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ISSN : 0254-8704
http : //www.jeb.co.in

J. Environ. Biol.
30(1), 151-154 (2009)
info@jeb.co.in

Antibacterial effects of minerals from ores indigenous to Korea

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(Received: April 30, 2008; Revised received: July 30, 2008; Accepted: August 10, 2008)

Abstract: *We tested the antibacterial properties of a mix of minerals consisting mainly of sericite, talc, and halloysite from Korea. The preparation showed clear growth inhibition of the gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa, and the gram-positive bacteria Staphylococcus aureus subsp. aureus, S. epidermidis, and Bacillus cereus, as well as the anaerobic bacterium Propionibacterium acnes. These results indicate that this preparation, made from ore minerals indigenous to Korea, could be used to develop new antibacterial reagents.*

Key words: *Ore minerals, Antibacterial effect, Gram-negative bacteria, Gram-positive bacteria*
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Introduction

The relationship between geologic materials and human health has been known for centuries. Chemical elements, minerals, rocks, soils, water, and air are the essential components of geologic environments, and humans have accumulated experiences and knowledge regarding the interactions among these components, including the beneficial and hazardous effects of some materials on humans, animals, and plants (Finkelman, 2006; Sugumar *et al.,* 2008). The first written reference to the use of ores and their curative effects for human health was "De Materia Medica" from 60 BC (Isabel Carretero, 2002; Park and Kim, 2002). Moreover, ancient Korean, Chinese, Egyptian, Islamic, and Greek texts describe the many therapeutic applications of various rocks and minerals and the many health problems that they may cause (Finkelman *et al*., 2005). According to the *Dongeuibogam,* the most celebrated Korean Materia Medica, 92 medicinal stones with notable pharmaceutical efficacy were described by Jun-Heo in 1613 (Hong, 1975).

The recognition that an intimate relationship between the environment, particularly geologic materials, and human health has led to the recent development of a new field of science called medical geology. Medical geology is a multidisciplinary scientific field shared by some distant scientific areas, such as earth sciences, environmental sciences, medicine, biology, and pharmacology (Gomes and Silva, 2007). The field attempts to assess health problems caused or exacerbated by geologic materials such as trace elements, rocks, minerals, natural dust, water, and geologic processes such as volcanic eruptions and earthquakes (Finkelman, 2006; Gomes and Silva, 2007).

Many ore minerals are currently being used as gastrointestinal protectors, laxatives, antidiarrheics, or excipients such as inert bases, emulsifiers, or lubricants in certain medicines (Isabel Carretero, 2002; Gomes and Silva, 2007). Some are being used in formulations for topical medicines in both dermopharmacy and dermocosmetics. The ore minerals used in pharmaceutical formulations are selected for their high specific area and sorptive capacity, rheological properties, chemical inertness, and low toxicity.

Talc has been used in oriental medicine to cure catharsis, dysentery, dry breast, poor nutrition, and chronic indigestion (Lee *et al*., 1999). Talc is a fine-powdered hydrous magnesium silicate with very good absorption capacity for oils and grease. Sericite is a layered silicate mineral, generally recognized as a fine white powder that is muscovite in form; it is widely used in alkali flux and cosmetics (Gomes and Silva, 2007; Joussein *et al*., 2005). Halloysite clay minerals are ubiquitous in soils and rocks, where these minerals occur in a variety of particle shapes and hydration states. Halloysite has a remarkable ability to absorb toxins and impurities from the surface of the skin (Joussein *et al*., 2005), and is thus especially beneficial for the treatment of acne and for removing dead skin cells from the skin surface (Viseras *et al*., 2007).

Although notable advances have recently been made in mineralogical studies (Bowman *et al*., 2003; Kang and Lee, 2008), systematic and multidisciplinary studies of the effects of ore minerals on the cell are not available. Here we present the first examination of the effects of Korean ore minerals on the growth of selected gram-positive and gram-negative bacteria. Our results could be used as the foundation for developing new antibacterial reagents derived from the geological environment.

Materials and Methods

Preparation of ore minerals: The ore minerals used were developed by NT and BT Co. Ltd. (Hongsung, Chungnam, Korea). Raw materials (mainly including halloysite, sericite, and talc) were mined, broken into small pieces, washed, and dried, and then ground into a fine powder. Minerals were isolated using a selector based on weight differences, and then metals were removed from the sample using magnetism. The isolated minerals were mixed and then treated with heat. The final preparation was used in this investigation.

