Molecular identification and corrosion behaviour of manganese oxidizers on orthodontic wires

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In the present study manganese oxidizing bacteria (MOB) were isolated and identified using molecular techniques (16S rRNA gene sequencing) and the electrochemical behaviour of these isolates on orthodontic wires was studied by employing polarization and impedance techniques. *Staphylococcus aureus* (EF516983), *Bacillus pumilus* (EF516984), *Planococcus rifitioensis* (EF516985), *Lysinibacillus boronitolerans* (EF516986), *B. fusiformis* (EF516987), *L. boronitolerans* (EF516988) and *B. thuringiensis* (EF516989) were identified as manganese oxidizers in orthodontic appliances. It is interesting to note that ten control samples did not have any manganese oxidizers. It reveals that stainless steel enhances the proliferation of manganese oxidizers and accelerates the corrosion of orthodontic wires. Since manganese is toxic to human beings and causes enhancement of nervous disorder, an in-depth study is needed in future.

Keywords: Corrosion, electrochemical studies, manganese oxidizing bacteria, orthodontic wires, 16S rRNA sequencing.

STAINLESS alloys have been used as orthodontic wires with a wide range of applications in both fixed and removable appliances[1]. Corrosion on orthodontic materials may be caused by an electrolyte such as saliva. Saliva has several viruses, bacteria, yeast and fungi and their products, such as organic acids and enzymes, epithelial cells, food debris and components from gingival crevicular fluid[1–3]. Factors such as temperature, quantity and quality of saliva, plaque, pH, proteins, physical and chemical properties of food and liquids, and oral health conditions may influence corrosion[4]. Matasa[5] explained the corrosion of orthodontic appliances which may be uniform, localized or pitting, crevice and inter-granular. During the past few years, there has been a broadening of interest in the use of implantable materials, viz. metals, ceramics and polymers, and devices in reconstructive oral surgery[6,7]. Hence, the orthodontic wires were evaluated by employing chemical and mechanical factors by various investigations[8–16]. Nickel was reported to be moderately cytotoxic, while chromium was considered to have little cytotoxicity in a human cell culture study[17–19]. Apart from nickel and chromium, manganese (0.48–20%) is also present in orthodontic wires[20]. Release of metallic element from almost all types of alloy has been documented. But no literature has been found regarding the action of manganese in saliva. Generally 5–7 ppm is needed for physiological activity[21] of human beings. Proteins depending on manganese present in the saliva were reported by many investigators[22,23]. If manganese becomes excess, it enhances the possibility of nervous disorder[24]. Hence, the importance of manganese oxidizer in the oral cavity is important in the medical field. The interrelationship between materials and bacterial distribution is unknown in orthodontic literature. Though several corrosion studies and those on the electrochemical behaviour of dental alloys have been carried out, no study has been carried out in the presence of manganese oxidizing bacteria (MOB) on orthodontic appliances, one of the most important bacterial communities involved in microbiologically influenced corrosion. The present study focuses on the identification of manganese oxidizers by 16S rRNA gene sequencing and electrochemical behaviour of MOB on orthodontic wires.

Saliva samples were collected from 15 to 20 aged persons wearing stainless steel orthodontic wire. The 12 h old biofilm was gently scrapped using a sterile brush and subsequently mouth-washed using sterile mineral water and collected. Similarly, samples were collected from persons with normal healthy teeth in the age group of 15–20 years without any orthodontic wires, to serve as control for comparative study. The food habit of the patients has not been considered in the present work. Mn agar (Hi-media, Mumbai) was used to count MOB. The biochemical characterization of bacterial isolates was done based on *Bergey's Manual of Determinative Bacteriology*[25].

