Killer Clays!

Natural antibacterial clay minerals

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Introduction

Clay minerals have been used in medicinal applications since Aboriginal times (Carretaro, 2002; Wilson, 2003). Clay poultices are used to heal wounds; people eat clays to coat stomach linings and soothe indigestion; animals eat clay containing trace elements (e.g., As) that can kill worms. The reasons that various clay minerals are effective as medicines may be as variable as the ailment, but by studying the mechanisms by which clay minerals heal, we may gain insight to a variety of diseases and potential cures.

The Romans first recorded studies on medicinal uses of clay minerals in 60 BC (Carretero, 2002) and Aristotle first mentioned the practice of eating clays (Mahaney et al., 2000). Most early research focused on the physical properties of clay minerals, which benefit digestion or protect and cleanse the skin. Smectite clay minerals can adsorb dissolved and suspended substances such as toxins, bacteria and viruses, while kaolinite and palygorskite are primarily used to soothe the digestive tract (Carretero, 2002). The physical adsorption of water and organic matter is the most common attribute of the healing properties of clays, however the chemical interaction of clay minerals with bacteria has received less attention.

We have only begun to investigate the clay chemical properties that may be important in medicine, but our results indicate that particular natural clay minerals can have striking and very specific effects on microbial populations. The effects can range from enhanced microbial growth to

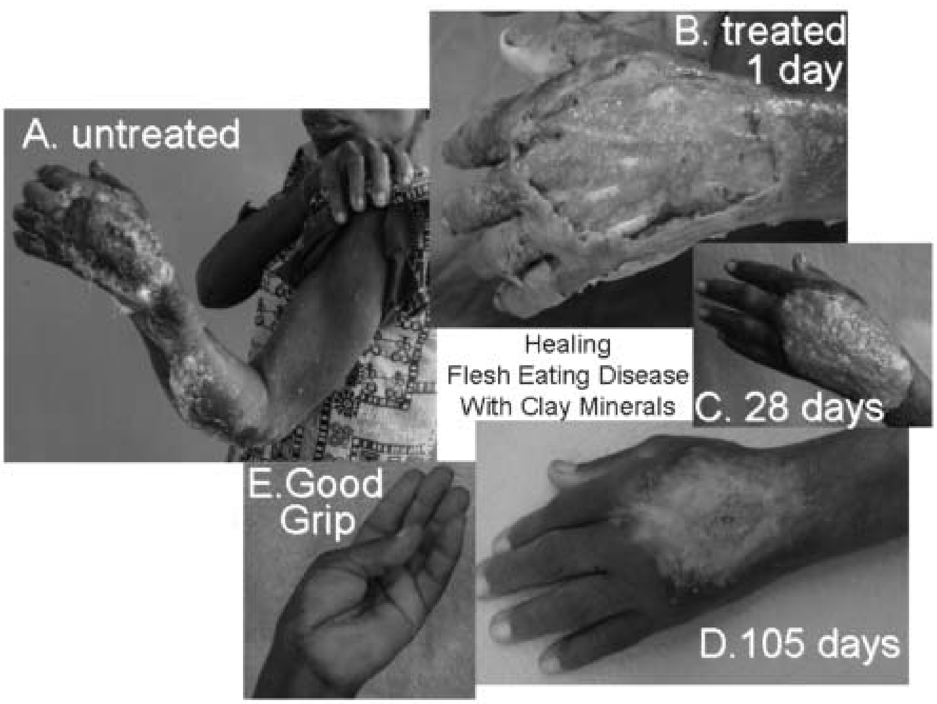

Fig.1 Buruli ulcer is a Mycobacterial infection that destroys fatty tissues under the skin (A). Treatment of the skin with a French green clay (B) kills bacterial infection and promotes healing (C,D,E).

