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Short Communication

Effects of microbes on color changes of red lead in murals

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The mineral pigments (especially red lead) in Dunhuang murals have changed their colors. For example, most red lead (Pb_3O_4) has changed to dark brown or black materials that have been identified as PbO_2 (e.g., Xu et al., 1983). This change is harmful to murals; thus it has become the central problem in the conservation of Dunhuang murals. Much work has been done to understand the mechanisms involved in the change of red lead on Dunhuang murals (e.g., Sun, 1985), but there is still no reliable evidence for it. Li and Michalski (1989) proved that red lead would gradually change to white lead [$Pb_3(OH)_2(CO_3)_2$] if the necessary light and humidity were provided, but no PbO_2 has been produced under their experimental conditions. The study of Dunhuang murals focuses on the real reason that caused the change of red lead. Oxalates were generally found in many color-changed murals (e.g., Li, 1990), but no further studies have been made on where the oxalates come from and what their effects are. In our studies, we proved that oxalates are evidence of a biological action and that microorganisms (especially *Cladosporium* and *Flavobacterium*) play a great part in the changing course of red lead.

Extracting of lead compounds. The compounds of lead were extracted from cells of *Flavobacterium* with various solutions as follows: 1 mol/L NaCl for lead compound with protein, diethyl ether for stearic lead, and 0.6 mol/L HCl+0.09 mol/L H_2O_2 for PbO_2 or oxalic lead (e.g., Pendias and Pendias, 1984).

Other methods. The crystal structure of red lead on the surface of imitated wall paintings and other pigment layers was analyzed with D/max-2400 X-ray Diffractometer (XRD) (RICAKU, Tokyo, Japan), The components of lead and oxalate were assayed with 170sx FT-IR ray spectrophotometer (Nicolet, WI, USA). The contents of the lead were determined by WFX-1D atom absorption spectrometry (3rd Analytic Apparatus corporation, Beijing, China). The changes of the crystal shapes of red lead were photographed by a TM-400 Electronmicroscope (PHILIP, Rotterdam, Netherlands), and the elements were assayed with Energy Dispersive Spectrometry (EDS) of TM-400.

Microbes inhibiting the surfaces of Dunhuang murals. We investigated the microbes on the surface of Dunhuang murals, and 5 genera of bacteria, 2 species of actinomyces, and 5 genera of fungi were found. All these microbes were tested to learn their effects during color changes. The results showed that strain 435 of *Cladosporium herbarum* and that strain 205 of *Flavobacterium* sp. have distinct effects. *C. herbarum* was distributed widely on the mural surfaces, and strain 435 of *C. herbarum* was isolated from grotto No. 435. *Flavobacterium* sp. could also be found in many grottoes. Strain 205 of *Flavobacterium* sp. was isolated from grotto No. 205. (Detailed results are reported in another paper.)

Effects of fungi on changes of red lead. In ancient murals, bone glue, a type of protein, was used as a bonding reagent (e.g., Li, 1990). It is crucial to the stability of pigments in the surface of murals, so the effects of all fungi strains isolated from Dunhuang murals on bone glue were investigated. The results show that all could grow on bone glue and that *C. herbarum* strain 435 could decompose bone glue quickly into amino acid or small molecular peptides after 50–70 h when it was inoculated in 2% bone glue solution (Fig. 1).

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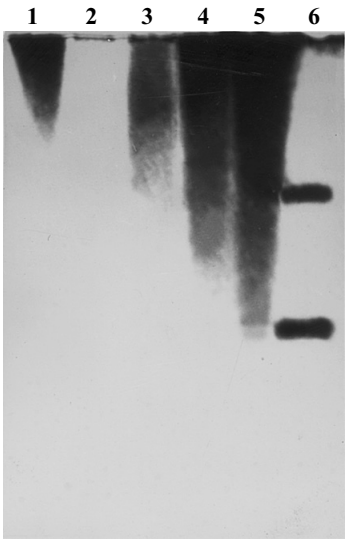


Fig. 1. Photograph of decomposing bone glue by *C. herbarum* strain 435.