The genomic DNA of the isolate was isolated as described earlier[26]. Tris-EDTA (TE) buffer and lysozyme were added to the pelleted cells and incubated for 30 min at room temperature. SDS and proteinase K were added and incubated at 55°C for 2 h. DNA was extracted with phenol, chloroform and iso-amyl alcohol, and precipitated with ethanol and dissolved in TE buffer. Polymerase chain reaction (PCR) was performed with a final volume of 50 μl in 0.2 ml thin-walled tubes. The primers[27] used for PCR amplification of the 16S rRNA gene were: 8F 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 1490R 5′-GGT TAC CTT GTT ACG ACT T-3′ (Sigma Genosys). Each reaction mixture contained 2 μl of template DNA (100 ng), 0.5 μM of two primers, and 25 μl of Enzyme Master Mix (Bioron). The PCR programme consisted of an initial denaturation step at 94°C for 5 min, followed by 34 cycles of DNA denaturation at 92°C for 30 s, primer annealing at 50°C for 1 min, and primer extension at 72°C for 2 min carried out in thermal cycler (Thermo Hybaid). After the last cycle, a final extension at 72°C for 20 min was added. The PCR products were purified using QIAquick PCR purification kit, as described by the manufacturer and cloned using QIAGEN PCR cloning

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*For correspondence. (e-mail: biocorrcecri@gmail.com) plus kit, as described by the manufacturer. Clones were selected and isolated plasmids with insert were sequenced with M13 sequencing primers using an automated sequencer ABI Biosystems.

The nucleotide database was searched with the sequences obtained using NCBI BLAST (Blastn) tool (http://www.ncbi.nlm.nih.gov/BLAST). The full length sequences obtained were matched with previously published sequences available in NCBI using BLAST[28]. Multiple sequence analysis was carried out using CLUSTAL W[29], and further NJ plot[30] and PHYLODRAW[31] were employed for constructing a phylogenetic tree. A bootstrap analysis was performed to validate the reproducibility of the branching pattern.

Manganese was estimated in the saliva samples (24 h old) collected at 6 a.m. before brushing from two orthodontic patients and two normal healthy persons (control), employing the atomic absorption spectrophotometer (VARIAN Model SPECTRAA 220).

Since the appropriate composition of SS-19 gauge wire could not be obtained from the manufacturer, the surface chemical composition of the wire was analysed using energy dispersive X-ray spectroscopy (EDX model: Noran System SIX; Thermo Electron Corporation).

SS-19 gauge wires were used for electrochemical evaluation. The appropriate composition of wires could not be obtained from the manufacturers. The wires were mounted with araldite in plastic straws, leaving only a small fixed area (1.42×10^{-3} sq. cm) for exposure to the medium; electrical contact was taken from the other end. The open circuit potential was monitored for a period of 5 h in the presence/absence of bacteria with respect to saturated calomel electrode (SCE) as the reference electrode, using a digital multimeter of high impedance for electrochemical evaluation.

Polarization was done using the above wires. The specimens were immersed for 12 h at 37°C in sterile as well as mixed bacteria-inoculated artificial saliva. Conventional three electrode cell assembly was used for polarization measurements. Cathodic polarization experiments were conducted in artificial saliva with and without bacteria using a computer-controlled potentiostat (PGP 201, Potentiostat with voltameter-1 software) in a 100 ml polarization cell. A three-electrode set-up was used consisting of test coupon as the working electrode, SCE as the reference and a platinum electrode as the auxilary. The test coupon was first immersed in the corrosion cell for ten minutes to allow equilibrium with the electrolyte. Cathodic polarization was initiated at the coupon OCP and polarized to –1000 mV vs SCE at a scan rate of 1800 mV/h. IR drop compensation was not needed since this was a high-conductivity electrolyte. As described above, polarization measurements were carried out for the orthodontic wire material. Anodic polarization was also initiated at the coupon from OCP and polarized to +1000 mV vs SCE at the scan rate of 1800 mV/h.

Impedance measurements were carried out using the above wires. The specimen was immersed for 12 h at 37°C in sterile as well as mixed bacteria inoculated in artificial saliva. Impedance studies were carried out using computer-controlled EG&G electrochemical impedance analyser (Model M6310) with software M 398. After attainment of a steady state potential, an AC signal of 10 mV amplitude was applied and impedance values were measured for frequencies ranging from 0.01 to 100 kHz. The values of R_{ct} were obtained from the Bode plots. The electrochemical studies were done by employing mixed cultures of the seven isolates in the cell density of 10^4/ml.

