steel analysed by field emission scanning electron microscopy and physicochemical methods. J. Ind. Microbiol. Biotechnol. 35, 751-760.

Ray, R., Lizewski, S., Fitzgerald, L. A., Little, B. and Ringeisen, B. R. 2010. Methods for imaging *Shewanella oneidensis* MR-1 nanofilaments. J. Microbiol. Methods 82, 187-191.

Rinaudo, C. D., Rosini, R., Galeotti, C. L., Berti, F., Necchi, F., Reguzzi, V., Ghezzo, C., Telford, J. L., Grandi, G. and Maione, D. 2010. Specific involvement of pilus type 2a in biofilm formation in group B *Streptococcus*. PLoS One 5, e9216.

Rohde, H., Frankenberger, S., Zähringer, U. and Mack, D. 2010. Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to *Staphylococcus epidermidis* biofilm formation and pathogenesis of biomaterial-associated infections. Eur. J. Cell Biol. 89, 103-111.

Ryder, C., Byrd, M. and Wozniak, D. J. 2007. Role of polysaccharides in *Pseudomonas aeruginosa* biofilm development. Review. Curr. Opin. Microbiol. 10, 644-648.

Rättö, M., Suihko, M. L. and Siika-aho, M. 2005. Polysaccharide-producing bacteria isolated from paper machine slime deposits. J. Ind. Microbiol. Biotechnol. 32, 109-114.

Saarimaa, C., Peltola, M., Raulio, M., Neu, T. R., Salkinoja-Salonen, M. S. and Neubauer, P. 2006. Characterization of Adhesion Threads of *Deinococcus geothermalis* as Type IV Pili. J. Bacteriol. 188, 7016-7021.

Sanborn, J. R. 1933. Development and Control of Microorganisms in a Pulp and Paper Mill System. J. Bacteriol. 26, 373-378.

Scheutz, F. and Strockbine, N. A. 2005. Genus I *Escherichia* Castellani and Chalmers 1919. In: Garrity, G. M., Brenner, D. J., Krieg, N. R. and Staley, J. T. (Eds.), Bergey's Manual of Systematic Bacteriology, *The Proteobacteria*, Part B *The Gammaproteobacteria*. Springer, New York, USA, pp. 607-624.

Schierholz, J. M., Steinhauser, H., Rump, A. F., Berkels, R. and Pulverer, G. 1997. Controlled release of antibiotics from biomedical polyurethanes: morphological and structural features. Biomaterials 18, 839-844.

Schleifer, K. H. and Kandler, O. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. 36, 407-477.

Schleifer, K. H. and Kloos, W. E. 1975. Isolation and Characterization of *Staphylococci* from Human Skin I. Amended Descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and Descriptions of Three New Species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, and *Staphylococcus xylosus*. Int. J. Syst. Bacteriol. 25, 50-61.

Sen, K. and Rodgers, M. 2004. Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: a PCR identification. J. Appl. Microbiol. 97, 1077-1086.

Sharma, M. and Anand, S. K. 2002. Characterization of constitutive microflora of biofilms in dairy processing lines. Food Microbiol. 19, 627-636.

Shi, Z., Neoh, K. G., Kang, E. T. and Wang, W. 2006. Antibacterial and mechanical properties of bone cement impregnated with chitosan nanoparticles. Biomaterials 27, 2440-2449.

Shime-Hattori, A., Iida, T., Arita, M., Park, K. S., Kodama, T. and Honda, T. 2006. Two type IV pili of *Vibrio parahaemolyticus* play different roles in biofilm formation. FEMS Microbiol. Lett. 264, 89-97.

Shively, J. M. 1974. Inclusion Bodies of Prokaryotes. Review. Annu. Rev. Microbiol. 28, 167-188.

Silagyi, K., Kim, S., Martin Lo, Y. and Wei, C. 2009. Production of biofilm and quorum sensing by *Escherichia coli* O157:H7 and its transfer from contact surfaces to meat, poultry, ready-to-eat deli, and produce products. Food Microbiol. 26, 514-519.

