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ORIGINAL PAPER

Detection and quantitation of colored deposit-forming *Meiothermus* spp. in paper industry processes and end products

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Abstract Colored biofilms cause problems in paper industry. In this work we used real-time PCR to detect and to quantitate members of the genus *Meiothermus* from the process samples and end products from 24 machines manufacturing pulp, paper and board in four countries. The results obtained from 200 samples showed the importance of members of the genus *Meiothermus* as ubiquitous biofoulers in paper machines. This genus was the dominant biofouler in some mills. From $\leq 10^4$ to 10^{11} copies of *Meiothermus* 16S rRNA genes were found per gram of process deposit (wet weight). *Meiothermus* spp. were found in paper and board products with colored defects and connection between deposit-forming microbes and end-product spots was shown. 16S rRNA gene sequences of 29 biofilm producing bacterial isolates from different mills were determined. Based on sequence data, 25 of the isolates were assigned to the genus *Meiothermus*, with *Meiothermus silvanus* and *M. ruber* as the most frequent species.

Keywords Biofilm · Paper machine · 16S rRNA · Quantitative RT-PCR · *Meiothermus*

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Introduction

Bacteria can cause several problems in paper machines. A major part of the problems is related to biofilms growing on machine surfaces [2, 10, 12]. In recent years, published information on the bacterial species living in paper machines has increased [5, 23, 26]. *Deinococcus geothermalis* was the first primary biofilm former described in paper process environments [11]. Colored bacteria from paper mill slimes have also been studied [12, 19].

The first attempts for quantitative assessment of the microbial taxa living in paper machines were based on whole-cell fatty acids, ATP contents, or plate counting of culturable cells [25, 26]. A major disadvantage of these methods is the labor intensiveness. In addition, rich culture media that were usually used for plate counting may give biased results, because many slow-growing biofilm-forming bacteria do not grow or are overgrown by fast growers on such media [12].

This paper describes quantitative research based on DNA methods, on bacteria present on paper mill process surfaces and end products of paper industry.

The quantitative PCR method was developed for genus *Meiothermus*, identified by Kolari et al. [12] as an important biofilm former in paper industry. The results show that *Meiothermus* spp. are widespread in paper industry, major biofoulers in paper machines as well as responsible for the formation of end-product defects.

Materials and methods

Bacterial strains

The bacterial strains used in this study (Table 1) were obtained from Prof. Mirja Salkinoja-Salonen's culture collection and HAMBI, the Culture Collection of the Faculty of Agriculture and Forestry, University of Helsinki, Finland.

Table 1 Strains used in this study

Non-target reference strains
Escherichia coli MT102[a]
Geobacillus stearothermophilus DSM1550
Lactobacillus fermentum ATCC14931
Lactobacillus salivarius ATCC11742^T
Roseomonas gilardii ATCC49956^T
Stenotrophomonas maltophilia DSM50170^T
Streptococcus rattus BHT-2[b]
Thermomonas haemolytica DSM13605^T
Xanthomonas campestris DSM3585^T
Meiothermus type strains used in method development
Meiothermus cerbereus DSM11376^T
M. chliarophilus DSM9957^T
M. ruber DSM1279^T
M. silvanus DSM9946^T
M. taiwanensis DSM14542^T
Novel isolates used in method development
Meiothermus chliarophilus[c] S2-bf-R2A-7
M. ruber[c] E-steel-R2A-2
M. silvanus[c] B-R2A5-50-4
Burkholderia multivorans [c] F28L1
Deinococcus geothermalis[c] E50051
Pseudomonas boreopolis[c] S2-s-PMWA-6
Pseudoxanthomonas taiwanensis[c] J-M

[a] Obtained from Otto Geisenberger

[b] Hillman et al.[8]

