# THERAPEUTIC MUD AND CYANOBACTERIA

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**Summary**

Mud therapy is one of the most commonly used non-pharmacological approach for many rheumatic pathologies, and is also used as a co-adjuvant in drug therapy. The mechanism of action of mud therapy is not completely understood, but most probably the beneficial effects could be due to mechanical, physical and chemical factors. The therapeutic effects of the "mature" mud are related to its organic components with special regards to the presence of cyanobacteria and micro-algae. Recent studies have proven that the glycoglycerolipids in cyanobacteria have several biological activities, such as anti-inflammatory effects.

**Key Words**: mud, cyanobacteria, glycoglycerolipids, balneotherapy

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## Thermal mud

Thermal mud is a mixture of clay and thermal water colonized by thermophilic bacterial species with a specific pharmacological character [l-3]. It is used as a natural therapy, practiced from ancient times. Extensive use of hot springs can be traced back more than 4,000 years. Plinius (23-79 AD) mentioned the use of the mud from the thermal ponds, drawing special attention to the great benefit provided in cases of chronic inflammatory changes, arthritis and rheumatism. The beneficial effect of mud pack or bath treatment has been hitherto ascribed to heat but now, after investigation, it is also ascribed to a specific anti-inflammatory action related to a so-called "maturation" process. During this process several colonizing thermophilic microorganisms grow, modifying the physical and chemical characteristics of the thermal mud [3, 4], thus optimizing the effectiveness of the mud pack treatment. The advantage of mud treatment is that it retains moisture and coolness for longer periods than cold water packs or compresses. The cold moisture in the mud packs relaxes the pores of the skin, draws the blood into the surface, relieves inner congestion and pain, promotes heat radiation and elimination of morbid matter. Mud pack treatment thus cannot be considered as a simple "alternative" natural remedy for the cure of chronic osteoarthrosis, but has the status of a true pharmacological treatment with clinically observable effects.

## Maturation process of thermal mud

The "maturation" (the heat treatment) of thermal mud makes the mud suitable to be used for mud-pack treatment of patient. Maturation takes place in special tanks built in stainless steel. Within each tank a mixer fitted with two rotating blades is installed to ensure appropriate section of the mixing mud and prevent compaction (fig. 1). The maturation process starts when the thermal water is mixed with clay. Each tank has a hot thermal water pipe at the top, which flows continuously over the mud at a constant level. In addition, the mud is transferred from one container to another; so that the constant rotation allows for a more uniform ripening of the mud because the mud is in contact with thermal water. Maturation time depends on the full colonization of the mud by thermophilic microorganisms, but it is generally 50-60 days, during which thermal water constantly flows over the mud at a temperature of about 60°C.

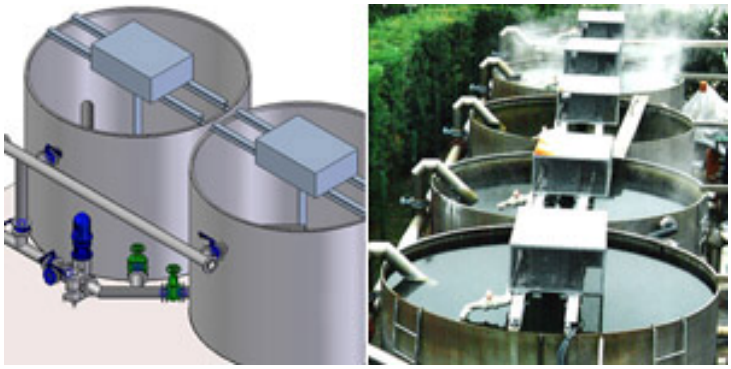

Fig. 1 Special tanks in stainless steel.

Non-pathogenic thermophilous microbial community grows during maturation, giving rise to extensive physical-chemical modifications of the mud, enriching it with valuable and highly anti-inflammatory active principles. The high temperature in the maturation process leads to the development of the active elements typically found in thermal mud. It is significant that only mature mud has therapeutic characteristics that allow its use for mud-packs. In fact, after maturation, several effects can be valuated to verify the quality of mature mud. For example:

- the changes of viscoelastic mud assessed at the beginning and end of the process of aging (also said RTM Index)
- measurement of free surface of the mud and its polar and dispersed components and of the ability of the mud to adhere to the skin;
- effectiveness of a functional mud mature, measurement of energy.

The maturation implies several changes of the physicochemical properties, i.e. increase of the thermo-insulating power and hydration capacity of the mud, together with biochemical changes, the most important of which is the production of several molecules. Galzigna et al. (1995) consider the maturation, as the process of colonization of the mud due to the growth of thermophilic bacteria and algae, in fact some physical changes of the mineral matrix are observed after mud maturation (i.e. the production of an active anti-inflammatory principle chemically related to a sulfo-glycolipid, also found by others [7] as a catabolite of thermophilic blue-green algae). These authors measured thermal mud maturation by conductimetry and viscosimetry of the changes in its physical and biochemical characteristics [5] with the production of molecules that have anti-inflammatory properties, capable to pass the rat skin barrier.