Bacteria and culture: *Escherichia coli* were cultured at 37°C for 24 hr in LB (Luria-Bertani) broth before the assay. LB media consisted of 1% bacto tryptone, 0.5% bacto yeast extract and 1% sodium chloride, with pH adjusted to 7.0. *Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus*, and *Pseudomonas aeruginosa* were cultured at 37°C for 24 hr in nutrient broth before the assay. Nutrient media consisted of 0.3% beef extract and 0.5% peptone, with pH adjusted to 6.8. *Propionibacterium acnes* were cultured at 37°C for 3-4 days in GAM (Gifu anaerobic medium) broth (Nissui, Japan) under anaerobic conditions before the assay. GAM medium consisted of 3% GAM broth and 1.5% agar, with pH adjusted to 7.1 (Leeja and Thoppil, 2007).

Antibacterial assays: The bacterial strains were *Escherichia coli*, *Staphylococcus aureus* subsp. *aureus, S. epidermidis, Bacillus cereus*, *Pseudomonas aeruginosa*, and *Propionibacterium acnes*. The antibacterial activity of the mineral preparation was evaluated using a liquid growth inhibition assay (Campagna *et al*., 2004) in sterile 96-well polypropylene microplates with LB broth and nutrient broth as growth media, except for *P. acnes*, for which GAM medium was used. A standard inoculum of 16 hr culture cells was added at a final concentration of 10^5 colony-forming units CFU ml^{-1}. Control wells contained all components except the mineral preparation. Experiments were performed in duplicate. After incubation of the microplates for 24 hr at 37°C, the absorbance of the assay mixture was determined at 620 nm and then corrected by subtracting the absorbance of the minerals alone. For colony-counting experiments, 50 µl of each well, after incubation for 24 hr at 37°C, were spread on a plate of LB, nutrient, and GAM media containing 1.5% agar. The agar plates were examined daily for the formation of colonies, and colony numbers were documented (Lee *et al*., 2007; Toroglu, 2007).

Results and Discussion

With the increasing resistance of microbial organisms to multiple antibiotics, and the continuing emphasis on healthcare, many researchers have attempted to develop new, effective, and environmentally-friendly antibacterial reagents. Many ore minerals such as halloysites, sericite, talc, kaolinite, and palygorskite have been used for therapeutic purposes, because they have high absorptive capacity, chemical inertness, and low or no toxicity for humans (Gomes and Silva, 2007). These minerals are used as active ingredients in gastrointestinal protectors, osmotic oral laxatives, and antidiarrheics,

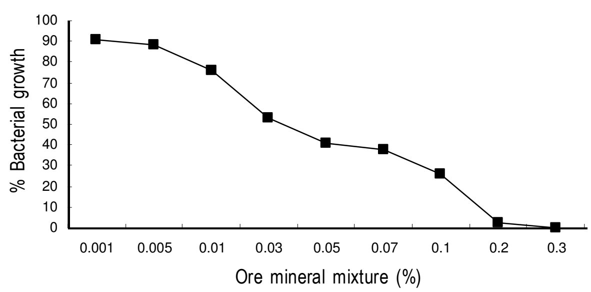

Fig. 1: Effects of mineral preparation on the growth of *Escherichia coli*

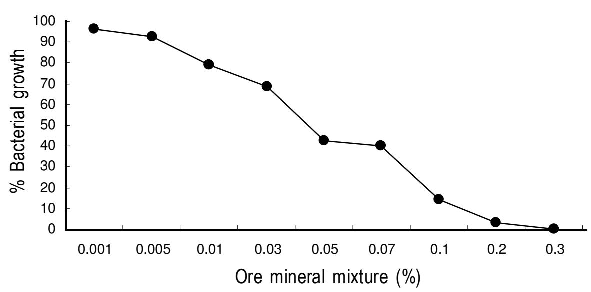

Fig. 2: Effects of mineral preparation on the growth of *Staphylococcus aureus* subsp *aureus*

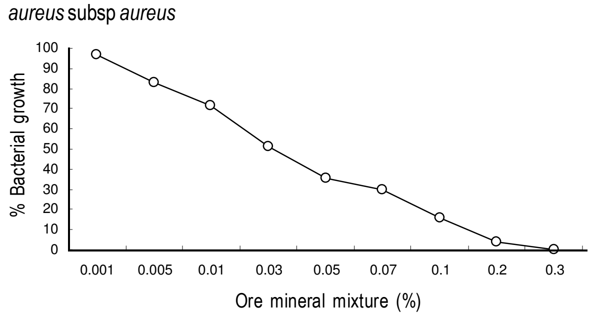

Fig. 3: Effects of mineral preparation on the growth of *Staphylocuccus epidermidis*