[c] Identification is based on BLAST; see Table 6

Extraction and purification of DNA

The samples (ca. 50 mg wet wt. of cultured bacteria or industrial deposits) were incubated in FastPrep Lysing Matrix E tubes (Q Biogene, Irvine, CA, USA) in Bacterial Lysis buffer with Proteinase K from the MagNA Pure LC DNA Isolation Kit III Bacteria & Fungi (Roche Diagnostics, Penzberg, Germany) for 30 min at 65°C. After chilling, phenol-chloroform (1:1) was added and FastPrep™ FP120 instrument (Bio101 Savant Instruments Inc., Holbrook, NY, USA) used for homogenization. Samples were extracted with phenol–chloroform again, and DNA was isolated from the supernatants (15,700*g*, 5 min) using the KingFisher (ThermoElectron, Waltham, MA, USA) instrument with lysis/binding buffer, magnetic particle suspension, washing buffers I, II and III and elution buffer from the MagNA Pure kit. Run program was constructed according to the manufacturer's instructions. Reagent blank without sample material was included. Concentration of DNA was measured fluorometrically using PicoGreen® dsDNA Quantitation Reagent and Kits (Molecular Probes, Eugene, OR, USA) according to the manufacturer's instructions.

16S rRNA gene sequencing

The PCR amplification of 16S rRNA gene was done in two parts using primer pairs pA–pF′ and pD–pH′ [6]. Sequencing reactions were done using BigDye® Terminator v3.1 Cycle Sequencing Kit and Applied Biosystems ABI3700 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Consensus sequences were formed using Staden Package [22] and compared to databases with the BLAST program [1].

Primer design

Meiothermus genus-specific primers were designed using DNAMAN-program Version 5.2.9 (Lynnon Biosoft, QC, Canada) and the ARB sequence database in ARB (ssujun02), which included 17 217 checked bacterial 16S rRNA gene sequences [13]. The size of the amplicon was 147 bp.

Table 2 Primers used in this study

Primer	Specificity	Sequence (5′–3′)	16S rRNA gene target site[a]
MeioF692	Genus *Meiothermus*	GAA ATG CGC AGA TAC CGG A	692–711
MeioR821	Genus *Meiothermus*	TGT CGG ACA CCC AGC ACT	821–839
pA	Domain Eubacteria	AGA GTT TGA TCC TGG CTC AG	8–28
pD	Domain Eubacteria	CCA GCA GCC GCG GTA ATA C	517–536
pF′	Domain Eubacteria	ACG AGC TGA CGA CAG CCA TG	1,053–1,073
pH′	Domain Eubacteria	AAG GAG GTG ATC CAG CCG CA	1,522–1,542

Reference for primers pA, pD, pD′, pE, pF′ and pH′: Edwards et al. [6]

[a] Nucleotide numbering is as for *E. coli*.

Quantitative real-time PCR for the domain *Eubacteria* and genus *Meiothermus*

The LightCycler Quantitative real-time PCR (QRT-PCR) machine (Roche Diagnostics, Penzberg, Germany) was used. The reaction volume was 20 µl per capillary, including 2 µl of DNA and 0.3 µM of each primer. For the domain *Eubacteria*, primers pE and pF′ (Table 2, product size 167 bp) and SYBR Premix Ex Taq (Takara Bio Inc., Shiga, Japan) (10 µl/reaction) were used with the temperature program of 30 s at 95°C and 40 cycles of 5 s at 95°C followed by annealing and extension for 20 s at 60°C. For genus *Meiothermus*, LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics) (2 µl/reaction) was used with the temperature program of 10 min at 95°C and 40 cycles of 10 s at 95°C followed by annealing for 2 s at 58°C and extension step of 6 s at 72°C. Both QRT-PCR kits contained dNTPs, DNA polymerase enzyme, buffer and SYBR Green I fluorescent dye. Fluorescence data was acquired at the end of each extension step. The cycle threshold values (Ct values) were determined by the second derivative maximum method using LightCycler software (version 3.5). For melting curve analysis of the products the temperature was raised from 63 to 98°C and the melting temperatures were determined with the LightCycler software.

The external calibration curves for QRT-PCR were prepared with three replicates using DNA from *D. geothermalis* E50051 and *Meiothermus silvanus* DSM9946^T for the domain *Eubacteria* and the genus *Meiothermus*, respectively. Gene copy numbers in calibration samples were calculated assuming genome size of 3.2 Mb and the copy number of 16S rRNA genes/genome as 2 for *D. geothermalis* and 2.5 Mb and 2 for *M. silvanus*. Amplification efficiency, *E*, was calculated from the slope of each calibration curve using the equation $E = 10^{-1/\text{slope}}$.