C. herbarum strain 435 was inoculated in bone glue solution of different content and cultured for 60 h. The results of electrophoresis of supernatant in PAGE gel was shown. Lane I: 20 g/L bone glue; lane 2 to lane 5: inoculating *C. herbarum* strain 435 in 20 g/L, 50 g/L, 80 g/L, 150 g/L bone glue solution, respectively; lane 6: protein marker (upper to lower: BSA 66,000 Da, lysozyme 14,300 Da).

To understand the protective effects of bone glue on red lead and the role of *Cladosporium* in the changes of red lead, *C. herbarum* strain 435 was inoculated in 0.2 g, 0.8 g, or 1.5 g bone glue mixed with 0.2 g red lead and cultured under light (4,000 lx) for 30 days. The change of red lead was then analyzed by XRD. An interesting phenomenon was noted: the red lead showed no change, even under illumination, when enough bone glue was provided. However, it would change to white lead partly or completely when bone glue was scarce or decomposed by *C. herbarum* strain 435 (Fig. 2).

C. herbarum strain 435 was inoculated into bone glue solution (20 g/L containing 2 g/L red lead) and cultured under dark (treatment I) or light (4,000 lx) (treatment II) for 30 days. Red lead solution (control I: 2 g/L red lead dissolved with distilled water) and bone glue solution (control II: 20 g/L bone glue containing 2 g/L red lead) were treated under light (4,000 lx) for 30 days without inoculation. The lead content of supernatant and the component of sediment were then assayed by atom absorption spectrometry and XRD, respectively. The results show that the lead content in the solution and the content of white lead was enhanced after being inoculated with *C. herbarum* strain 435. Under light, little white lead was produced in control II, but white lead was the main component of sediment in treatment II. Even more, a slight amount of white lead was produced in treatment I, though it was cultured in darkness (Table 1).

C. herbarum strain 435 was inoculated on imitated mural (baked clay cake with a layer of a mixture of red lead and bone glue on the surface) and cultured for 30 days under light. The shape of red lead was then observed by an SEM (scanning electron microscope), and the change of components was assayed by IR. The results show that the crystals of red lead were damaged after *C. herbarum* strain 435 was grown, and some crystals containing calcium (analysis by EDS) could be discovered near the mycelium of *C. herbarum* (Fig. 3). Distinct peaks of calcium oxalate were shown by the results of IR analysis of the substance scratched from the surface of an imitated mural (Fig. 4).

Effects of bacteria on the color of red lead. Bacteria isolated from Dunhuang murals were cultured in a medium containing 0.2% red lead. All strains were found to survive in red lead. Colonies of *Flavobacterium* sp. strain 205 took on a dark brown color after 50 h grown in red lead medium, but they had only a yellow color in the control without red lead.

Dark brown color colonies were collected for assaying lead content. The results showed that 40.3 mg/g dry cell lead had been accumulated in the cells of *Flavobacterium* sp. strain 205. When the lead was extracted with a variety of solutions, 27.9 mg/g dry cell lead could be extracted by a solution of 0.6 mol/L HCl+0.09 mol/L H_2O_2. The solution usually was used for extracting lead as a form of PbO_2 or $Pb(C_2H_4O)$ in cells. The pigment of *Flavobacterium* cells growing in a medium with or without red lead was extracted by acetone. Both extractions had the same yellow color and the same UV-visible light spectrum.

Dry cells were ground with distilled water. The supernatant was chromatographed with diatomite column, and the brown section was collected. The total lead contents of the supernatant and the collected section was 18.4 mg and 15.2 mg, respectively.

The existence of oxalate was discovered on murals at different sites, especially on murals in which the colors had badly changed. Del Monte and Sabbioni (1987) indicated that the formation of oxalate and the damage of murals and other historic relics are related with lichens. Although no traces of lichens were found in our investigation, many fungi were found in some humid grottoes of Dunhuang murals. Various fungi, actinomyces, and bacteria have been isolated from the mural surfaces. The formation of oxalate by these isolated fungi on the surfaces of imitated murals would be a good explanation for the existence of oxalate in ancient murals.