The enumeration of MOB is presented in Table 1. The count of MOB in the orthodontic appliance salivary sample was about 10^4. It is interesting to note that ten control samples collected from normal healthy individual did not have any manganese oxidizers. Seven morphologically different bacterial colonies were isolated from the saliva of persons with orthodontic appliances and analysed using biochemical and molecular tests. All the 7 MOB isolates were Gram-positive and rod-shaped. The hanging drop method employed was helpful in determining whether the bacterium was flagellate or non-flagellate, where one was found as non-motile. Spore-staining was useful in determining spore-forming, aerobic bacteria – *Bacillus* sp. All the isolates tested for catalase were found to be positive. It infers the fact that manganese serves as a co-factor for catalase in H_2O_2 decomposition. The results reveal that catalase activity influences the oxidization of manganese[32]. Isolated bacteria have been identified as *Staphylococcus aureus* (EF516983), *Bacillus pumilus* (EF516984), *Planococcus rifitioensis* (EF516985), *Lysinibacillus boronitolerans* (EF516986), *B. fusiformis* (EF516987), *L. boronitolerans* (EF516988) and *B. thuringiensis* (EF516989) (Table 2). Comparisons of 16S rRNA have proven to be useful for determining phylogenetic relationships among organisms from the level of domains to the level of moderately closely related species[33]. The results obtained in this study lead to the conclusion that the evolutionary relations (Figure 1) exhibited by the isolates are such that these organisms belong to the same eubacterial group. Generally, microorganisms are important agents in

Table 1. Enumeration of manganese oxidizers from seven orthodontic appliances and ten samples of control (without orthodontic wires)

Samples from orthodontic appliances	Bacterial counts (CFU/ml)
1	4.0×10^4
2	3.2×10^4
3	5.8×10^4
4	4.7×10^4
5	3.0×10^4
6	3.0×10^4
7	3.0×10^4
8–17 (Control without orthodontic wires)	Nil

Table 2. Identified manganese oxidizers in orthodontic appliances and accession numbers in GenBank

Isolation (Sl. no.)	Blast results	Accuracy of matches (%)	Accession no.
1	*Staphylococcus aureus*	99.9	EF516983
2	*Bacillus pumilus*	99	EF516984
3	*Planococcus rifitensis*	99	EF516985
4	*Lysinibacillus boronitolerans*	99.9	EF516986
5	*B. fusiformis*	99	EF516987
6	*L. boronitolerans*	99.9	EF516988
7	*B. thuringiensis*	99	EF516989

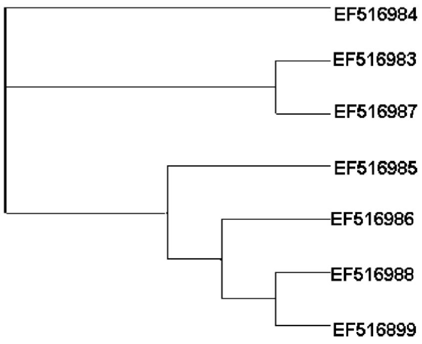

Figure 1. Phylogenetic tree for identification of manganese oxidizers collected from orthodontic appliances.

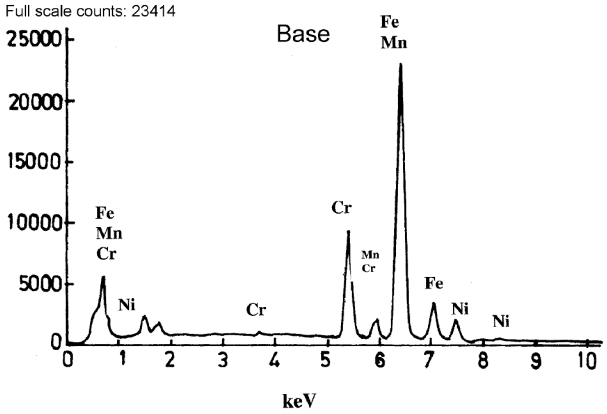

Figure 2. EDX analysis of surface chemical composition of the wire used in the electrochemical study.