The numbers of *Meiothermus* or *Eubacterial* 16S rRNA gene copies in the DNA isolated from paper machine samples were determined amplifying a 10-, 30- or 100-fold dilution of the DNA. Two reactions with 0.5 or 0.05 ng of *M. silvanus* DSM9946^T or 1 ng of *D. geothermalis* E50051 DNA as templates, were included in each run as positive controls. Based on the Ct values of the positive controls, LightCycler software was used to import the external calibration curve and applied to quantify the gene copy numbers in the DNA of the deposit samples. The Ct values obtained from deposit samples were converted into numbers of *Meiothermus* or *Eubacterial* 16S rRNA gene copies using the external calibration curve. PCR-grade H_2O and reagent blanks of the DNA extractions were included as negative controls.

Culture media

The culture media used were R2A and tryptic soy agar (Becton, Dickinson and company, Sparks, MD, USA), Castenholz trypticase yeast extract agar (DSMZ medium 86) and nutrient agar (Laboratorios Conda, Madrid, Spain).

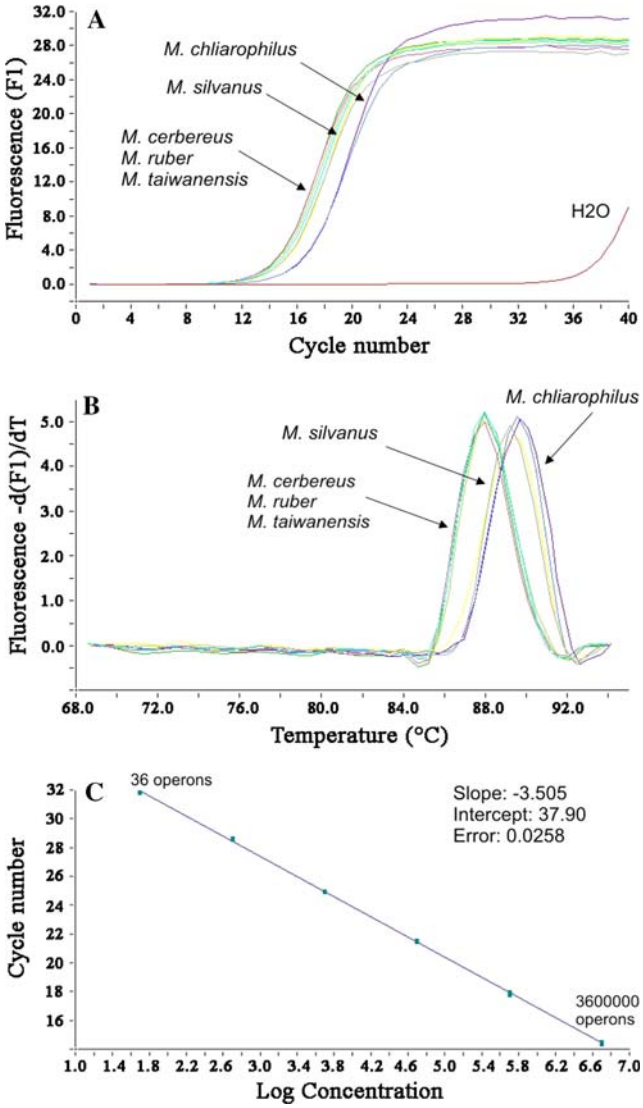

Fig. 1 Examples of QRT-PCR results when *Meiothermus* species described in Table 3 were amplified with primers MeioF692 and MeioR821. **a** Melting curves obtained for different *Meiothermus* species. Melting point was 88.0 ± 0.5°C for *M. cerbereus, M. ruber* and *M. taiwanensis* and 89.3 ± 0.6°C for *M. silvanus* and *M. chliarophilus*. **b** Amplification curves of different species of *Meiothermus*. Of each strain, 4 ng of genomic DNA was amplified with primers MeioF692 and MeioR821. Crossing points are shown in Table 3. **c** Calibration curve used for quantitation of unknown samples. The calibration curve was formed amplifying a dilution series 50 fg to 5 ng (36–360,000 16S rRNA gene copies) of genomic DNA from *M. silvanus* DSM9946^T. The amplification was placed between crossing points 14.5–32. Each dilution was amplified as three replicates