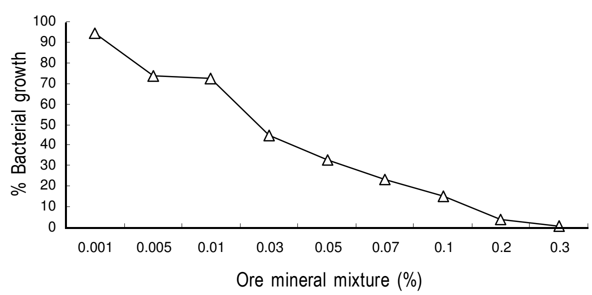

Fig. 4: Effects of mineral preparation on the growth of *Bacillus cereus*

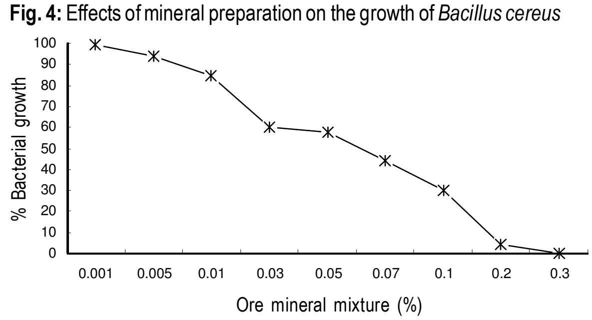

Fig. 5: Effects of mineral preparation on the growth of *Pseudomonas aeruginosa*

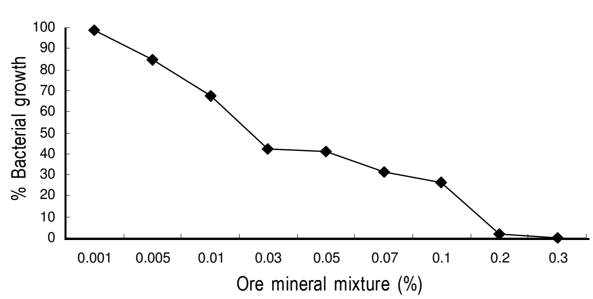

Fig. 6: Effects of mineral preparation on the growth of *Propionibacterium acnes*

Table - 1: Numbers of residual colonies for selective gram-positive and gram-negative bacteria in the presence of ore minerals

Strains		Concentration (%)	Residual colony
E. coli	Control	- -	2.1×10^6
	Mineral	0.1	5.5×10^5
		0.2	5.6×10^4
		0.3	0
S. aureus. sub sp. *aureus*	Control	- -	1.8×10^7
	Mineral	0.1	2.5×10^6
		0.2	5.7×10^5
		0.3	0
S. epidermidis	Control	- -	2.4×10^6
	Mineral	0.1	3.7×10^5
		0.2	8.6×10^4
		0.3	0
B. cereus	Control	- -	2.7×10^6
	Mineral	0.1	4.1×10^5
		0.2	9.7×10^4
		0.3	1.0×10^4
P. aeruginosa	Control	- -	3.2×10^5
	Mineral	0.1	9.5×10^4
		0.2	1.3×10^4
		0.3	0
P. acnes	Control	- -	2.8×10^6
	Mineral	0.1	7.3×10^5
		0.2	5.0×10^4
		0.3	0

and are recommended for treating acne and boils (Isabel Carretero, 2002; Gomes and Silva, 2007; Kang and Lee, 2008).

We found effective antibacterial effects of a mineral mix indigenous to Korea against all bacterial strains analyzed here. The antibacterial effects against several gram-positive and gram-negative bacteria were examined, using liquid growth inhibition assays and a colony-counting method.

As shown in Fig. 1, a significant reduction in the growth of the gram-negative bacterium *E. coli* was observed. A common bacterium in the lower intestine of warm-blooded animals, most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans and are occasionally responsible for costly product recalls (Vogt and Dippold, 2005). Treatment with 0.1% of the mineral preparation reduced *E. coli* growth by approximately 70%, while treatments of 0.2% and 0.3% almost completely inhibited *E. coli* growth. Table 1 shows the antibacterial effects of treatment, as evaluated by counting CFUs on solid agar plates. The 0.1% treatment reduced bacterial counts to 5.5×10^5 from an initial population of 2.1×10^6, corresponding to a ~70% reduction. The 0.2% treatment reduced bacterial counts to 5.6×10^4, corresponding to a ~97.3% reduction. No colonies were observed after treatment with 0.3% preparation.