Silver, S., Phung le, T. and Silver, G. 2006. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. J. Ind. Microbiol. Biotechnol. 33, 627-634.

Simpson, D. R. 2008. Biofilm processes in biologically active carbon water purification. Water Res. 42, 2839-2848.

Sinde, E. and Carballo, J. 2000. Attachment of *Salmonella* spp. and *Listeria monocytogenes* to stainless steel, rubber and polytetrafluorethylene: the influence of free energy and the effect of commercial sanitizers. Food Microbiol. 17, 439-447.

Singh, P. K., Schaefer, A. L., Parsek, M. R., Moninger, T. O., Welsh, M. J. and Greenberg, E. P. 2000. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature 407, 762-764.

Smit, G., Kijne, J. W. and Lugtenberg, B. J. 1989. Roles of flagella, lipopolysaccharide, and a Ca^{2+}-dependent cell surface protein in attachment of *Rhizobium leguminosarum* biovar *viciae* to pea root hair tips. J. Bacteriol. 171, 569-572.

Somerville, G. A., Beres, S. B., Fitzgerald, J. R., DeLeo, F. R., Cole, R. L., Hoff, J. S. and Musser, J. M. 2002. In vitro serial passage of *Staphylococcus aureus*: changes in physiology, virulence factor production, and agr nucleotide sequence. J. Bacteriol. 184, 1430-1437.

Stapper, A. P., Narasimhan, G., Ohman, D. E., Barakat, J., Hentzer, M., Molin, S., Kharazmi, A., Hoiby, N. and Mathee, K. 2004. Alginate production affects *Pseudomonas aeruginosa* biofilm development and architecture, but is not essential for biofilm formation. J. Med. Microbiol. 53, 679-690.

Stewart, P. S. and William Costerton, J. 2001. Antibiotic resistance of bacteria in biofilms. Review. The Lancet 358, 135-138.

Stickler, D. J., Lear, J. C., Morris, N. S., Macleod, S. M., Downer, A., Cadd, D. H. and Feast, W. J. 2006. Observations on the adherence of *Proteus mirabilis* onto polymer surfaces. J. Appl. Microbiol. 100, 1028-1033.

Stoodley, P., Sauer, K., Davies, D. G. and Costerton, J. W. 2002. Biofilms as complex differentiated communities. Review. Annu. Rev. Microbiol. 56, 187-209.

Stoodley, P., Lewandowski, Z., Boyle, J. D. and Lappin-Scott, H. M. 1999. Structural deformation of bacterial biofilms caused by short-term fluctuations in fluid shear: an in situ investigation of biofilm rheology. Biotechnol. Bioeng. 65, 83-92.

Stoodley, P., Dodds, I., Boyle, J. D. and Lappin-Scott, H. 1998. Influence of hydrodynamics and nutrients on biofilm structure. J. Appl. Microbiol. 85, 19S.

Storgårds, E., Juvonen, R. and Mäntylä, T. 2007. Functional materials - a possibility to improve brewery hygiene? Proceedings of the 31st European Brewery Convention Congress, Venice, 6-10 May, Nürnberg, Fachverlag Hans Carl (CD-ROM) 158, 1385-1392.

Suihko, M. L. and Skyttä, E. 2009. Characterisation of aerobically grown non-spore-forming bacteria from paper mill pulps containing recycled fibres. J. Ind. Microbiol. Biotechnol. 36, 53-64.

Suihko, M. L., Sinkko, H., Partanen, L., Mattila-Sandholm, T., Salkinoja-Salonen, M. and Raaska, L. 2004. Description of heterotrophic bacteria occurring in paper mills and paper products. J. Appl. Microbiol. 97, 1228-1235.

Sunada, K., Watanabe, T. and Hashimoto, K. 2003a. Studies on photokilling of bacteria on TiO_2 thin film. J. Photochem. Photobiol. A. 156, 227-233.

Sunada, K., Watanabe, T. and Hashimoto, K. 2003b. Bactericidal Activity of Copper-Deposited TiO_2 Thin Film under Weak UV Light Illumination. Environ. Sci. Technol. 37, 4785-4789.

