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Cultural and biochemical diversity of pink-pigmented bacteria isolated from paper mill slimes

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A study of 25 paper mill slime deposits and one additive revealed nine pink-pigmented bacterial isolates, eight of which were different from pink-pigmented bacteria identified in the paper industry in the middle 1900s. The pink-pigmented bacteria described previously in pulp and paper included *Micrococcus agilis*, *Bacillus subtilis*, *Serratia* sp. and *Alcaligenes viscosus*. With the exception of one isolate, *Micrococcus* sp., these isolates possessed many cultural, biochemical and chemical properties which were different from the ones previously reported for paper mills. Eight of these bacteria were Gram-negative rods or filamentous, aerobic and positive for catalase production. Two isolates were methylotrophic, oxidizing methanol and identified as *Methylobacterium zatmanii*. Cellular fatty acid analysis and other characteristics showed one isolate to be *Roseomonas* sp. Using 16S rRNA gene sequencing, one isolate which was a Gram-negative rod was identified as *Deionococcus grandis*. Four bacteria had cells that were long or filamentous and these were isolated from mills with pink slime problems. The identity of one of the filamentous bacteria was determined by 16S rRNA gene sequencing to be close to *Flectobacillus* sp. strain MWH38. Most of the isolates were susceptible to 11 industrial biocides. *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 74–80.

Keywords: pink slime; pink-pigmented bacteria; paper mill slime; biocides; *Flectobacillus* sp.; *Methylobacterium* sp.; *Roseomonas* sp.; *Deionococcus grandis*

Introduction

The uncontrolled growth of microorganisms in paper mills results in the formation of deposits called slime. Slime formation on paper machines can lead to many problems [28], which ultimately impair the efficiency of the papermaking process as well as the quality of the finished product. One such undesirable consequence is the production of pink stains in paper, which adversely affects quality. Pink slime was recognized in the middle 1900s as the cause of pink stains in paper and this was traced to certain pink-pigmented fungi [13,14,29] and bacteria [1,29]. The pink bacteria believed to have caused the problem included *Serratia marcescens*, *Serratia plymuthica*, a strain of *Bacillus subtilis*, *Micrococcus agilis* and *Alcaligenes viscosus*.

Since that period, published information about the nature of bacteria that cause pink slime in the pulp and paper industry has been scanty even though the problem persists in some paper mills [2]. It is also interesting that in the intervening period there have been changes and advances in papermaking technology. Paper-machine speed has increased and with these increases have come different kinds of chemicals to help solve drainage, retention and deposit problems [18,31]. There has been a shift of papermaking from acid to alkaline or neutral conditions. The possible microbiological implications of this shift have been described [7,12]. Environmental concerns have led many mills to close their white water systems. This, in turn, has resulted in an increase in the supply of nutrients necessary to support the growth of microorganisms [4,17,27]. The use of post-consumer recycled fiber for the manufacture of paper has increased recently. This has affected the types of microorganisms found on the paper machine since this source of fiber tends to be microbiologically contaminated. Additionally, biocides and other programs used to control microorganisms have moved from harsh and toxic compounds to compounds which are relatively less toxic to humans and the environment [6].

All of these changes are expected to affect the kinds and number of microorganisms that grow in the paper mill systems. Information about changes in microbial populations on the paper machine will not only help in understanding how these organisms are controlled but could also give us some insight as to whether or not there is a potential health risk.

This work was done to gain an understanding of the nature of pink bacteria that constitute pink slime in some modern paper machines and the papermaking environment and also to compare the minimum inhibitory concentrations of some of the biocides that are currently used to control bacteria in paper mills.

Materials and methods

Biocides

All the biocides used were commercial products used in industrial microorganism control. With the exception of the one identified below, they were all obtained from Buckman Laboratories International, Memphis, TN. The active ingredients in the formulations are identified as follows: (A) 2-bromo-4′-hydroxyacetophenone; (B) a mixture of 2-(thiocyanomethylthio)-benzothiazole and methylene-bis(thiocyanate); (C) 2-(thiocyanomethylthio)benzothiazole; (D) a mixture of disodium cyanodithioimidocarbonate and potassium *N*-methyldithiocarbamate; (E) methylene-bis(thiocyanate); (F) 1,4-bisbromoacetoxy-2-butene; (G) a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and methyl-4-isothiazolin-3-one; (H) 2-bromo-2-nitro-

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propane-1,3-diol, obtained from Angus Chemical Company, Northbrook, IL, USA; (I) 2,2-dibromo-3-nitrilopropionamide; (J) potassium dimethyldithiocarbamate; (K) 3,5-dimethyl-1,3,5(2*H*)-tetrahydrothiadiazine-2-thione.