The results of our experiments show that many species of fungi, especially *C. herbarum* strain 435, had the effect of decomposing on bone glue. Although bone glue was decomposed, red lead on a mural lost

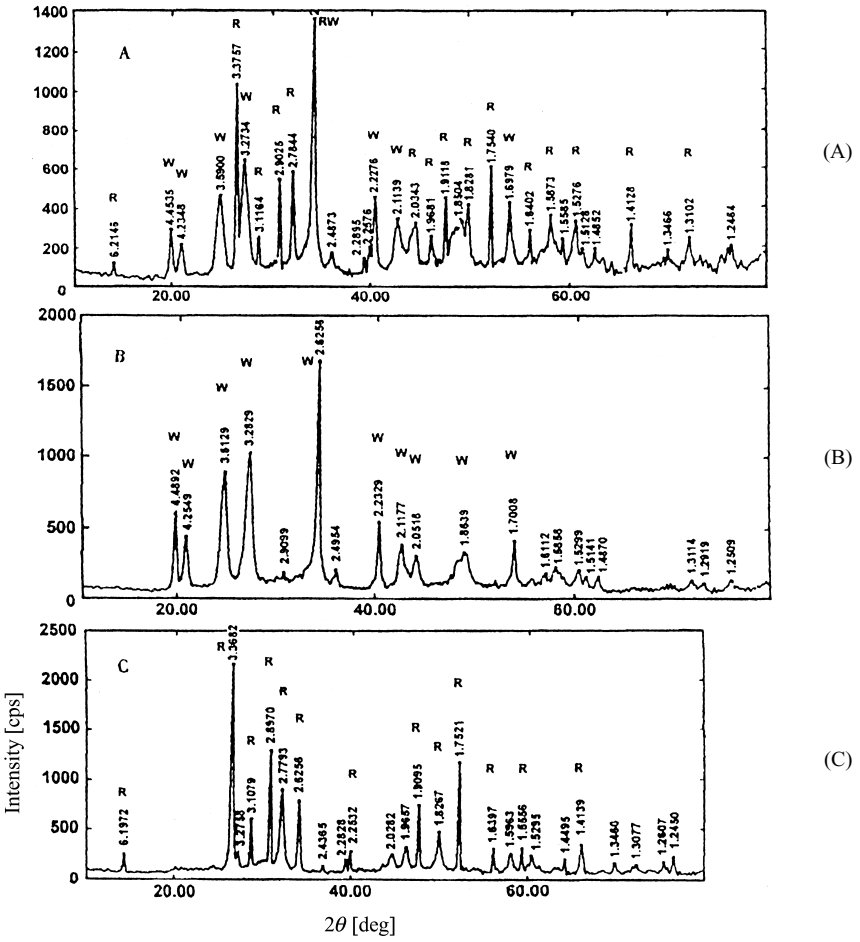


Fig. 2. Effects of bone glue and *C. herbarum* strain 435 on color change of red lead.
A. Mixture of 0.2 g bone glue and 0.2 g red lead. B. Mixture of 0.2 g bone glue and 0.2 g red lead inoculated *C. herbarum* strain 435. C. Mixture of 0.8 g bone glue and 0.2 g red lead inoculated *C. herbarum* strain 435 (R: red lead; W: white lead).

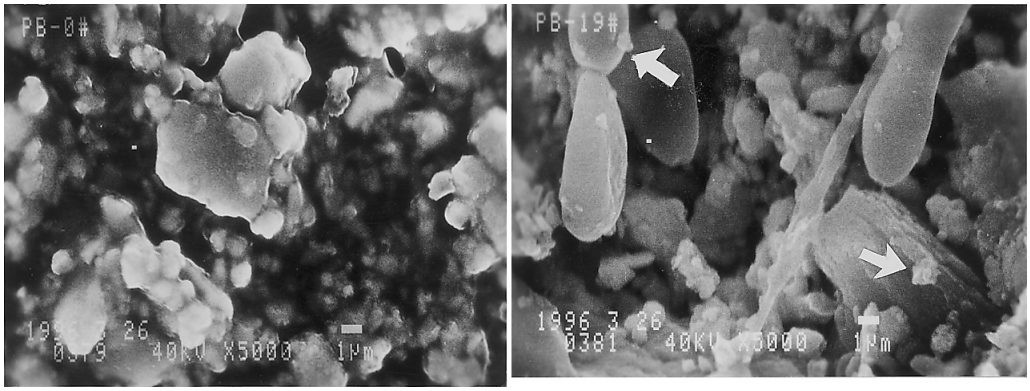
Table 1. Effect of *C. herbarum* strain 435 on red lead under different conditions.