Suominen, I., Sproer, C., Kampfer, P., Rainey, F. A., Lounatmaa, K. and Salkinoja-Salonen, M. 2003. *Paenibacillus stellifer* sp. nov., a cyclodextrin-producing species isolated from paperboard. Int. J. Syst. Evol. Microbiol. 53, 1369-1374.

Sutherland, I. W. 2001. The biofilm matrix - an immobilized but dynamic microbial environment. Review. Trends Microbiol. 9, 222-227.

Szewzyk, U., Szewzyk, R., Manz, W. and Schleifer, K. 2000. Microbiological safety of drinking water. Review. Annu. Rev. Microbiol. 54, 81-127.

Tacket, C. O., Taylor, R. K., Losonsky, G., Lim, Y., Nataro, J. P., Kaper, J. B. and Levine, M. M. 1998. Investigation of the roles of toxin-coregulated pili and mannose-sensitive hemagglutinin pili in the pathogenesis of *Vibrio cholerae* O139 infection. Infect. Immun. 66, 692-695.

Tenreiro, S., Nobre, M. F. and da Costa, M. S. 1995. *Thermus silvanus* sp. nov. and *Thermus chliarophilus* sp. nov., two new species related to thermus ruber but with lower growth temperatures. Int. J. Syst. Bacteriol. 45, 633-639.

Thelin, K. H. and Taylor, R. K. 1996. Toxin-coregulated pilus, but not mannose-sensitive hemagglutinin, is required for colonization by *Vibrio cholerae* O1 El Tor biotype and O139 strains. Infect. Immun. 64, 2853-2856.

Thierry, S., Macarie, H., Iizuka, T., Geissdorfer, W., Assih, E. A., Spanevello, M., Verhe, F., Thomas, P., Fudou, R., Monroy, O., Labat, M. and Ouattara, A. S. 2004. *Pseudoxanthomonas mexicana* sp. nov. and *Pseudoxanthomonas japonensis* sp. nov., isolated from diverse environments, and emended descriptions of the genus *Pseudoxanthomonas* Finkmann *et al.* 2000 and of its type species. Int. J. Syst. Evol. Microbiol. 54, 2245-2255.

Thomas, V. C., Thurlow, L. R., Boyle, D. and Hancock, L. E. 2008. Regulation of autolysis-dependent extracellular DNA release by *Enterococcus faecalis* extracellular proteases influences biofilm development. J. Bacteriol. 190, 5690-5698.

Thormann, K. M., Saville, R. M., Shukla, S., Pelletier, D. A. and Spormann, A. M. 2004. Initial Phases of biofilm formation in *Shewanella oneidensis* MR-1. J. Bacteriol. 186, 8096-8104.

Thorpe, P. H. 1987. Microbial corrosion of stainless steel in paper machines. Appita J 40, 108-111.

Tian, B., Wang, H., Ma, X., Hu, Y., Sun, Z., Shen, S., Wang, F. and Hua, Y. 2010. Proteomic analysis of membrane proteins from a radioresistant and moderate thermophilic bacterium *Deinococcus geothermalis*. Mol. Biosyst 6, 2068-2077.

Tiirola, M., Lahtinen, T., Vuento, M. and Oker-Blom, C. 2009. Early succession of bacterial biofilms in paper machines. J. Ind. Microbiol. Biotechnol. 36, 929-937.

Tijssen, J. P., Beekes, H. W. and Van Steveninck, J. 1982. Localization of polyphosphates in *Saccharomyces fragilis*, as revealed by 4',6-diamidino-2-phenylindole fluorescence. Biochim. Biophys. Acta 721, 394-398.

Tiller, J. C., Liao, C. J., Lewis, K. and Klibanov, A. M. 2001. Designing surfaces that kill bacteria on contact. Proc. Natl. Acad. Sci. U. S. A. 98, 5981-5985.