In their studies the process of maturation of thermal mud was followed as a measure of the production of such a compound, and it is completed within 60 days as indicated by the production of the sulfolipid fraction and by the physico-chemical changes of the mud. It is interesting to note that the initial lag phase in its production corresponds to the extraction of the highest amount of chlorophyll a. This may indicate a switch in the production of photosynthesizing pigments toward a phase of growth in which a prevalent utilization of the sulphur present in the system occurs [l]. The effect of mud packs on patients was objectively assessed on the basis of serum analyses changes, whereas the process of maturation was shown to be necessary to optimize the therapeutic value of the treatment [5].

## Cyanobacteria and therapeutic effects

Cyanobacteria, also known as blue-green algae, belong to a phylum of photosynthesing bacteria (fig. 2).

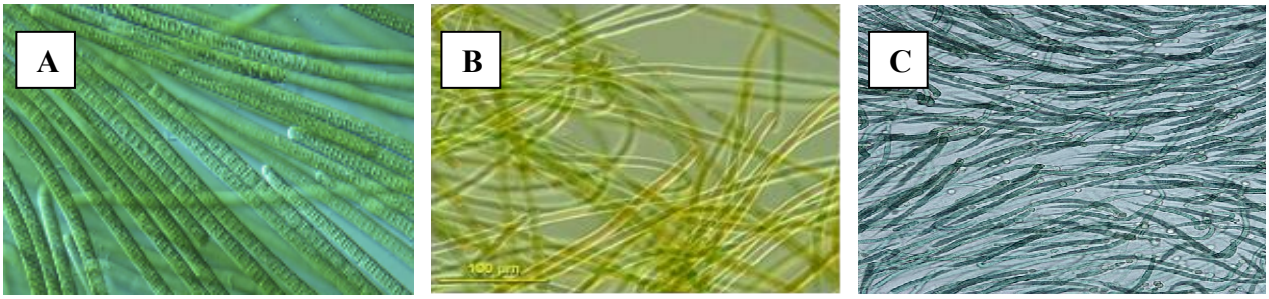


Fig. 2. Different types/species of Cyanobacteria: (A) *Tolypothrix;* (B) *Oscillatoria;* (C) *Rivularia*.

Blue-greens are not true algae. They have no nucleus and no chloroplast, in most forms the photosynthetic machinery is enclosed into folds of the cell membrane, called thylakoids. The blue-greens are widely distributed over land and in water, especially in environments where no other vegetation exists. Their fossils, called stromatolites, have been discovered in 1961 in Shark Bay, Western Australia, and have been identified to be over three billion years old. Cyanobacteria were probably the primary producers of organic compounds and the first organisms to release molecular oxygen, $O_2$, into the primitive atmosphere. In fact photosynthesis in cyanobacteria generally uses water as an electron donor, though some may also use hydrogen sulfide as occurs among other photosynthetic bacteria [9,10].

The majority of blue-greens are aerobic photoautotrophs, their life processes requiring only oxygen, light and inorganic substances. The process of nitrogen fixation and the occurrence of gas vesicles are especially important for the success of species of Cyanobacteria. They can live in extremes temperatures (-60°C to 85°C), and a few species are halophilic or salt tolerant. They can also grow in full sunlight or in almost complete darkness. They are often the first organisms to colonize bare areas of rock and soil, as seen after cataclysmic volcanic explosion (e.g. Krakatoa, Indonesia in 1883). Unlike more advanced organisms, these organisms do not need any substance produced by other organisms [10].

At the onset of adverse environmental conditions, some blue-greens algae can develop akinetes (modified cells). Akinetes contain large reserves of carbohydrates, which allow these organisms to tolerate adverse conditions such as the complete drying of ponds or cold winter temperatures.

Thermal hot springs are environments in which the combination of high temperature with $H_2S$ or acidic conditions decreases the biodiversity enormously. Cyanobacterial mats are most common in hot springs at near neutral or alkaline conditions. Thermal springs that contain sulfide may limit mats formation of thermophilic cyanobacteria that do not tollerate the combination of high temperature and high levels of sulfide. At moderate concentrations of sulfide, mats of *Oscillatoria spp*. have been shown to lower the sulfide concentration through anoxygenic photosynthesis. Another strategy is found in the so-called inverted mat. Here mats of the anoxygenic phototroph Chloroflexus overlay the cyanobacterial mat [11].

Muds of thermal springs have been studied in terms of microorganism's colonization, and it has been found that there is an extensive growth of diatoms, and, subsequently, the growth of blue-green algae which form a sort of continuous film on the surface.

The most numerous group of cyanobacteria present in mud belongs to *Phormidium* genus (fig. 3).