the oxidation and deposition of iron and manganese. The manganese-oxidizing group is a phylogenetically diverse assemblage, which is characterized by the ability to catalyse the oxidation of divalent, soluble Mn(II) to insoluble manganese. The present study reveals that the collected saliva from orthodontic patients contained manganese in the range between 4.9 and 5.2 μg/l. Manganese was not present in two normal healthy persons. Estimation of manganese in saliva for a large number of samples has to be made. Three among the seven MOB isolates had swarming properties on the agar surface. Among MOB, those with gliding motility are significant and most of them belong to sheathed and budding bacterial groups[34,35]. However, the spore-forming genus *Bacillus* was commonly seen on all the samples studied, where the Mn concentration in biofilms was also abundant[36]. This observation further confirms the earlier report indicating that the *Bacillius* sp. requires more Mn for sporulation than during vegetative growth. Besides, the *Bacillus* sp. is involved in manganese oxidation[35]. The involvement of *Pseudomonas* and *Vibrio* belonging to the Gram-negative group, in Mn oxidation could be seen on selected substrates. Hence, it is inferred that stainless steel orthodontic wire enhances the proliferation of manganese oxidizers. This observation further confirms the earlier report by Eisenstadt *et al.*[37], who showed that *Bacillius* sp. requires more Mn for sporulation than during vegetative growth.

Table 3. Surface chemical composition of wire

Element	Weight (%)	Atom (%)
Cr	17.55	18.68
Mn	0.91	0.92
Fe	73.87	73.18
Ni	7.67	7.23
Total	100.00	100.00

Surface chemical composition of the wire is presented in Table 3 and Figure 2. The wire contained 73.18% iron, 18.68% chromium, 7.23% nickel and 0.92% manganese. It confirms that the presence of manganese may influence distribution of MOB.

Potential–time behaviour of stainless steel wires in the presence of MOB was studied in the range between –200 mV and –110 vs SCE. In the absence of microbes, the initial potential was –175 mV, which increased to –90 mV vs SCE. Microbes attack the oxide film formed on the surface of stainless steel and shift the potential to negative value.

Anodic and cathodic polarization results are presented in Figure 3. The breakdown potential in the control system was about +600 mV vs SCE. While inoculating the bacteria the breakdown potential was in the range between +420 and +500 mV. This indicates that the manganese oxidizers enhance corrosion (pitting probability) by the breakdown of the passive film. The passivation current (*ip*) in control system in two experiments was more or less the same. Fluctuation of the passivation cur-

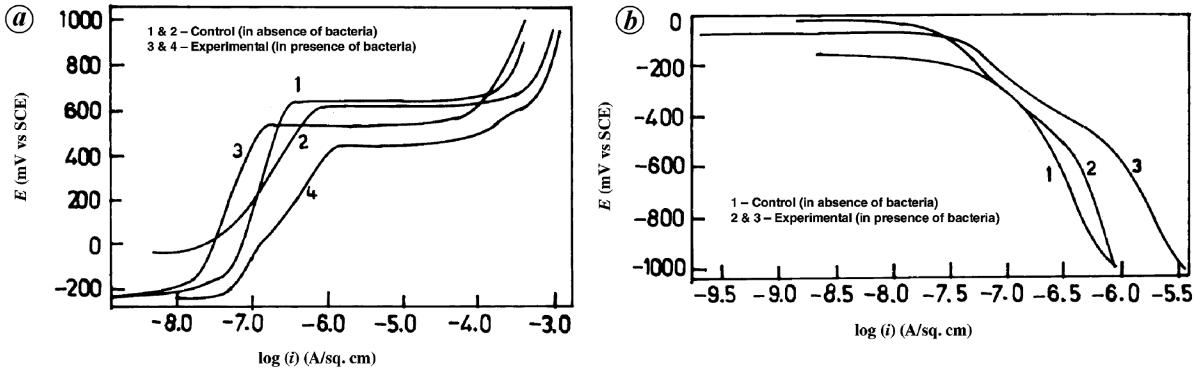

Figure 3. Anodic (*a*) and cathodic (*b*) polarization curves for SS orthodontic wire in the presence and absence of manganese oxidizing bacteria.