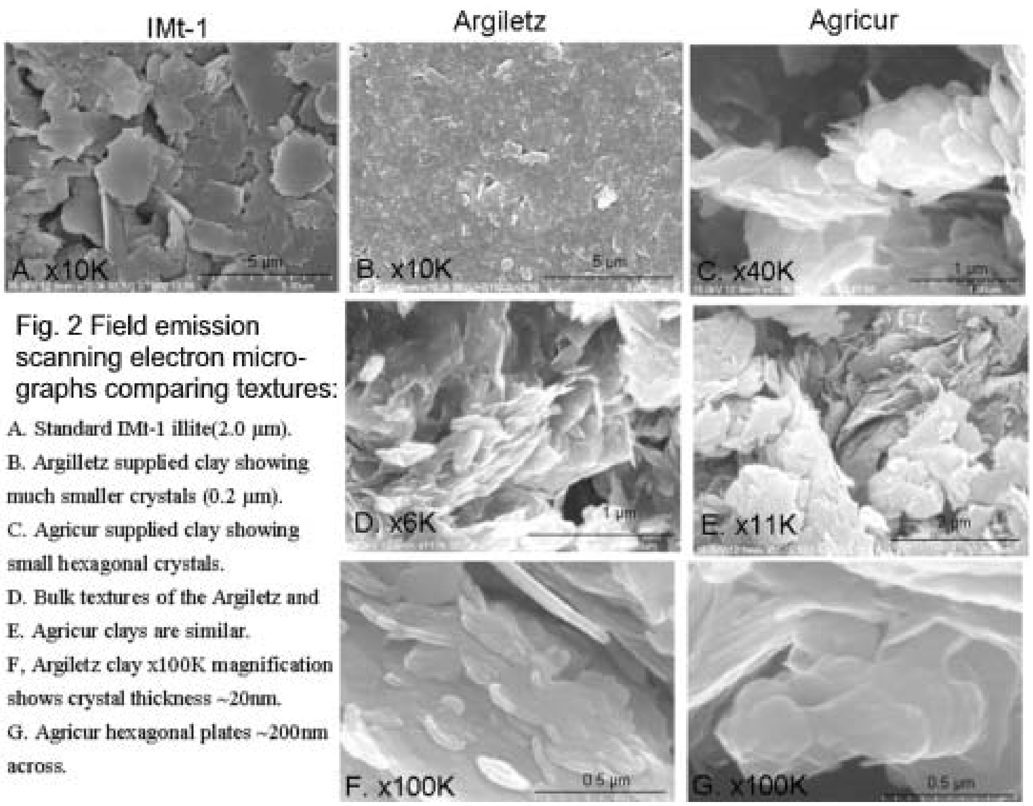

Fig. 2 Field emission scanning electron micrographs comparing textures:
A. Standard IMt-1 illite(2.0 µm).
B. Argilletz supplied clay showing much smaller crystals (0.2 µm).
C. Agricur supplied clay showing small hexagonal crystals.
D. Bulk textures of the Argiletz and
E. Agricur clays are similar.
F, Argiletz clay x100K magnification shows crystal thickness ~20nm.
G. Agricur hexagonal plates ~200nm across.

complete sterilization. Even minerals with similar structure and bulk crystal chemistries can have opposite effects on a particular microbial population. Here we present evidence that natural clay minerals can be effective antimicrobial agents. The fascinating puzzle is why? With a reverse engineering approach we hope to unravel the chemical secrets of the medicinal clays.

Background

In 2002, Line Brunet de Courssou, a French humanitarian working in the Ivory Coast of Africa, presented to the World Health Organization the results of her research on treatment of Buruli ulcer with clay minerals. Buruli ulcer is a flesh eating disease that is borne by *Mycobacterium ulcerans*, a bacterium related to leprosy and tuberculosis. *Mycobacterium ulcerans* produces a lipid toxin, mycolactone, which destroys the fatty tissues under the skin (George et al., 1998; 2002). The infection is immuno-suppressant, so no pain is felt as the mycobacterium grows and destroys adipocytes (fat tissue). No white blood cells are called to attack the disease, so no inflammation occurs.