Isolation of organisms

Twenty-five samples were aseptically collected from various paper mills, placed in sterile Ziploc bags and transported to the laboratory under ambient conditions. In the laboratory, samples were analyzed as follows: 3 g of each sample were added to 30 ml 0.85% sterile saline and thoroughly mixed by vortexing them. The mixed samples were spread-plated on plate count agar (Difco Laboratories, Detroit, MI, USA), actinomycete isolation agar (Difco Laboratories, Detroit, MI, USA) and potato dextrose agar (Difco Laboratories, Detroit, MI, USA) and the plates were incubated at 25°C or 30°C for 1 week. Pink or red-pigmented colonies that appeared on these plates were carefully picked and re-streaked on Plate Count Agar to check for purity. Isolated pink bacterial colonies were maintained on plate count Agar.

Cultural and biochemical/physiological studies

Gram stain (Hucker modification) was made from cultures on plate count agar after 18–24 h of growth. Spore test (Schaeffer–Fulton method) was made on 5–7 day old cultures. The oxidase test was done with SpotTest oxidase reagent (Difco). Acid production from various carbohydrates was determined after 7 days of growth of the organisms on the OF basal medium according to the formula of Hugh and Leifson [16]. Acetate differential medium (Becton Dickinson and Company, Cockeysville, MD, USA) and Simmons citrate agar (Difco Laboratories, Detroit, MI, USA) were used to determine the ability of the organisms to utilize acetate and citrate respectively. Esculin hydrolysis, phenylalanine deaminase, DNase, and urease production were determined with media purchased from Difco. Starch hydrolysis was determined with starch agar containing (g/l) 23 g nutrient agar, 10 g soluble starch and 1 l of deionized water. Indole nitrite medium (BBL) was used for both indole production and nitrate reduction tests. Growth at various temperatures was determined in nutrient broth held in a water bath at the specified temperature and observed daily for 7 days. For growth at 4°C, the culture was placed in a refrigerator. The ability of the organisms to grow in sodium chloride was studied by inoculating the respective bacteria in sterile nutrient broth containing 6% (w/v) sodium chloride at 30°C and observing for the presence or absence of growth daily for 7 days. Hydrogen sulfide production was studied using triple sugar iron agar (TSI).

Utilization of carbon sources

The basal medium of Palleroni and Doudoroff [23] was used to study how the isolates utilized various carbon sources. The medium was solidified with 1.5% (w/v) purified agar (BBL). The carbon sources were filter sterilized and added to the basal medium at a final concentration of 0.1% (w/v). Bacterial suspensions were prepared from a 2-day-old culture and used as the inoculum. This was done by using a sterile cotton applicator to carefully remove bacterial cells from the surface of the agar plate in such a way as to avoid carry-over of nutrients from the plate. The cells were then mixed by vortexing the suspension in 0.85% sterile saline. Sterile cotton swabs were used for inoculation with one swab used for each plate. To determine that carry-over of nutrients from the bacterial suspension was not a problem, the mineral salts–agar base was inoculated with the respective bacteria. Inoculated plates were then incubated at 30°C and examined after 1- and 2-week intervals.

Microscopy

The morphology of the bacterial cell was studied with a Nikon Labophot microscope and with a scanning electron microscope. To prepare samples for the scanning electron microscope, a pure culture of bacteria was swabbed on trypticase soy agar and then three 1/2-in. squares of sterile paper were aseptically placed on the agar surface. The petri dishes were incubated for 2–3 days at 30°C. After incubation, the pieces of filter paper were placed in small bottles for processing. Processing began with the samples being rinsed in 0.1 M cacodylate buffer followed by fixation in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least 24 h in a refrigerator. After gutaraldehyde fixation, the cells were rinsed three times in 0.1 M cacodylate buffer for 10 min per rinse. Post-fixation was done for 1 h in 1.5% osmium tetroxide in 0.1 M cacodylate buffer followed by three rinses in the same buffer. The cells were then dehydrated using a graded series of ethanol: 30%, 50%, 70%, 85%, 95%, 100% and 100%. The samples were critical-point dried, mounted on specimen stubs and sputter-coated with gold–pallidium.