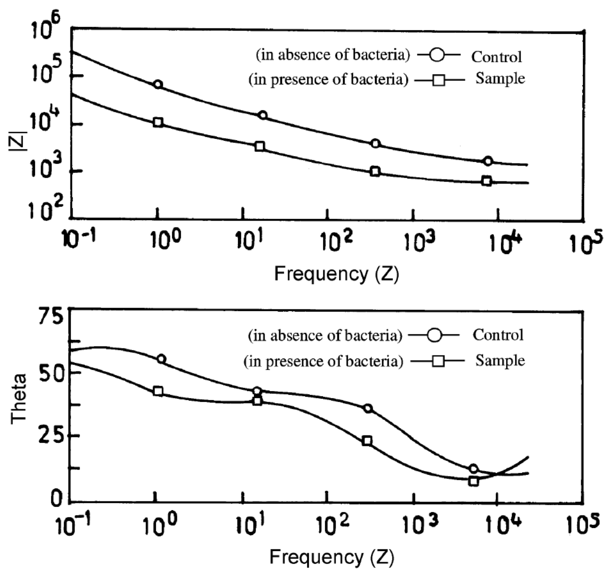

Figure 4. Impedance curve for stainless steel in the presence and absence of bacteria.

Table 4. Impedance studies for stainless steel orthodontic wires in the presence and absence of bacteria

System	Solution resistance (R_s) Ohm.cm^2	Charge transfer resistance (R_{ct}) Ohm.cm^2
Control	700	2×10^5
Bacterial inoculated system	300	2×10^4

rent in the experimental system depends upon the multiplication and attachment of bacteria on stainless steel.

Cathodic reduction current for stainless steel in the absence of bacteria was lower when compared to the bacteria-inoculated system. Higher cathodic current was noticed between –625 and –700 mV in the bacterial-inoculated system, which indicates chromium reduction.

$$Cr^{++} + 2e^- \rightarrow Cr \qquad -625 \text{ mV vs SCE.}$$

Since bacteria produce H_2O_2 during respiration, they also enhance the reduction reaction by consumption of higher current. Hence, bacteria accelerate oxygen reduction compared to the control[16]. Thompson *et al.*[38] studied the corrosion behaviour of 2205 duplex stainless steel with 0.9% sodium chloride. They found that SS-2205 had a longer passivation range than SS316L. Kao and Huang[39] studied the corrosion behaviour of orthodontic metal brackets at various pH values. A comparative evaluation of the growth of microorganisms on the surface of various orthodontic materials was made by Uppendar Kumar[40]. The electrochemical behaviour of orthodontic materials has been studied in the presence of mixed cultures of heterotrophic and chemolithotrophic bacteria. However, no study has been carried out on electrochemical behaviour of manganese oxidizers on orthodontic wires.

Change transfer resistance (R_{ct}) value for stainless steel in the presence and absence of MOB is presented in Figure 4 and Table 4. R_{ct} of control system was 2×10^5 Ohm.cm^2 and in the presence of the bacterial system, R_{ct} was 2×10^4 Ohm.cm^2. This indicates that bacteria enhance corrosion by differential aeration cells. Dexter and Maruthamuthu[41] reported that biogenic MnO_2 acts as a cathode to the parent metal, which creates an electrochemical cell on materials and accelerates the corrosion process. Hence the role of MOB on the corrosion process is important. Laurent *et al.*[42] noticed a slight reduction in polarization resistance on precious alloy and an increase with non-precious alloy in the presence of *Actinomyces viscosus*. Manganese is one of the toxic essential trace elements not only necessary for humans to survive, but it is also toxic when high concentrations are present in the human body. The recommended (5 mg) daily allowance is needed for physiological activity; when the uptake is too high, health hazards occur. The uptake of manganese by humans takes place through food such as spinach, tea, soybeans, eggs, rice, nuts, oysters, etc. After absorption in the body, manganese is transported through the blood to the liver, kidneys, pancreas and endocrine glands. Manganese affects mainly the respiratory tract and the nervous system; symptoms are hallucination, forgetfulness and nerve damage[24]. Huang *et al.*[43] estimated different metal ions released from new and recycled stainless steel brackets in the saliva. They noticed 121.9 μg/ml of manganese in pH 4 at 48 weeks immersion and 104 μg/ml in pH 7 at 48 week immersion. Nowadays, both rural and urban people use stainless steel alloys as orthodontic wires. The maximum content of manganese in the stainless steel wire and soldering material is in the range 0.48–20% (refs 20 and 44). Hence it is assumed that manganese may affect human health through oxidation by bacteria. The present study reveals the need for further investigations regarding the application of orthodontic wires.