The cause of the mycobacterial infection is still under investigation, with some indication that the mycobacterium, common in swampy soils, is transmitted by aquatic insects (Portaels, 1999). In several cases the infection stems from sites that resemble bites (Travis, 1999).

Brunet de Courrsou (2002) documented numerous case studies of healing the disease by daily applications of the French clay that she had known and used on wounds since childhood. An example of the stages of healing by clay can be seen in the photo mosaic shown in Figure 1 (excerpted from www.burulibusters.com). Destroyed tissue (Fig.1A) is easily removed by one treatment with the green clay mineral poultice (Fig. 1B exposing raw muscle and bone. The progression of healing shown (Fig. 1C,D,E) occurs when the clay poultice is changed daily. After 3–4 months the infection is healed with soft supple scarring and a return of normal motor function. This is the only known cure for Buruli ulcer.The alternative is surgical removal or amputation.

Two green clay samples from France were used in the Buruli ulcer treatment, from two different suppliers. The source of the clay minerals was not revealed, but they are thought to be altered volcanic ash deposits from the Massif Central. It was immediately apparent that one of the clay samples was not effective in killing mycobacterium, but was more suited to promotion of skin granulation after the

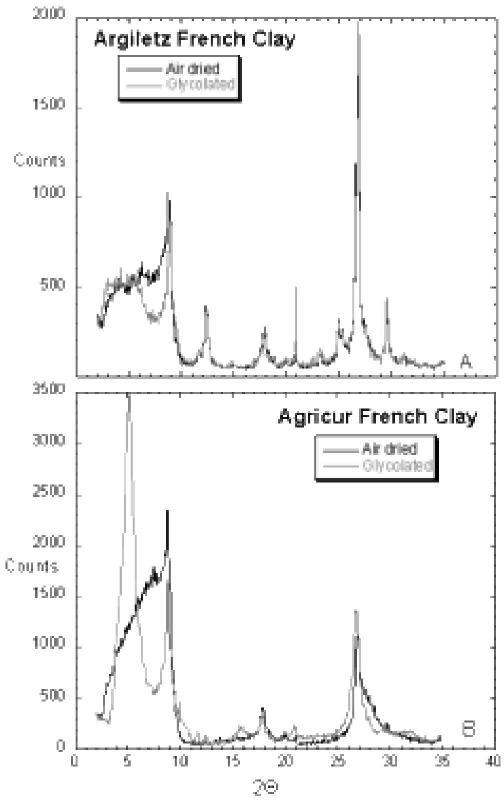

Fig. 3 X-ray diffraction spectra of the Argiletz and Agricur supplied clay samples used for healing.

mycobacterium were killed. These observations remain unexplained.

Clay Mineralogy and Chemistry

Samples of the two clay minerals from France, supplied by Argiletz and Agricur, were compared texturally, mineralogically and chemically. Figure 2 shows the texture of the two French clay minerals, compared to a clay mineral standard illite, IMt-1 (CMS, Source Clay Repository). The crystals are hexagonal with texture similar to IMt-1 illite, but with a much smaller particle diameter (avg. 200nm).)

X-ray diffraction analyses of the French clay samples were done for bulk powders, and for the 2.0µm fraction on oriented clay mounts, air-dried and ethylene glycolated (Fig. 3). The samples are not pure, but are predominantly clay with the Argiletz sample containing 63% and the Agricur sample containing 78% clay. A comparison of the bulk mineralogy is shown in Figure 4. Non-clay minerals in these samples include quartz, calcite, (Na, K)-feldspar. The Argiletz sample also contained kaolinite and chlorite (~3%). Estimates of the clay surface area are 90.5 m^2/g for Argiletz and 115.4 m^2/g for the Agricur clay.

Major element analyses of the <2.0 µm fraction of each French clay (Table 1) indicates that they both contain 6–7 wt % total Fe. The only other major chemical differences are that the Argiletz clay is enriched in Ca and the Agricur contains more K.