Results

Quantitative real-time PCR for *Meiothermus* genus

The genomic DNA from eight *Meiothermus* strains (Table 1) was amplified using the primers MeioF692 and MeioR821 (Table 2). When DNA from *M. cerbereus*, *M. ruber* and *M. taiwanensis* (four strains) was used as templates a product with melting temperature of 88.0 ± 0.5°C was obtained. DNA from *M. silvanus* and *M. chliarophilus* (four strains) yielded products that melted at 89.3 ± 0.6°C (Fig. 1b; Table 3). Primer dimers were not formed when the PCR reaction included ≥50 fg of genomic DNA. When 4 ng of genomic DNA from each of the strains was amplified, the crossing points obtained with QRT-PCR were 14.5–15 (Fig. 1a; Table 3), excepting *M. chliarophilus* where it was 16.5. The efficiency of *M. chliarophilus* as template was somewhat lower than that of the other species. However, the difference to the other species was smaller than 1 log unit. *M. silvanus* was selected as a calibration species for further studies.

The calibration curve obtained with *M. silvanus* DSM9946^T genomic DNA is shown in Fig. 1c. The curve was log linear from 50 fg to 5 ng genomic DNA per reaction, yielding crossing points of 32–14.5. Fifty femtograms of the template DNA corresponds approximately to 36 16S rRNA gene copies, assuming the genome size as 2.5 Mb and the number of 16S rRNA gene copies as 2 per genome. The amplification efficiency ($E = 10^{-1/\text{slope}}$) of genomic DNA from *M. silvanus* DSM9946^T was 1.93. *E* was also determined for a process sample (L, Table 4) and found equal to that obtained for pure culture of *M. silvanus* DSM9946^T DNA, indicating absence of inhibitory substances in the process sample DNA.

Specificity of the primers

The specificity of the designed primers MeioF692 and MeioR821 was investigated using selected target and non-target reference strains listed in Table 1. Table 3 shows that for the non-target species crossing points were ≥30 and the melting temperatures of the amplicons differed from those obtained for DNA of *Meiothermus* spp. When 0.5 or 0.05 ng genomic DNA from *M. silvanus* DSM9946^T was added to 1 ng of genomic DNA of a non-target species in a PCR reaction, the melting peak of the amplicon coincided with that of *Meiothermus* DNA in all cases (results not shown). Based on these results, the designed primers were specific to the genus *Meiothermus* and the calibration curve obtained with DNA from *M. silvanus* DSM9946T can be used to quantify *Meiothermus* DNA in mill samples.

Table 3 Amplification of DNA from bacterial strains to test primers MeioF692 and MeioR821

Target genus: *Meiothermus*	PCR outcome	
	Crossing point	Melting temperature (°C)
Strain		
Meiothermus cerbereus DSM11376^T	14.3	87.9 ± 0.5
M. chliarophilus DSM9957^T	16.6	89.5 ± 0.5
M. chliarophilus S2-bf-R2A-7	16.3	89.6 ± 0.5
M. ruber DSM1279^T	14.4	88.0 ± 0.5
M. ruber E-steel-R2A-2	14.8	88.0 ± 0.5
M. silvanus DSM9946^T	15.1	89.0 ± 0.5
M. silvanus B-R2A5–50-4	14.9	89.0 ± 0.5
M. taiwanensis DSM14542^T	14.6	88.1 ± 0.5
Non-target taxa		
Burkholderia multivorans F28L1	33.4	80.3
Deinococcus geothermalis E50051	>36	79.4
Escherichia coli MT102	29.7	Multiple
Geobacillus stearothermophilus DSM1550	30.0	85.6
Lactobacillus fermentum ATCC14931	>36	Multiple
L. salivarius ATCC11742^T	>36	80.7
Pseudomonas boreopolis S2-s-PMWA-6	>36	Multiple
Pseudoxanthomonas taiwanensis J-M	34.2	Multiple
Roseomonas gilardii ATCC49956^T	>36	Multiple
Stenotrophomonas maltophilia DSM50170^T	32.4	Multiple
Streptococcus rattus BHT-2	31.5	86.8
Thermomonas haemolytica DSM13605^T	>36	Multiple
Xanthomonas campestris DSM3585^T	>36	Multiple