The samples were examined with a Hitachi S-2460N scanning electron microscope.

Fatty acid analysis

Cellular fatty acids were prepared as described previously [5] and were analyzed using a Hewlett-Packard gas chromatograph equipped with a flame ionization detector, an autosampler, an integrator, a computer and a 5% methyl phenyl silicone capillary column (Hewlett-Packard Co., Palo Alto, CA, USA). To identify the bacteria, the fatty acid profile generated for each organism was compared with the bacterial identification database, TSBA, version 4.10 (MIDI Labs, Newark, DE, USA).

16S rRNA gene sequence and data analysis

16S rRNA gene sequences of isolates P3 and Td were generated using the protocol in the MicroSeq 16S rRNA Gene Kit (PE Biosystems, Foster City, CA, USA). Sequence analysis was performed using PE Applied Biosystems MicroSeq microbial analysis and database. In the case where there was no match, databases of GenBank and Ribosomal Database Project were searched for a possible match.

Biocide testing

The biocides were evaluated in dilute nutrient broth (2.5 g/l) supplemented with 0.1% glucose. The nutrient broth was added to test tubes in 5-ml amounts and autoclaved at 121°C for 20 min. Biocides in the desired concentrations were then added to 5 ml of sterile broth. To prepare an inoculum, an isolate was grown for 48 h on plate count agar. A suspension of the culture was made in sterile 0.85% sterile saline and then 100 μl of the inoculum was added to the tubes containing the biocides. The final bacterial concentration ranged from $(2.0 \text{ to } 5.2) \times 10^6$ cfu/ml. The tubes were incubated at 30°C and the minimum inhibitory concentration (MIC), based on visual inspection of growth or no growth, was recorded after 24 h. MIC was defined as the lowest

Table 1 Sources of pink bacterial isolates

Isolate	Source	Indication of pink slime?
A1	unknown[a]	Yes
A2	white water flume	Yes
A3	white water flume	Yes
Tb	white water flume	Yes
P3	underfoil	No
Q6	white water filter	No
Cl	kaolin clay	No
Td	former save-all pan	Yes
N3	underfoil	No

[a]Location of sampling was not specified.

concentration of the biocide at which there was no visible bacterial growth.

Results and discussion

Nine pink or red bacteria were isolated from the pulp and paper mill environment. Five of the isolates (A1, A2, A3, Tb, Td)) came from deposits at mills that were having problems with pink slime. Three isolates (N3, P3, Q6) were isolated from slime deposits taken from mills that were not experiencing pink slime problems at that time. The remaining isolate, Cl, was isolated from a kaolin clay sample. Specific areas in the mill from where each sample was secured can be found in Table 1.

The results of the growth and biochemical studies are summarized in Tables 2 and 3. All bacteria grew aerobically and produced nondiffusible red or pink pigment. All were Gram-negative rods except N3 which was a Gram-positive coccus. All were catalase positive and all grew well in nutrient broth at 30°C. None of the isolates produced spores. Using cultural, biochemical and chemical characteristics, the isolates were differentiated into five groups.

Isolate N3 was composed of Gram-positive cocci with cells arranged in fours and twos, oxidase positive with orange to red colonies and identified as *Micrococcus* sp. [30]. *M. agilis* has been described previously [29] as one of the bacteria that made up the flora of pink bacteria in paper mills.

Isolates Tb and Cl grew oxidatively on methanol. Isolate Tb was a short rod with cell length ranging from 1 to 2 μm and a cell width of about 0.5 μm. Cell length for Cl ranged from 2 to 3 μm and the