1. Brantley, W. A., Iijima, M. and Grentzer, T. H., Temperature modulated DSC study of phase transformations in nickel–titanium orthodontic wires. *Thermochim. Acta*, 2002, **393**, 329–337.
2. Martinez, J. R. and Barker, S., Ion transport and water movement. *Arch. Oral Biol.*, 1987, **32**, 843–847.
3. Eriksson, K., Ostin, A. and Levin, J. O., Quantification of melatonin in human saliva by liquid chromatography tandem mass spectrometry using stable dilution. *J. Chromatogr. B*, 2003, **794**, 115–123.
4. Maijer, R. and Smith, D. C., Biodegradation of the orthodontic bracket systems. *Am. J. Orthod. Dentofacial Orthop.*, 1986, **90**, 195–198.
5. Matasa, C. G., Adhesion and its ten commandments. *Am. J. Orthod. Dentofacial Orthop.*, 1989, **95**, 355–356.
6. Can, R. W., Assen, P. and Kramer, E. J., *Materials Science and Technology: A Comprehensive Treatment* (ed. Williams, D. F.), Wiley-VC, Weineim, 1991, vol. 14.
7. Williams, D. F., Implantable prostheses and reconstructive materials in oral and maxillofacial surgery. *J. Dent.*, 1986, **14**, 185–190.
8. Leung, V. W. H. and Darvell, B. W., Artificial saliva for *in vitro* studies for dental materials. *J. Dent.*, 1997, **25**, 474–484.
9. Brune, D., Metal release from dental biomaterials. *Biomaterials*, 1986, **7**, 163–175.
10. Staffolani, N., Damioni, F., Lilli, C., Guerra, M., Staffolani, N. J., Castro, S. B. and Loci, P., Ion release from orthodontic appliances. *J. Dent.*, 1999, **27**, 449–454.
11. Gal, J. Y., Fovert, Y. and Adip-Yadzi, M., About synthetic saliva for *in vitro* studies. *Talanta*, 2001, **53**, 1103–1115.
12. Kim, K. and Oh, K., Corrosion properties on dental restorative alloys. In Proceedings of International Conference on Electrochemistry, SAEST, Chennai, 2002.
13. Elagli, K., Traisnel, M. and Hildebrand, H. F., Electrochemical behaviour of titanium and dental alloys in artificial saliva. *Electrochim. Acta*, 1993, **38**, 1769–1774.
14. Ameer, M. A., Khamis, E. and Al-Motlaq, M., Electrochemical behaviour of non-precious dental alloys in bleaching agents. *Electrochim. Acta*, 2004, **50**, 141–148.
15. Duffo, G. S. and Quezada Castillo, E., Development of artificial saliva solutions for studying the corrosion behaviour of dental alloys. *Corrosion*, 2004, **60**, 594–602.
16. Maruthamuthu, S., Rajasekar, A., Sathiyanarayanan, S., Muthukumar, N. and Palaniswamy, N., Electrochemical behaviour of microbes on orthodontic wires. *Curr. Sci.*, 2005, **89**, 988–996.
17. Lin, S. Y., Hsu, C. C., Hsu, C. H., Lee, T. H., Chou, M. Y. and Huang, H. H., Orthodontic materials. In General Session of International Association for Dental Research, 2001.
18. Lee, C. S., Choc, C. H., Ko, Y. N. and Chou, N. C., Orthodontic materials. In General Session of International Association for Dental Research, 2003.
19. Ijima, M., Ohno, H., Kawashima, I., Endo, K., Brantley, W. A. and Mizoguchi, I., Micro X-ray diffraction study of super elastic-nickel–titanium orthodontic wires at different temperatures and stresses. *Biomaterials*, 2002, **23**, 1769–1774.
20. Sestini, S. *et al.*, *In vitro* toxicity evaluation of silver soldering, electrical resistance and laser welding of orthodontic wires. *Eur. J. Orthod.*, 2006, **28**, 567–572.
21. Crossgrove, J. and Zheng, W., Manganese toxicity upon overexposure. *NMR Biomed.*, 2004, **17**, 544–553.
22. Shukla, G. S. and Singhal, R. L., The present status of biological effects of toxic metals in the environment: Lead, cadmium, and manganese. *Can. J. Physiol. Pharmacol.*, 1984, **6**, 1015–1031.
23. Tabak, L. A., Levine, M. J., Mandal, I. D. and Ellison, S. A., Role of salivary mucins in the protection of the oral cavitity. *J. Oral. Pathol.*, 1982, **11**, 1–17.
24. Levis, H. S., Brown, S. A., Song, J. X., McCauley, S. R., Boake, C., Contant, H. and Kotaria, K. J., Depression and posttraumatic stress disorder at three months after mild to moderate traumatic brain injury. *J. Clin. Exp. Neuropsychol.*, 2001, **23**, 754–769.
25. Holt, J. G., Kreig, N. R., Sneath, P. H. A. and Stanely, J. T., In *Bergey's Manual of Determinative Bacteriology* (ed. Williams, S. T.), Williams and Wilkins, Maryland, 1994.
26. Wawer, C. and Muyzer, G., Genetic diversity of *Desulfovibrio* spp. in environmental samples analysed by denaturating gradient get electrophoresis of hydrogenase gene frangments. *Appl. Environ. Microbiol.*, 1995, **61**, 2203–2210.
27. Teske, A. *et al.*, Microbial diversity of hydrothermal sediments in the Guaymas Basin: Evidence for anaerobic methanotrophic communities. *Appl. Environ. Microbiol.*, 2002, **68**, 1994–2007.
28. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zang, Z., Miller, W. and Lipman, D. J., Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.*, 1995, **25**, 3389–3402.
29. Higgins, D., Thompson, J., Gibson, T., Thompson, J. D., Higgins, D. G. and Gibson, T. J., CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, 1994, **22**, 4673–4680.
30. Saitou, N. and Nei, M., The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 1987, **4**, 406–425.
31. Choi, J. H., Jung, H. Y., Kim, H. S. and Cho, H. G., PhyloDraw: A phylogenetic tree drawing system. *Bioinformatics*, 2000, **16**, 1056–1058.
32. Kehres, D. G. and Maguire, M. E., Emerging themes in manganese in bacteria. *FEMS Microbiol. Rev.*, 2003, **27**, 263–290.
33. Stackebrant, E. and Goebel, B. M., Taxonomic note: A place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.*, 1994, **44**, 846–849.
34. Ghirose, W. C., Biology of iron and manganese depositing bacteria. *Annu. Rev. Microbiol.*, 1984, **38**, 515–550.
35. Nealson, K. H. and Saffarini, D., Iron and manganese in aerobic respiration: Environment significance, physiology and regulation. *Annu. Rev. Microbiol.*, 1994, **48**, 311–343.
36. Palaniswamy, S., Maruthamuthu, S., Manickam, S. T. and Rajendran, A., Microfouling of manganese-oxidizing bacteria in Tuticorin harbour waters. *Curr. Sci.*, 2002, **82**, 865–869.
37. Eisenstadt, E., Fisher, S. and Der, C. L., Silver, manganese transport in *Bacillus subtilis* W23 during growth and sporulation. *J. Bacteriol.*, 1973, **113**, 1363–1372.