Four nanograms of meiothermus and 1 ng of non-target DNA were used per reaction. To verify the correct amplification product, melting curves of the products were checked and the products were run in a 1.5% agarose gel

Multiple multiple peaks in melting curve

Table 4 Quantification of DNA from process and deposit samples using universal and *Meiothermus*-specific primers.

Machine code and sampling site		*Meiothermus* primers	Universal primers
		16S rRNA gene copies g^{-1} sample	
Fine paper machines			
A	Wire section, splash area	4×10^6	2×10^{10}
T	Disc filter, lid	7×10^5	6×10^9
U	Framework of wire section	8×10^5	4×10^8
SS		1×10^9	3×10^9
A, T, U	Wire sections, headboxes, filtrate tank ($n = 9$)	$<10^5$	
Board machines			
K	Shower water tank	1×10^{10}	1×10^{10}
K	Cloudy filtrate tank	2×10^9	3×10^9
L	Wire section of top layer	6×10^{10}	5×10^{10}
No.1	Wire section of inner layer	2×10^8	4×10^9
H	Clear filtrate tank	4×10^8	2×10^{10}
H	Wire pit of back layer	3×10^7	3×10^{10}
Three other machines	Wire sections, tanks for filtrates, broke system ($n = 14$)	$<10^6$	
Label and packaging paper			
J	Wire section	2×10^7	1×10^{10}
MKc	Clear filtrate tank	7×10^7	2×10^{10}
USc	AES screen	4×10^9	3×10^{10}
USc	Wire section	6×10^7	2×10^9
J	Wire section, kratzer, circulation water tank ($n = 7$)	$<10^6$	
Newsprint and magazine paper machines			
N	Wire section ($n = 2$)	2×10^7–3×10^7	5×10^9–7×10^9
N	Circulation water tank	2×10^9	1×10^9
R	Wire section ($n = 11$)	1×10^6–3×10^8	1×10^9–2×10^{11}
R	White water silo ($n = 5$)	5×10^5–3×10^9	8×10^9–5×10^{10}
R	Wire pit ($n = 4$)	9×10^6–8×10^7	3×10^9–5×10^{10}
R	Press water tray ($n = 2$)	9×10^6–4×10^7	2×10^{10}–3×10^{10}
R	Bow screen ($n = 2$)	3×10^9–8×10^9	1×10^{10}–3×10^{10}
R	Reject tank machine screen ($n = 2$)	3×10^6	4×10^9–9×10^9
R	Broke thickener ($n = 2$)	4×10^5–4×10^7	3×10^8–9×10^{10}
R	Disc filter ($n = 4$)	9×10^5–2×10^{10}	5×10^9–2×10^{11}
R	Recovered fiber chest ($n = 2$)	7×10^6–2×10^7	7×10^7–5×10^8
R	Reject of centrifugal cleaner	4×10^4	3×10^8
R	Reject storage tank ($n = 2$)	9×10^5–3×10^8	6×10^8–8×10^8
R	Wire water	1×10^4	3×10^7
R	Shower water ($n = 2$)	2×10^3–2×10^4	2×10^4–3×10^5
R	Circulation water ($n = 2$)	3×10^4–4×10^4	7×10^6–3×10^8
R	Circulation water tank ($n = 2$)	5×10^6–1×10^9	6×10^9–1×10^{10}
R	Clear filtrate tank ($n = 4$)	8×10^8–7×10^9	8×10^9–1×10^{11}
R	Super clear filtrate tank ($n = 4$)	$<3 \times 10^5$–4×10^8	2×10^{10}–1×10^{11}
R	Cloudy filtrate tank ($n = 2$)	6×10^8–1×10^9	4×10^9–8×10^9
R	Bentonite slurry mixer ($n = 2$)	$<1 \times 10^4$–7×10^5	3×10^5–2×10^7
R	Broke (n = 2)	$<5 \times 10^4$–1×10^5	5×10^7–2×10^8
R	Clear filtrate tank ($n = 3$)	$<7 \times 10^5$–4×10^6	6×10^9–2×10^{10}
R	Head box ($n = 4$)	2×10^9–4×10^{10}	2×10^{10}–4×10^{10}
R	Circulation water tank ($n = 3$)	$<2 \times 10^6$–9×10^8	1×10^{10}–2×10^{10}
R	Wire channel	6×10^5	2×10^{10}
R	Clear filtrate	6×10^3	2×10^7
KV	Disc filter	2×10^{10}	4×10^{10}
D	Wire section	8×10^7	1×10^{10}
E	Wire section ($n = 2$)	1×10^{11}–2×10^{11}	4×10^{10}–1×10^{11}
E	Press section	2×10^7	1×10^{10}