Toledo-Arana, A., Valle, J., Solano, C., Arrizubieta, M. J., Cucarella, C., Lamata, M., Amorena, B., Leiva, J., Penades, J. R. and Lasa, I. 2001. The enterococcal surface protein, Esp, is involved in *Enterococcus faecalis* biofilm formation. Appl. Environ. Microbiol. 67, 4538-4545.

Tsitko, I., Rahkila, R., Priha, O., Ali-Vehmas, T., Terefework, Z., Soini, H. and Salkinoja-Salonen, M. S. 2006. Isolation and automated ribotyping of *Mycobacterium lentiflavum* from drinking water distribution system and clinical specimens. FEMS Microbiol. Lett. 256, 236-243.

Unden, G. and Bongaerts, J. 1997. Alternative respiratory pathways of *Escherichia coli*: energetics and transcriptional regulation in response to electron acceptors. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1320, 217-234.

Uutela, P., Mattila, K., Carpén, L., Raaska, L., Hakkarainen, T. and Salkinoja-Salonen, M. 2003. Biogenic thiosulfate and oxalate in paper machine deposits connected to corrosion of stainless steels. Int. Biodeterior. Biodegrad. 51, 19-28.

Vacheethasanee, K., Temenoff, J. S., Higashi, J. M., Gary, A., Anderson, J. M., Bayston, R. and Marchant, R. E. 1998. Bacterial surface properties of clinically isolated *Staphylococcus epidermidis* strains determine adhesion on polyethylene. J. Biomed. Mater. Res. 42, 425-432.

Väisänen, O. M., Mentu, J. and Salkinoja-Salonen, M. S. 1991. Bacteria in food packaging paper and board. J. Appl. Bacteriol. 71, 130-133.

Väisänen, O. M., Nurmiaho-Lassila, E. L., Marmo, S. A. and Salkinoja-Salonen, M. S. 1994. Structure and composition of biological slimes on paper and board machines. Appl. Environ. Microbiol. 60, 641-653.

Väisänen, O. M., Weber, A., Bennasar, A., Rainey, F. A., Busse, H. J. and Salkinoja-Salonen, M. S. 1998. Microbial communities of printing paper machines. J. Appl. Microbiol. 84, 1069-1084.

van Campenhout, L., Iserentant, D. and Verachtert, H. 1998. On-line measurement of the microbial impacts on the germination of barley during malting. J. Inst. Brew. 104, 197-202.

van Campenhout, L., Shen, H. Y., Iserentant, D. and Verachtert, H. 1999. The gas environment of germinating barley in various microbial states during malting. Proc. Biochem. 34, 929-937.

van Houdt, R., Aertsen, A., Jansen, A., Quintana, A. L. and Michiels, C. W. 2004. Biofilm formation and cell-to-cell signalling in Gram-negative bacteria isolated from a food processing environment. J. Appl. Microbiol. 96, 177-184.

van Loosdrecht, M. C., Lyklema, J., Norde, W. and Zehnder, A. J. 1990. Influence of interfaces on microbial activity. Review. Microbiol. Rev. 54, 75-87.

Varga, J. J., Therit, B. and Melville, S. B. 2008. Type IV pili and the CcpA protein are needed for maximal biofilm formation by the gram-positive anaerobic pathogen *Clostridium perfringens*. Infect. Immun. 76, 4944-4951.

Verran, J. and Boyd, R. D. 2001. The relationship between substratum surface roughness and microbiological and organic soiling: A review. Biofouling 17, 59-71.

Vidal, O., Longin, R., Prigent-Combaret, C., Dorel, C., Hooreman, M. and Lejeune, P. 1998. Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: involvement of a new ompR allele that increases curli expression. J. Bacteriol. 180, 2442-2449.

Vilain, S., Pretorius, J. M., Theron, J. and Brozel, V. S. 2009. DNA as an adhesin: *Bacillus cereus* requires extracellular DNA to form biofilms. Appl. Environ. Microbiol. 75, 2861-2868.

Vlassov, V. V., Laktionov, P. P. and Rykova, E. Y. 2007. Extracellular nucleic acids. Review. Bioessays 29, 654-667.