38. Thompson, J. Y., Bayne, S. C. and Swift, E. J., Dental materials citations: Part A, January to June 1996. *Dent. Mater.*, 1996, **12**, 272–286.
39. Kao, C. T. and Huang, H. T., Orthodontic materials. In General Session of International Association for Dental Research, 2003.
40. Uppendar Kumar, A comparative evaluation of growth of microorganism on the surface of various orthodontic bonding materials. *J. Indian Orthod. Sci.*, 1998, **31**, 47–52.
41. Dexter, S. C. and Maruthamuthu, S., Response of passive alloys with n- and p-type passive films to manganese to biofilms. *Corrosion*, 2001, paper no. 01256, 1–15.
42. Laurent, F., Grossgogeat, B., Reclaru, L., Dalard, F. and Lissac, M., Comparison of corrosion behaviour in presence of oral bacteria. *Biomaterials*, 2001, **22**, 2273–2282.
43. Huang, T. H., Ding, S. J., Min, Y. and Kao, C. T., Metal ion release from new and recycled stainless brackets. *Eur. J. Orthod.*, 2004, **26**, 171–177.
44. Speck, K. M. and Fracker, A. C., Anodic polarization behaviour of Ti–Ni and Ti–6Al–4V in simulated physiological solution. *J. Dent. Res.*, 1980, **59**, 1590–1595.