Detection of eubacterial and genus *Meiothermus* 16S rRNA gene copies in paper industry samples

The prevalence of *Meiothermus* in paper and board machines was investigated from 138 samples, including deposits, process waters and raw materials collected from 24 paper and board machines in four countries (Table 4). Twenty-five of the 138 samples were found to contain a large number (10^9–10^{11} g^{-1}) of *Meiothermus* 16S rRNA gene copies. In addition, 62 further

Table 4 continued

Machine code and sampling site		*Meiothermus* primers	Universal primers
		16S rRNA gene copies g^{-1} sample	
E		3×10^7	1×10^{10}
O, R	Wire area, carbonate preparation tank ($n = 3$)	$<10^6$	
R	Waters and raw materials ($n = 6$)	$<10^3$	
Others			
C	Wire section, pulp dryer	5×10^{10}	5×10^{10}
MKa	Wire section, laminating paper	7×10^6	5×10^8
Two tissue paper machines	Wire sections, filtrate tank, disc filter ($n = 5$)	$<10^6$	

Deposit samples were scraped off from surfaces of paper and board machines and stored at −20°C until used. The detection limit was $\sim 10^5$ Meiothermus 16S rRNA gene copies g^{-1} wet weight when 100 mg of the deposit sample was analyzed and diluted 1:10 for QRT-PCR. The result was interpreted below detection limit in cases where the observed crossing point exceeded 32 cycles. The investigated machines located in China, Finland, Sweden and USA

samples exceeded the threshold of detection for *Meiothermus*. The numbers of eubacterial 16S rRNA gene copies in the samples were measured using universal primers pE and pF′ (Table 2). When the number of eubacterial 16S rRNA gene copies (Table 4) is compared to that of *Meiothermus* 16S rRNA gene copy number, it is seen that in 16 of the examined deposits, *Meiothermus* was a major species, representing over 30% of the total eubacterial 16S rRNA gene copies.

Meiothermus was present in samples obtained from 18 out of the 24 machines investigated, demonstrating the widespread presence of *Meiothermus* species in paper and pulp industry processes. It was found in the mills from all countries investigated.

Amplification of eubacterial and genus *Meiothermus* 16S rRNA gene copies from paper and board products

The PCR method was used to analyze the role of microorganisms in the quality defects visible as aberrant colored spots or holes in 18 paper and board products from two machines (L and R). A reference sample, taken from non-defective area of the sampled paper product, was investigated for comparison.

Of the 18 investigated defect samples, 9 were found to contain *Meiothermus* DNA (Table 5). They contained 2×10^6–4×10^7 copies of *Meiothermus* 16S rRNA genes g^{-1} of paper. In defect-free areas of the same papers the number of *Meiothermus* 16S rRNA gene copies was $<10^6$ g^{-1}. Results in Tables 4 and 5 show that *Meiothermus* containing deposits are important in the formation of end-product defects in paper industry.

In machine R, *Meiothermus* was frequent in slime deposits (Table 4). Paper product samples from this machine contained frequently 10^8 eubacterial 16S rRNA genes g^{-1} paper, in absence of visible paper quality defects. In samples of defective paper from the same machine, the amounts of bacterial 16S rRNA genes exceeded, often tenfold, those in defect-free paper, suggesting bacterial origin of the defects.