Staphylococcus aureus subsp. *aureus* is the most common cause of staph infection in humans. It is a spherical bacterium, commonly found on the skin or in the nose, but it may also infect soft tissue, respiratory organs, bones, joints, the endovascular system, and wounds (Fuente *et al*., 1985). The bacterium can cause a range of illnesses, from minor skin infections such as pimples, impetigo, boils, furuncles, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, and septicemia (Fuente *et al*., 1985). Treatment with 0.1% preparation reduced the growth of this gram-positive bacterium by approximately 85% (Fig. 2). Treatment with 0.2% reduced bacterial counts to 5.7×10^5 from an initial population of 1.8×10^7, corresponding to a ~96.8% reduction. Treatment with 0.3% completely inhibited bacterial growth, and no colonies were observed (Table 1).

Staphylococcus epidermidis is a common constituent of the commensal microflora of human skin. However, it also causes opportunistic infections and is considered a major nosocomial pathogen (Vuong and Otto, 2002). Among the coagulase-negative staphylococci (CoNS), *S. epidermidis* represents one of the organisms most commonly associated with superficial skin infections in humans (Akiyama *et al*., 1998). Furthermore, this organism is thought to act as a reservoir for antibiotic resistance for the major staphylococcal pathogen, *S. aureus* (McLaws *et al*., 2008). Treatment with 0.1% preparation reduced *S. epidermidis* growth by about 80% (Fig. 3). Treatment with 0.2% reduced bacterial counts to 8.6×10^4, and no colonies survived in the presence of 0.3% preparation (Table 1).

Bacillus cereus is an environmentally ubiquitous, gram-positive, spore-forming bacillus responsible for two distinct food-borne disease syndromes as well as other manifestations of pathogenicity (Jackson, 1991). Treatment with 0.1% preparation reduced bacterial growth by approximately 80%, as shown in Fig. 4. Treatments with 0.2% and 0.3% reduced bacterial counts to 9.7 x 10^4 and 1.0 x 10^4, respectively, from an initial population of 2.7 x 10^6 (Table 1).

Pseudomonas aeruginosa is a common environmental gram-negative bacillus that is an opportunistic pathogen under certain conditions. The ubiquitous occurrence of *P. aeruginosa* in the environment is due to several factors, including its ability to colonize multiple environmental niches and use many environmental compounds as energy sources (Lyczak *et al*., 2000). Nearly all clinical cases of *P. aeruginosa* infection have compromised host defense (Lyczak *et al*., 2000). Treatment with 0.1% preparation reduced bacterial growth by approximately 70%, as shown in Fig. 5. Treatment with 0.2% preparation reduced bacterial counts to 1.3 x 10^4 from an initial population of 3.2 x 10^5, and no colonies were observed after treatment with 0.3% preparation (Table 1).

Propionibacterium acnes is a commensal microorganism found in sebum-rich skin that plays a role in acne inflammation by stimulating keratinocytes to produce a number of proinflammatory cytokines (Choi *et al*., 2008). Acne vulgaris is an extremely common disorder affecting many adolescents and adults throughout their lifetimes. The pathogenesis of acne is multifactorial and is thought to involve excess sebum, follicular hyperkeratinization, bacterial colonization, and inflammation (Amin *et al*., 2007). Treatment with 0.1% preparation reduced bacterial growth by approximately 70%, as shown in Fig. 6. Treatment with 0.2% reduced bacterial counts to 5.0 x 10^4 from an initial population of 2.8 x 10^6, corresponding to a ~97% reduction. Treatment with 0.3% preparation completely inhibited the growth of *P. acnes* (Table 1).

Collectively, these results show that this preparation of ore minerals from Korea could be used to control many species of bacteria, including *Escherichia coli*, *Staphylococcus aureus* subsp. *aureus, S. epidermidis, Bacillus cereus*, *Pseudomonas aeruginosa* and *Propionibacterium acnes*. Therefore, this antibacterial ore mix may be developed as new antibacterial materials, such as sanitary antibacterial ceramics, antibacterial tiles, antibacterial architectural coatings, antibacterial wallpaper, *etc*. However, the mechanism of action is not yet known, and detailed comparative examinations on bacterial strain specificity are required to determine the antibacterial efficiency of these minerals before commercialization.

Acknowledgments

This research was financially supporting by the Ministry of Education, Science Technology (MEST) and Korea Industrial Technology Foundation (KOTEF) through the Human Resource Training Project for Regional Innovation.

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