Table 2 Morphological and biochemical characteristics of isolates

	Isolates								
Test	A1	A2	Tb	A3	N3	P3	Q6	Cl	Td
Morphology	R/F	R/F	R	R/F	C	R	R	R	R/F
Gram reaction	−	−	−	−	+	−	−	−	−
Pigment	p/r	p/r	p/r	p/r	o/r	p/r	p/r	p/r	p/r
Oxidase	+	+	+	+	+	−	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
Motility	nd	+	+	+	nd	−	+	+	−
Spore	−	−	−	−	−	−	−	−	
Nitrate reduction	−	−	−	−	−	−	+	−	−
Nitrite reduction	−	−	−	−	−	−	−	−	−
Indole	−	−	−	−	−	−	−	−	−
Urease	−	−	+	−	−	−	+	+	−
Simmons citrate	−	−	+	−	−	−	−	−	−
Sodium acetate	nd	−	+	−	nd	−	+	−	−
H_2S	−	−	−	−	−	−	−	−	−
Hydrolysis									
Starch	+	nd	−	nd	−	+	−	−	+
Esculin	+	+	−	+	nd	+	+	−	+
Phenylalanine deaminase	−	−	−	−	−	−	−	−	−
DNase	−	−	−	−	nd	−	−	−	−
Growth									
25°C	+	+	+	+	+	+	+	+	+
30°C	+	+	+	+	+	+	+	+	+
42°C	−	−	+	−	nd	+	+	−	−
On MacConkey	−	−	−	−	−	−	−	−	−
On cetrimide	−	−	−	−	nd	−	−	−	−
Nutrient broth	+	+	+	+	+	+	+	+	+
NB+6% NaCl	−	−	−	−	nd	−	−	−	−
Acid from (OF base)									
Glucose	nd	−	−	−	−	−	−	−	+
Arabinose	nd	−	−	−	−	−	−	−	+
Xylose	nd	+	−	−	−	−	−	−	+
Methanol (1%)	nd	−	+	−	−	−	−	+	−
Lactose	nd	+	−	−	−	−	−	−	+
Sucrose	nd	+	−	−	−	−	−	−	+
Maltose	nd	+	−	−	−	−	−	−	+
Mannitol	nd	−	−	−	−	−	−	−	−
Raffinose	nd	−	−	−	nd	−	−	−	+

p/r=pink/red; R=rods, F=filamentous, C=cocci; +=positive reaction; −=negative reaction; nd=not determined; OF base=oxidative–fermentative base; NB=Nutrient broth; o/r=orange/red.

Table 3 Utilization of carbon sources by five pink-pigmented bacteria

	Isolates				
Carbon source	Tb	P3	Q6	Cl	Td
Methanol	+	–	–	+	–
Glucose	–	–	–	–	+
D-Xylose	–	–	–	–	+
D-Lactose	–	–	–	–	+
D-Mannitol	–	–	–	–	–
Raffinose	–	–	–	–	+
Glycerol	+	–	+	+	ND
Sodium formate	+	–	–	+	–
L-Arabinose	+	–	–	–	+
Sucrose	–	–	–	–	+
Maltose	–	–	–	–	+
D-Fructose	+	–	+	+	+

ND = Not determined; + = Positive; – = Negative.

cell width was about 1 μm. Both were positive for urease production. Isolate Tb grew on Simmons citrate and acetate but isolate Cl did not. Both organisms were motile. Vacuoles were observed in the cells of Cl but not in Tb. The cellular fatty acid profile (Table 4) for these two bacteria were nearly identical. The ability of these two organisms to grow oxidatively on methanol as the sole carbon source and their fatty acid profile together with other biochemical and cultural characteristics summarized in Tables 2 and 3, enabled them to be identified as *Methylobacterium zatmanii* [8,32].

Species of *Methylobacterium* are common and have been isolated from a number of environments including leaf surfaces [3], hospital environments [9], and lakes [24]. However, the presence of such organisms in paper mill deposits has not been reported previously.

Water could be one of the sources of *Methylobacterium* in paper mill deposits. Water is one of the major ingredients used to make paper, and water from a variety of sources including lakes, rivers, wells and springs is used. Incoming water is usually treated with chlorine or other oxidizing biocides; but even where this is the case, some *Methylobacterium* species could resist disinfection as evidenced by the significant number of *Methylobacterium* species in many water sources observed by Hiraishi *et al.* [11] to be resistant to chlorine.

Isolate Q6 was composed of Gram-negative rods with cell length ranging from 1 to 2 μm and width 0.5 to 1 μm. This isolate was motile, urease and oxidase positive. It did not utilize methanol as the sole source of carbon. The cellular fatty acid profile and other characteristics (Tables 2 and 3) observed for this isolate placed Q6 in the genus *Roseomonas*. Organisms of this taxon were recently named *Roseomonas* [26] to differentiate them from *Methylobacterium*, which are able to oxidize methanol. *Roseomonas* species have been isolated primarily in the hospital environment [21,26] and this is the first time they have been reported in the paper mill environment.