Sample L-LT1 contained practically no 16S rRNA gene copies other than those of *Meiothermus*. Also, a deposit sample from the same board machine L was *Meiothermus* positive, and contained over 10^{10}

Table 5 PCR detection of *Meiothermus* and eubacterial DNA in paper and board products

Sample	16S rRNA gene copy number g^{-1} of paper or board			
	Meiothermus primers		Primers pE and pF'	
	Spot	Clean	Spot	Clean
L-LT1	4×10^7		3×10^7	
H-050805-2	6×10^6	$<2 \times 10^5$	4×10^9	7×10^7
R-120905-2	4×10^6	9×10^5	5×10^9	3×10^8
R-120905-4	8×10^5	$<6 \times 10^5$	4×10^8	2×10^8
R-140905-2	9×10^6	$<2 \times 10^5$	3×10^8	2×10^8
R-140905-4	2×10^6	$<6 \times 10^5$	3×10^9	2×10^8
R-140905-6	1×10^7	$<2 \times 10^5$	1×10^9	2×10^8
R-140905-12	5×10^6	$<2 \times 10^5$	2×10^9	2×10^8
R-151105-2	6×10^6	$<2 \times 10^5$	1×10^{10}	1×10^8
R ($n = 9$)	$<3 \times 10^6$	$<7 \times 10^5$	3×10^8–3×10^9	2×10^7–3×10^8

Meiothermus 16S rRNA gene copies g^{-1} (Table 4). This is practically the same as the number of eubacterial 16S rRNA gene copies (= amplicons obtained using universal primers pE and pF′) in the same sample. These results indicate that the spot in the board sample L-LT1 originated from the same source as the deposit sample L.

Different species of *Meiothermus* in paper machines

16S rRNA gene sequences obtained from 25 presumptive *Meiothermus* sp. strains from six different paper and board machines were determined (Table 6). Based on sequence comparisons, most isolates were closely related to *M. silvanus* strain GY−10 or *M. ruber* strain SPS-241. One strain (S2-bf-R2A-7) was closely related to *M. chliarophilus.* Also, paper machine isolates of *Meiothermus* spp. represented only narrow phylogenic diversity (Table 6).

Discussion

Meiothermus spp., first described as paper machine contaminants by Kolari et al. [12], were in the present work shown to be common biofoulers in paper industry. This was revealed by quantitative PCR, developed in the present work for the fast quantification of this genus. *Meiothermus* was found from 18 out of the 24 investigated machines and from all four countries studied, demonstrating wide distribution of the genus in paper mills of the world. Almost one-fifth of the deposits

Table 6 16S rRNA gene sequences of presumptive *Meiothermus* and other isolates from paper and board machines

Strain	Accession number	Isolation source
Sequence closely related to *M. chliarophilus* DSM9957^T (X84212) (similarity 99.8%)		
S2-bf-R2A-7	AJ864717	Disc filter
Sequences closely related to *M. ruber* SPS-241 (AJ871172.1) (similarity 98.8–99.9%)		
L-s-R2A-4C.1	AM229087	Wire section
L-s-R2A-4B.2.1	AM229088	Wire section
L-s-R2A-4B.2.3	AM229089	Wire section
L-s-PMW-4B.2.1	AM229090	Wire section
L-s-PMW-4B.2.2	AM229091	Wire section
L-s-PMW-4C.1	AM229092	Wire section
L-s-PMW-11.1	AM229093	Disc filter
L-bf-PMW-16.2.2	AM229094	Press felt
L-bf-PV-PMW-3B.1	AM229095	Wire section
L-s-R2A-3B.2	AM229096	Wire section
L-bf-PV-PMW-3B.2.3	AM229097	Wire section
L-bf-PMW-16.1.2.1	AM229098	Press felt
JN41005	AM229099	
Sequence closely related to *M. ruber* SPS-242 (AJ871173.1) (similarity 98.8%)		
C-PA5-5c[a]	AM229102	
Sequence closely related to *M. ruber* 16106 (MRY13597) (similarity 99.6%)		
E-steel-R2A-2[a]	AJ864718	
Sequences closely related to *M. silvanus* GY-10 (MSY13599) (similarity 99.9%)		
L-s-R2A-4C.2	AM229086	Wire section
B-maf-R2A-1[a]	AM229100	
B-maf-R2A5-3[a]	AM229101	
C-jv-PA-1[a]	AM229103	Cooling water
B-R2A-5[a]	AM229104	
B-R2A-6[a]	AM229105	
B-R2A5-50-1[a]	AM229106	
B-R2A5-50-3[a]	AM229107	
B-R2A5-50-4[a]	AJ864719	
Sequence closely related to *Burkholderia multivorans* ATCC17616 (AB092606) (99.5%)		
F28L1	AJ864720 (1484 nt)	
Sequence closely related to *Deinococcus geothermalis* E50053 (AJ000002) (99.9%)		
E50051	AJ864721 (1461 nt)	
Sequence closely related to *Pseudomonas boreopolis* ATCC33662^T (AB021391) (99.5%)		
S2-s-PMWA-6	AJ864722 (1497 nt)	
Sequence closely related to *Pseudoxanthomonas taiwanensis* ATCCBAA404^T (AF427039) (similarity 99.9%)		
J-M	AJ864723 (1499 nt)	