	Condition of culture	Content of soluble lead (mg/L)	Component of sediment
Control I	dark		RD
Control II	light	22.9	RD with little WD
Treatment I	dark	28.6	RD with little WD
Treatment II	light	31.7	WD, RD

Control I: 2 g/L red lead solution; control II, treatment I and treatment II: 20 g/L bone glue solution containing 2 g/L red lead; treatments I and II were inoculated with *C. herbarum* strain 435. All these solutions were cultivated under 4,000 lx light or dark for 30 days.
RD: red lead; WD: white lead.

the protective effect of bone glue (e.g., Kenjo, 1984). On the other hand, the metabolites of these molds, such as oxalic acid, would react with red lead slowly. As a result, the crystals of red lead were damaged and the contents of soluble lead increased, changing to white lead when met with suitable light and humid conditions. Oxalic acid also reacted with the calcite of clay and limestone to form the calcium oxalates.

Flavobacterium sp. strain 205 isolated from Dunhuang murals was inoculated in a medium containing red lead, and the color of colonies showed dark brown. Two reasons may have been why the cells of *Flavobacterium* change their color to dark brown. One is that lead taken into cells induced some special organic pigment, such as carotenoid (e.g., Buchanan and Gibbons, 1974); another reason is that some kind of lead compound in the cells has a dark brown color. According to the results of an assay, a great deal of lead had accumulated in the cells of *Flavobacterium* sp. strain 205, and the material having a dark brown



(A) (B)

Fig. 3. SEM photograph of effects of *C. herbarum* strain 435 on red lead.
A. Imitated mural without *C. herbarum* strain 435 (×5,000). B. Imitated mural inoculated *C. herbarum* strain 435 (×5,000, high calcium content shown in arrow).

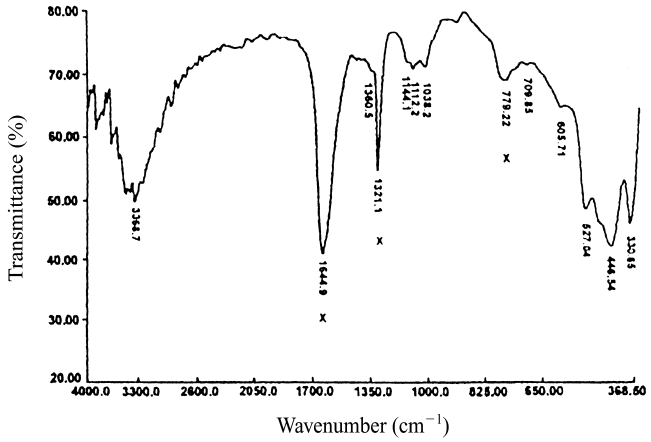


Fig. 4. Oxalate formation of *C. herbarum* strain 435 in imitated mutal (IR).
1,645, 1,321, 780 cm^{-1} show the peaks of calcium oxalate.

color was a lead compound, but not organic pigments, because it had a high content of lead even after being chromatographed, and it could not be extracted by acetone or other organic solvent. Furthermore, the experiments extracting lead from the cells of *Flavobacterium* sp. by various extract solutions showed that PbO_2 or $Pb(C_2O_4O)$ is the main form of lead in the dark brown cells of *Flavobacterium* sp. strain 205. These results may suggest that the lead accessing the cells of *Flavobacterium* sp. strain 205 was oxidized; consequently, the dark brown color of PbO_2 appeared.

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