Trace elements were measured by Secondary Ion Mass Spectrometry. Analyses of the two French clays are shown (Fig. 5) in comparison to Clay Mineral Standards IMt-1 illite and SWy-1 smectite. The trace elements are within the range of values observed for the illite and smectite examples, with the exception of high Ba in the Argiletz sample. Similarly, the rare earth elements (REE), compared to C1 chondrite values, (Fig. 6) show trends common to most shales worldwide (Fauer, 1991) with values between that of IMt-1 illite and SWy-1 smectite.

Microbial Experiments

Because our laboratory is not authorized to work with pathogens such as *Mycobacterium ulcerans*, we tested the effect of various clay minerals on common *Escherichia coli* (wild-type strain "B," *E. coli* Genetic Stock Center #5365) to see if we could simulate the effect documented by Brunet de Courssou (2002). Microbial analysis of the Argiletz clay by the suppliers (Institut De Recherche Microbiologique) showed that the clay contained ~$5x10^5$ natural bacteria per gram as well as 400 mold organisms per gram. None of these bacteria were colonized during our experiments.

Experiments were performed using the two French clay samples, along with clay mineral standards IMt-1 illite, SWy-1 montmorillonite, and KGa-1 kaolinite from the Clay Minerals Repository (Purdue, IN). A duplicate experiment was performed on the two French clay samples to verify the results of the initial experiment. In the second experiment we added the Muloorina Illite, from Australia, which is

Argiletz (supplier analysis)			Agricur (microprobe analysis)		
Oxide	Wt %	Wt% Norm	Oxide	Wt %	Wt% Norm
SiO_2	50.8	59.8	SiO_2	60.0	61.8
TiO_2			TiO_2	0.2	0.2
Al_2O_3	20.0	23.5	Al_2O_3	18.6	19.2
Fe_2O_3	5.8	6.8	Fe_2O_3	6.1	7.0
MgO	0.0	0.0	MgO	4.8	4.9
MnO	0.0	0.0	MnO	0.1	0.1
CaO	6.4	7.5	CaO	1.2	1.3
Na_2O	0.1	0.1	Na_2O	2.9	3.0
K_2O	1.9	2.2	K_2O	3.4	3.5
Sum=	85.0		Sum=	97.2	
LOI	3.08				
org.	0.612				
C	4.73				
S	0.209				
P	0.152				
Cl	0.014				

Table 1. Major element analyses of the two French clay samples

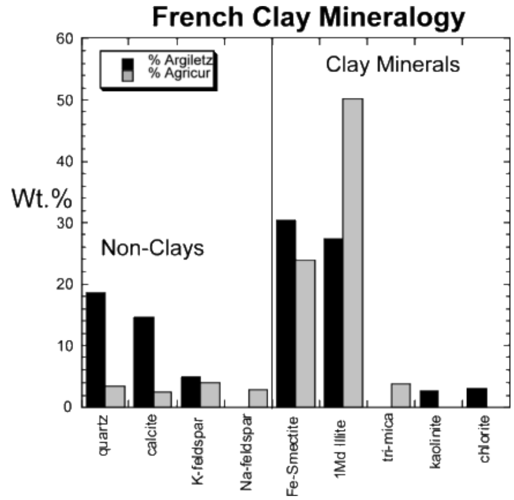

Fig. 4. Mineralogical results interpreted from XRD using Rockjock (Eberl, 2003)

similar in texture, crystal size and chemistry to the French clays.

The *E. coli* were grown in Luria-Bertani (LB) broth (a high organic (15g/l), saline (10g NaCl/l) solution of pH = 7.3) and diluted in LB broth to produce an initial population of ~50 organisms/l. We mixed 200 mg of clay with 400µl of this *E. coli* "starter culture" and incubated the mixture at 37°C (body temperature) for 6–9 hrs. After incubation, dilutions of the samples were prepared and spread on LB agar plates for quantification. When spread on agar plates, single organisms grow to individual colonies countable by eye (Fig.7). Controls of unadulterated starter culture were incubated along with the experimental clay mixtures and quantified by plate counts to show the expected amount of *E. coli* growth without clay during the experiment. By comparing the number of *E. coli* produced in each clay suspension with the control, we can evaluate how the clay minerals affect E. coli growth.