The lengths of the sequenced 16S rRNA genes were 1,438–1,445 bp; exceptions indicated in the table

[a] Kolari et al. [12]

contained $\geq 10^9$ *Meiothermus* 16S rRNA gene copies g^{-1} and in over 10% of the samples *Meiothermus* was dominant comprising more than 30% of total bacterial 16S rRNA genes. *Meiothermus* spp. thus can be considered as a major former of biofilms in paper machines.

The highest amount of *Meiothermus* 16S rRNA gene copies detected in this work in deposits was 10^{11} g^{-1} wet weight. In some deposits it looked like *Meiothermus* was the only bacterial taxon present, i.e. *Meiothermus* spp. seem capable of forming almost monophyletic high-density biofilms in paper machines.

We demonstrated in this paper that DNA methods are useful for establishing whether or not end-product defects are of bacterial origin: when the number of 16S rRNA gene copies in a spotted area exceeds that in spotless reference area of the same paper product, bacteria are likely the reason. This invites for searching the machine wet-end and raw materials to disclose the area from which the defect-causing bacteria originated. To our knowledge, this is the first time the connection between the bacterial DNA content and spots in end products is demonstrated. DNA methods have previously been used with paper industry samples by Priha et al. [21]. They applied *Bacillus cereus*-specific primers [7] to assess the presence of this species in paper industry products.

The total number of 16S rRNA gene copies ranged from 10^7 to 10^{11} g^{-1} in different samples. In this study we used universal primers pE and pF′ [6] for eubacterial quantification because these primers have successfully been used for 16S rRNA gene sequencing in our and other studies. Other universal primers for bacteria have also been published [9, 15, 16]. For calculations we used genome sizes of 3.2 and 2.5 Mb for *D. geothermalis* and *M. silvanus*, respectively, and the 16S rRNA gene copy number/genome was assumed as 2 for both species. The actual numbers are currently not available. It also is well known that amplification efficiencies may vary between genera and species [27], inducing uncertainty in the outcome of Q-PCR. However, this uncertainty is unlikely to be higher than that involved in the use of culturing methods.

Meiothermus species have earlier been described from hot fermentors fed with wastewater [18] and spent nuclear fuel storage systems [14] as well as inhabiting thermal springs. Their optimal growth temperature is 50–65°C and pH 7–8.5 [3, 4, 17, 20, 24], which are common in paper machine water systems. *M. chliarophilus* and *M. timidus* are known as starch degraders [20]. Starch is a readily available carbon source for bacteria in paper machines. It is interesting that *Meiothermus* DNA was found in large amounts in an industrial environment, for example wire sections, disc filters and water tanks of paper machines. Other inhabitants of thermal springs have previously been isolated from paper machines as well [12, 23].

Of the 25 *Meiothermus* strains isolated from the paper industry, all except one were closely similar in 16S rRNA gene sequence to the species *M. ruber* or *M. silvanus*, indicating that these two species may represent the main *Meiothermus* contaminants in paper industry.

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