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Olfactory responses of banana pseudostem weevil, *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) to semiochemicals from conspecifics and host plant

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Electroantennogram and olfactometer bioassays were conducted to study the olfactory behaviour of banana pseudostem weevil (BSW), *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae), to semiochemicals from conspecifics and host plants. Hexane extracts of whole-body chemicals of male and female weevils, host plant (banana pseudostem sheath) and combinations of weevil extracts and host plant extracts were used as stimuli or odour source in both electrophysiological and behavioural tests. BSW weevils exhibited sex-specific differences in responsiveness towards stimulus extracts in both the assays and males showed greater responsiveness in all the experiments. Female weevils were not responsive to their own body extracts, but showed significant responses towards male extracts. Male weevils were responsive to both male and female extracts. The present study provides electrophysiological and behavioural evidence that the olfactory behaviour in *O. longicollis* weevils is more precisely mediated through the male specific volatiles (aggregation pheromone). These findings also provide necessary information for developing an ecologically safe semiochemical-based control method for *O. longicollis*.

Keywords: Banana pseudostem weevil, electroantennogram, olfactory responses, semiochemicals, volatile extracts.

ODOIPORUS LONGICOLLIS Olivier (Coleoptera: Curculionidae), also known as the banana pseudostem weevil (BSW), is one of the main pests in banana (*Musa paradisiaca* L.) plantations in South East Asia and all the banana-growing belts of India[1]. The female BSW punctures the outer leaf sheath of the pseudostem and lays eggs inside the sheath. The emerging larvae feed on the soft tissue of the pseudostem and make extensive tunnels ranging up to 8–10 cm depth until pupation. Extensive infestations of BSW make the pseudostem weak and thus reduce the rate of flowering of the plant and finally result in undersized fruiting or no fruiting at all[1]. It has been estimated that the stem weevil causes 10–90% yield loss depending on the infestation stage and management efficiency. Adult weevils, though scanty feeders, live up to 200 days and often show the tendency to remain in the pseudostem, but exhibit strong flight activity when they move from one host plant to another. The biology, ecology and chemical control of BSW has been previously studied in detail[2]. Because of the long lifespan of adults and endophytic behaviour of the larvae, conventional methods of control, especially chemical control using insecticides proved to be less effective. Additionally, insecticides can be harmful to non-target species and the residues may pollute the environment. Hence, it is necessary to develop alternative control methods that are safe for the environment and highly efficient for the management of *O. longicollis*.

Semiochemicals or insect behaviour modifying chemicals, which include pheromones, have been proved to provide better and selective pest control or management in the protection of crops and forests[3]. The use of aggregation pheromones, which attract both male and female insects, in association with host volatiles, has led to the development of mass trapping as a control strategy for several weevil species[4,5]. Literature also cites the successful management of weevil populations in cotton, coconut and sweet potato using aggregation pheromones[6–8]. Sordidin, the aggregation pheromone of *Cosmopolites sordidus*, another important pest of banana, closely related to *O. longicollis*, has been identified, synthesized and a commercial formulation has been developed and successfully used for the control of the pest[9–12]. To date, no reports are available providing information either about the existence of aggregation pheromone or about the chemical cues involved in BSW communication. Studies on insect behavioural bioassays and electrophysiology provide

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