Watnick, P. I. and Kolter, R. 1999. Steps in the development of a *Vibrio cholerae* El Tor biofilm. Mol. Microbiol. 34, 586-595.

Watnick, P. and Kolter, R. 2000. Biofilm, city of microbes. Review. J. Bacteriol. 182, 2675-2679.

Watnick, P. I., Fullner, K. J. and Kolter, R. 1999. A role for the mannose-sensitive hemagglutinin in biofilm formation by *Vibrio cholerae* El Tor. J. Bacteriol. 181, 3606-3609.

Wei, C., Lin, W. Y., Zainal, Z., Williams, N. E., Zhu, K., Kruzic, A. P., Smith, R. L. and Rajeshwar, K. 1994. Bactericidal Activity of TiO_2 Photocatalyst in Aqueous Media: Toward a Solar-Assisted Water Disinfection System . Environ Sci Technol 28, 934-938.

Whitchurch, C. B., Tolker-Nielsen, T., Ragas, P. C. and Mattick, J. S. 2002. Extracellular DNA required for bacterial biofilm formation. Science 295, 1487.

Whitehead, K. A. and Verran, J. 2006. The Effect of Surface Topography on the Retention of Microorganisms. Food Bioprod. Process. 84, 253-259.

Wimpenny, J., Manz, W. and Szewzyk, U. 2000. Heterogeneity in biofilms. FEMS Microbiol. Rev. 24, 661-671.

Wolf-Hall, C. E. 2007. Mold and mycotoxin problems encountered during malting and brewing. Int. J. Food Microbiol. 119, 89-94.

Wolfrum, E. J., Huang, J., Blake, D. M., Maness, P., Huang, Z., Fiest, J. and Jacoby, W. A. 2002. Photocatalytic Oxidation of Bacteria, Bacterial and Fungal Spores, and Model Biofilm Components to Carbon Dioxide on Titanium Dioxide-Coated Surfaces. Environ. sci. technol. 36, 3412-3419.

Wong, A. C. 1998. Biofilms in food processing environments. Review. J. Dairy Sci. 81, 2765-2770.

Wong, S. Y., Li, Q., Veselinovic, J., Kim, B. S., Klibanov, A. M. and Hammond, P. T. 2010. Bactericidal and virucidal ultrathin films assembled layer by layer from polycationic N-alkylated polyethylenimines and polyanions. Biomaterials 31, 4079-4087.

Wood, S. R., Kirkham, J., Marsh, P. D., Shore, R. C., Nattress, B. and Robinson, C. 2000. Architecture of intact natural human plaque biofilms studied by confocal laser scanning microscopy. J. Dent. Res. 79, 21-27.

Wozniak, D. J., Wyckoff, T. J., Starkey, M., Keyser, R., Azadi, P., O'Toole, G. A. and Parsek, M. R. 2003. Alginate is not a significant component of the extracellular polysaccharide matrix of PA14 and PAO1 *Pseudomonas aeruginosa* biofilms. Proc. Natl. Acad. Sci. U. S. A. 100, 7907-7912.

Yildiz, F. H. and Visick, K. L. 2009. *Vibrio* biofilms: so much the same yet so different. Review. Trends Microbiol. 17, 109-118.

Yoon, J. H., Kang, S. J., Oh, H. W. and Oh, T. K. 2006. *Stenotrophomonas dokdonensis* sp. nov., isolated from soil. Int. J. Syst. Evol. Microbiol. 56, 1363-1367.

Zhao, Q., Liu, Y., Wang, C., Wang, S., Peng, N. and Jeynes, C. 2008. Reduction of bacterial adhesion on ion-implanted stainless steel surfaces. Med. Eng. Phys. 30, 341-349.

ZoBell, C. E. and Allen, E. C. 1935. The Significance of Marine Bacteria in the Fouling of Submerged Surfaces. J. Bacteriol. 29, 239-251.

ZoBell, C. E. 1943. The Effect of Solid Surfaces upon Bacterial Activity. J. Bacteriol. 46, 39-56.