Isolate P3 was a Gram-negative rod, with most cells arranged in pairs and a few in chains (Figure 1). Cell length ranged from 1 to 2.5 μm and the cell width was 0.5 to 1 μm. Acid was not produced on OF media. None of the carbon sources studied was utilized as a sole source of carbon. Esculin and starch were hydrolyzed. Partial 16S rRNA gene alignment with GenBank database produced a match of 97% with *Deionococcus grandis*, accession number Y11329 [25]. This organism has been described [15,22] and a separate genus, *Deionobacter*, was initially proposed to differentiate it from *Deinococcus* sp., which are Gram-positive cocci. Based on evidence from ribosomal RNA, the taxonomy of this group was recently revised placing *Deionobacter* sp. in the genus *Deionococcus* [25]. Though *D. grandis* has been isolated from animal feces and freshwater fish [22], its occurrence in paper mill deposits has not been reported previously.

Isolate Td had long cells or were filamentous and unbranched. Most cells were straight with only a few of them slightly curved (Figure 2). Cell width was approximately 0.5 μm and the length ranged from 3 to 10 μm. Filaments greater than 10 μm were also observed. Catalase reaction was positive but weak. The cells were longer for freshly grown organisms and the length appeared to get shorter after prolonged incubation. Additionally, the pink coloration disappeared with older cultures. Under the microscope, stained cells tended to aggregate. This isolate was obtained from a paper mill that was having problems with pink slime. Potato dextrose agar, plate count agar, and actinomycete isolation agar all proved useful in isolating these organisms. Incubating samples on these media for 7 days at 25°C proved optimal in isolating these filamentous organisms. These organisms were aerobic, positive for oxidase, and they hydrolyzed starch and esculin. As shown in Table 3, Td utilized many compounds as sole sources of carbon. The

Table 4 Fatty acid composition of five pink-pigmented bacterial isolates

	Percent fatty acid in isolates				
Fatty acid	Tb	P3	Q6	CL	Td
13:1	–	–	–	–	1.03
14:0 *i*	–	–	–	–	0.26
14:0	–	–	–	–	6.06
14:0 2OH	–	–	–	–	1.53
15:0 *i* 3OH	–	–	–	–	3.39
15:0 *i*	–	4.66	–	–	13.48
15:0 *a*	–	–	–	–	0.91
15:1 ω6c	–	7.40	–	–	–
15:0	–	3.90	–	–	0.70
16:0	2.53	19.55	4.85	2.09	9.45
16:1ω7c/15 *i* 2OH	–	43.01	8.84	–	8.57
16:1 ω5c	–	1.88	0.43	–	40.92
16:1 ω9c	–	–	0.55	–	–
16:0 2OH	–	–	–	–	1.75
16:0 3OH	–	–	4.33	–	6.28
16:1 *i*/14:0 3OH	3.21	–	5.36	2.55	0.80
17:1 *i*/*a*	–	2.32		–	–
17:0 *i*	–	5.35	–	–	0.75
17:1 *a* ω9c	–	–	–	–	0.54
17:1 ω8c	–	1.99	–	–	–
17:1 ω6c	–	3.52	0.53	–	–
17:0	–	5.37	–	–	–
17:0 *i* 3OH	–	–	–	–	2.06
18:1 ω7c	88.77	1.03	70.05	90.17	–
18:1 ω9c	–	–	0.72	–	–
18:0	4.20	–	–	4.23	–
18:1 2OH	–	–	4.34	–	–
18:0 3OH	1.28	–	–	–	–
Unknown	–	–	–	0.97	1.03

The number to the left of the colon indicates the number of carbon atoms and the number on the right of the colon indicates the number of double bonds. The position of the double bond is indicated by ω#c. Thus, ω6c indicates that the double bond is located at position 6 from the acyl group. In straight chain fatty acids, *i* and *a* indicate iso and anteiso respectively. 2OH and 3OH indicate the positions of the hydroxyl group in hydroxylated fatty acids.