Evidence for Killer Clays

Examples of the *E. coli* plated in agar with clay minerals are compared to *E. coli* grown without clay (Fig. 7). In duplicate experiments, the Agricur clay caused complete sterilization of E. coli! The bar graph (Fig. 8) shows that the initial broth contained ~2x10^4 *E. coli*/ml. The control, incubated without clay, grew to 4.6x10^6 counts/ml. Relative to this amount, the Argiletz clay sample, both bulk and .0 m separate, apparently enhanced *E. coli* growth. However, the errors in this analysis (0.5x10^6) are significant. Other clay standards had no significant effect on the *E. coli* growth. The second experiment, which compared the French clays with Mulloorina illite, showed that the latter had no effect on *E. coli*.

Future Research

The mechanism of bacterial death has yet to be identified for these killer clays. One Fe-rich illite from France, Argiletz supplied, apparently kills *Mycobacterium ulcerans*, while the clay supplied by Agricur kills *E. coli*. It is the Agricur sample that was used to promote skin granulation in the treatment of Buruli ulcer. Perhaps it is the antibacterial properties of this particular clay that assist in the healing process.

Possible causes of the antibacterial properties of natural clay minerals need to be explored. It is known that certain man-made clay intercalates, such as transition metals, can impart antibacterial properties (Kostyniak et al., 2003; Top and Ülkü, 2004). We need to look for such trace elements (e.g., Ag, Cu, Mn, Zn, Fe) occurring naturally in the different clay minerals, and determine their specific interactions with cell membranes. Do the minerals rob

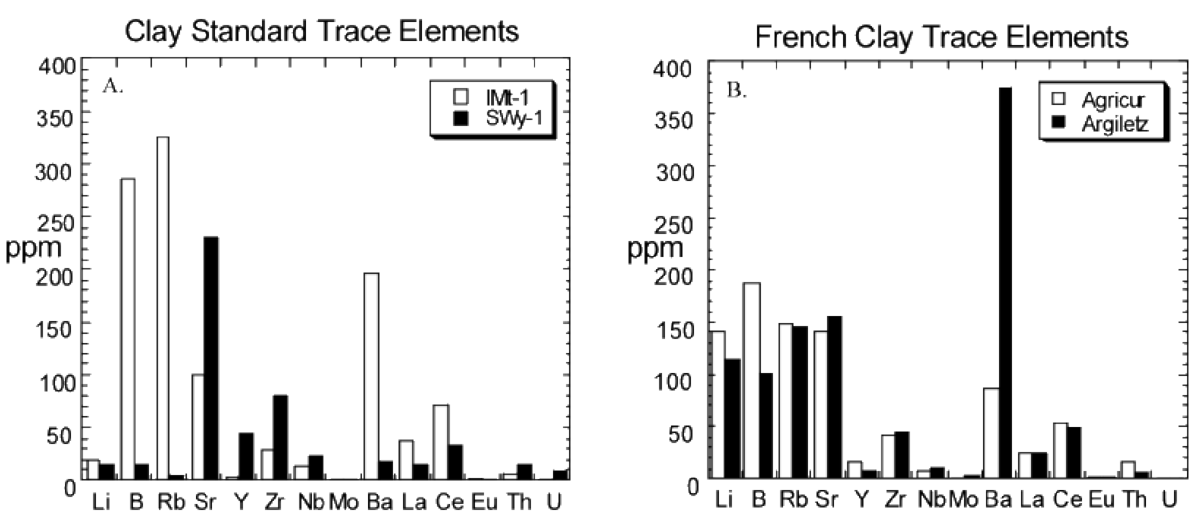

Fig. 5. Trace elements measured by Secondary Ion Mass Spectrometry. A) Illite and montmorillonite standards compared to B) French healing clays