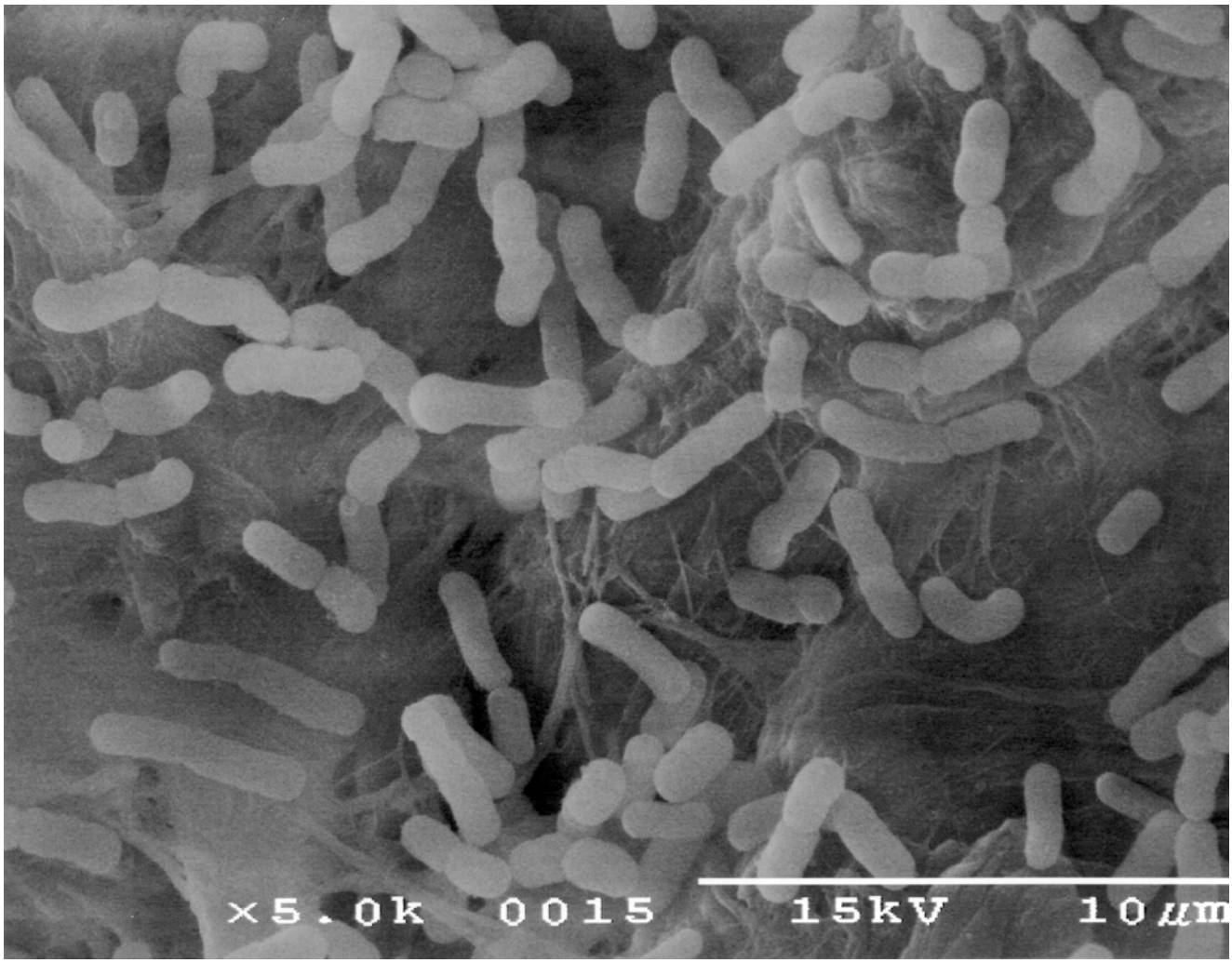

Figure 1 Scanning electron micrograph of isolate P3 (*D. grandis*). Cells are rod-shaped and occur mainly in pairs. Bar = 10 μm.

organism did not grow at a salt concentration of 6%. The organism grew very well at 30°C, relatively slowly at 25°C and failed to grow at either 4 or 42°C.

The cellular fatty acid profile for Td is shown in Table 4. Many different fatty acids were produced by this organism with the most prominent fatty acid being 16:1 ω5c.

Partial 16S rRNA gene alignment with Genbank database gave a match of 99% with *Flectobacillus* sp. strain MWH38, accession number AJ011917 [10]. This strain of *Flectobacillus*, while noted [10] to have some differences in cell morphology from *Flectobacillus major*, was however reported [10] to be related to the latter based on 16S rRNA gene sequencing. The straight cell morphology observed for Td resembled that reported for *Flectobacillus* strain MWH38 more than the curved cells of *Flectobacillus major* [20]. The carbon utilization pattern and other biochemical characteristics (Tables 2 and 3), however, were not different from those reported for *Flectobacillus major* [20].

Flectobacillus sp., as described previously, have been isolated from freshwaters [20] and eutrophic lake water [10] but their presence in paper mill deposits has never been published.

The observations made for the isolate, together with results published previously [20] for *Flectobacillus major*, indicate that the organism grows on a wide variety of carbohydrates. The paper mill, with its abundant supply of carbohydrates, would be a suitable habitat for such an organism. Though *Flectobacillus* sp. are reported to be unable to utilize cellulose [20], an abundant source of carbohydrates in paper mills, a number of cellulolytic organisms exist in this environment that could break cellulose into simpler forms, making them available to *Flectobacillus*.

Isolates A1, A2 and A3 were also long or filamentous but failed to grow after a few days of isolation and so could not be completely characterized. The partial characteristics observed for A1, A2 and A3 are shown in Table 2.

The MIC values for 11 biocides versus all nine isolates are shown in Table 5. In general, low MIC values were obtained for the biocides against most of the isolates. The MIC ranged from <0.1 to 40 ppm. At least 10 biocides provided MIC values <10 ppm for five or more of the isolates.

There was some variation in the way each biocide inhibited the isolates. In a number of cases, the MIC for a particular biocide varied for many of the isolates. Many of the isolates were from different genera of bacteria and as a result, their sensitivities to different biocides might be different. In addition, many of the