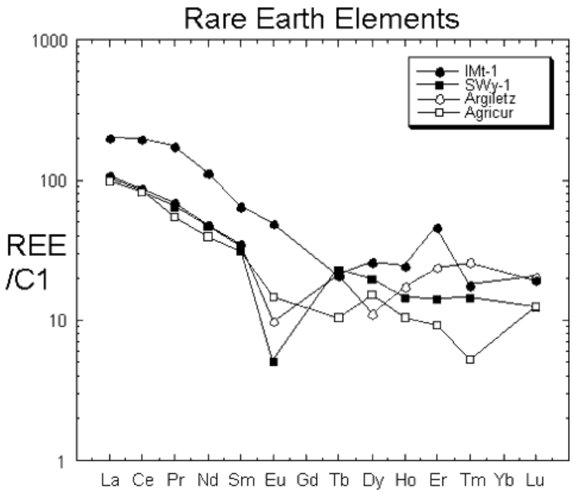

Fig. 6. Rare earth element patterns for the French healing clays compared to standard illite and montmorillonite.

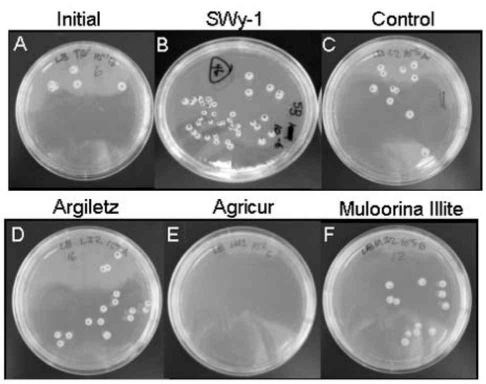

Fig. 7. Agar plates showing *E.coli* growth after incubation with and without clay minerals. A) Initial population, B) growth with montmorillonite SWy-1, C) Control sample grown without clay, D) Argiletz clay effective in killing Mycobacterium, E) Agricur clay showing complete sterilization of *E coli* F) Muloorina illite, chemically and physically similar to the latter, but ineffective as an antimicrobial agent for *E coli.*

bacteria of vital nutrients, or do they exchange toxic elements with cell membranes? The chemical reactions between clay minerals and bacteria represent a whole new field of study in understanding medicinal minerals. Our observations suggest that clay minerals may be more than just physical absorbents of toxins, bacteria and viruses (Carretero, 2002). They may eliminate bacterial colonies by specific chemical reactions not yet understood. Nano-scale properties of mineral surfaces are our primary interest. The variables we are exploring include trace elementexchange, surface free energy potential, pH,

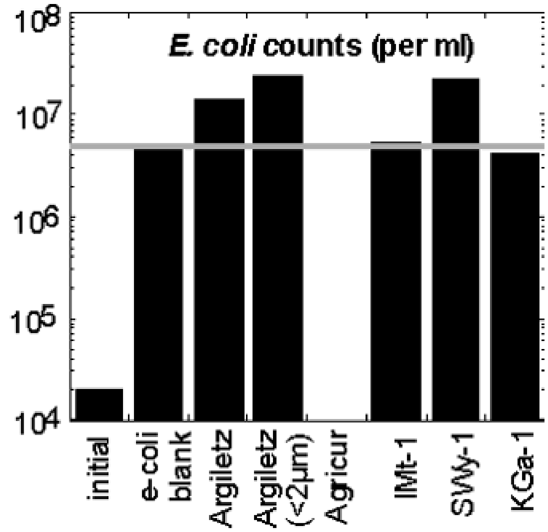

Fig. 8 Summary bar graph showing *E.Coli* growth in samples incubated with clay minerals, compared to a blank grown without clay (grey line). Note the lack of E. Coli

oxidation state, and how these vary with mineral morphology.

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