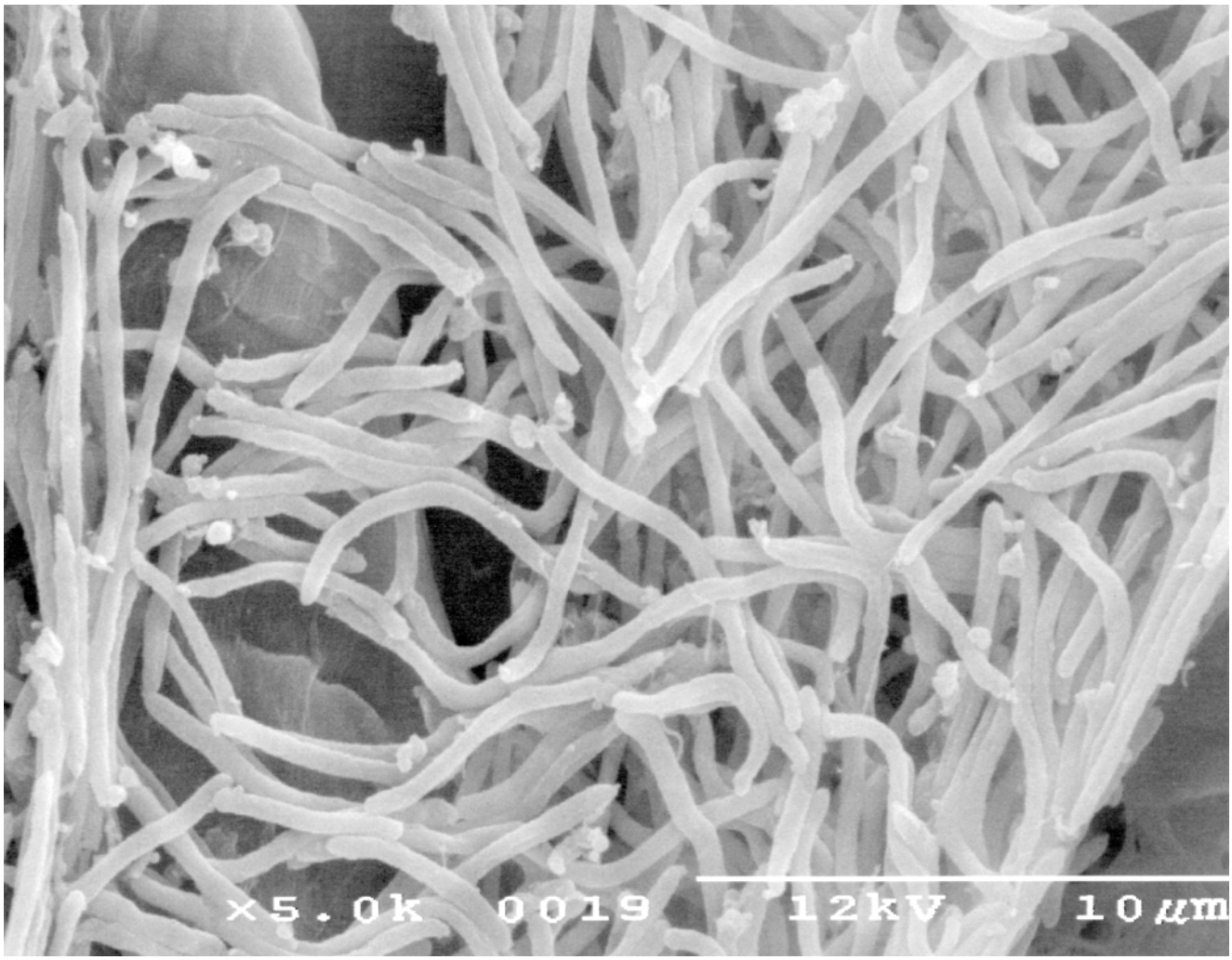

Figure 2 Scanning electron micrograph of isolate Td (*Flectobacillus* Sp). Note the mostly straight cellular morphology of this isolate. Bar = 10μm.

isolates were obtained from mills with varying processing conditions including the use of biocides and other chemicals that can influence the susceptibility of an organism to certain biocides.

This study has shown that pink-pigmented bacteria in paper mills are represented by different genera of bacteria, many of which have not been reported before for this environment. Two of these

Table 5 *In vitro* susceptibilities of the isolates to industrial biocides

	Isolates								
Biocides[a]	A1[b]	A2[b]	Tb[c]	A3[b]	N3[b]	P3[c]	Q6[c]	Cl[c]	Td[c]
A	0.3	0.3	2.5±0.71	0.3	1.5	0.75±0.0	0.3±0.0	0.75±0.00	0.75±0.00
B	1	1	0.5±0.0	1	1	2.0±0.0	0.4±0.14	0.5±0.00	0.83±0.24
C	0.75	1.5	0.75±0.0	nd	1.5	0.3±0.0	0.3±0.0	0.75±0.00	0.75±0.00
D	3.0	1.5	7.5±0.0	0.3	15	3.0±0.0	0.6±0.21	12.5±3.53	10.0±3.53
E	0.5	0.5	0.25±0.0	0.3	1	1±0.0	0.25±0.0	0.67±0.24	0.67±0.24
F	2	2	8±0.0	0.8	8	6.66±1.89	4.0±0.0	2±0.00	8.0±0.00
G	0.08	0.15	0.23±0.11	0.08	0.75	0.75±0.0	2.0±0.71	0.23±0.11	0.23±0.11
H	1	4	8±2.8	nd	4	10±0.0	1.33±0.47	40±0.00	8±2.83
I	5	2	13.33±4.71	2	20	4±1.41	2±0.0	13.33±4.71	5.0±0.00
J	12.5	0.5	0.75±0.35	nd	12.5	16.67±5.89	1±0.35	16.67±5.89	0.5±0.00
K	6	1.2	6±0.0	0.6	6	4.8±1.70	0.36±0.17	8.0±2.83	6.0±0.00

[a]See Materials and Methods section for description of biocide. Biocide concentrations are in parts per million of active ingredients.
[b]Data available for one study only. Organisms became nonviable.
[c]Data represent means±SD of three independent measurements.
nd, not determined.

bacteria, *Roseomonas* and *Methylobacterium* spp. have been found in the hospital environment and some species have been isolated from people with certain diseases [19,21,26]. More work would be needed to ascertain how widespread these two organisms are in paper mills before a meaningful conclusion about their potential health hazard could be made. Lastly, the information presented about pink-pigmented bacteria should be valuable in controlling these organisms in paper mills.

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