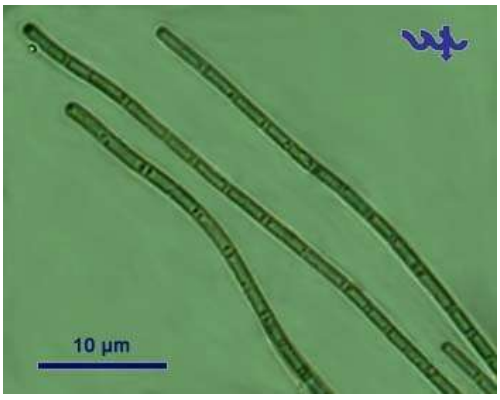


Fig. 3 *Phormidium*

Numerous studies have focused on the properties of the thermal muds of the "Euganean" hot springs. The Euganean mud baths maturation achieves a mature mud enriched in ETS-05 cyanobacterium with high anti-inflammatory activity. The discovery of cyanobacterium ETS-05, the anti-infiammatory activity of its glycolipids, the medicament with said glycolipids and the uniqueness maturation process have received a European patent. Now, this specific mud is classified as drug by the European Patent Office. In particular, the mud's therapeutic effects are determined by a high anti-infiammatory activity of glyceroglycolipids, by an increase of antioxidant defenses, by a reduction of IL-1 serum levels and by a protective action on cellular metabolism. In addition, these beneficial effects are go together with the absence of side effects on gastrointestinal tracts. Glycolipids are present in photosynthetic eucaryotic and prokaryotic organisms, in which they are associated with thylakoid membranes. In cyanobacteria, glycolipids are also associated with the heterocystous cell walls. The polar diacylglycerolipids content of this cyanobacterium consists of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG) (fig. 4) and phosphatidylglycerol. Recently, both natural and synthetic forms of these glycoglycerolipids have been shown to have specific biological activities, including anti-algal, antiviral, anti-tumour, anti-inflammatory and immunosuppressive activities, inhibition and promotion of cell growth, and protection against cell death [12]. Bruno A. et al. (2005) have determined the relative anti-inflammatory potencies of the purified MGDG, DGDG and SQDG from ETS-05 in vivo. MGDG, DGDG and SQDG inhibit croton-oil-induced ear oedema in mouse in a dose-dependent manner. MGDG are more effective than other glycoglycerolipids and referent anti-inflammatory drugs, indomethacin and betamethasone. All three lipid compounds show lower toxicity than the referent drugs. The activity of MGDG increases with the onset of the second, later phase of infiammation (4 to 24h) and the effect was gradually lost over the following 40 h. A comparison of these time-related effects on cyclooxygenase (COX) and nitric oxide synthase (NOS) activities suggest that MGDG inhibition of the second phase of carrageenan-induced oedema could be due to a selective effect on the induced COX2 activity and/or a selective towards endothelial NOS inhibition [12,13].

Lenti M. et al. (2009) have reported a study about the possible anti-inflammatory function of MGDG in cartilage using an avian articular cartilage model. They have showed that treatment of avian articular chondrocytes with the galactolipid MGDG suppresses the expression of the inflammation-induced proteins, suggesting a strong anti-inflammatory property of MGDG. MGDG shows also cell anti-proliferative activity, without interference with cell differentiation, suggesting a protective role for articular cartilage [13].

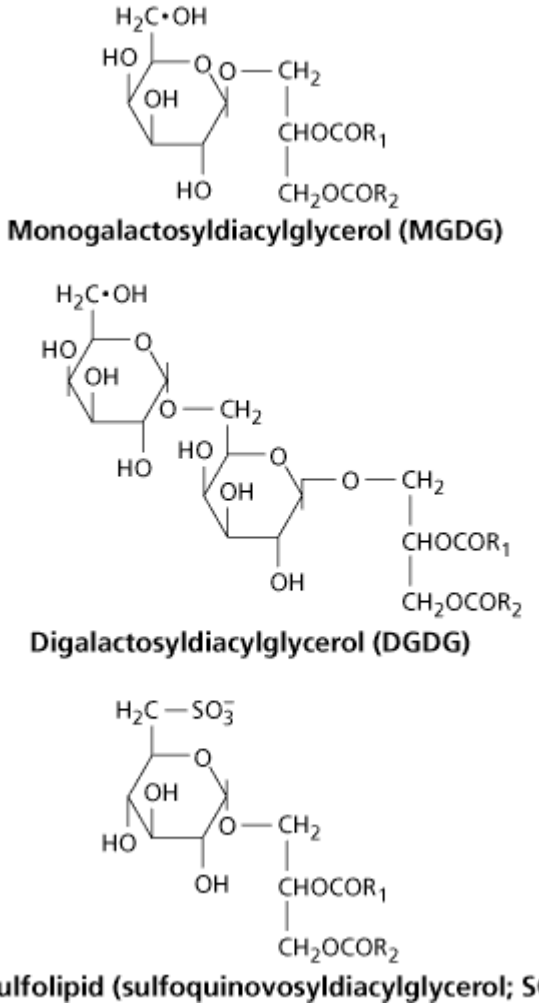


Fig. 4. Main structural components of the glycoglycerolipids used in this study (as isolated from the ETS-05 thermophilic blue-green alga): Monogalactosyldiacylglycerol (MGDG); digalactosyldiacylglycerol (DGDG); sulphoquinovosyldiacylglycerol (SQDG).

Furthermore, several studies have shown the efficacy of thermal therapy in psoriasis. Delfino M. et al. (2003) have investigated the efficacy of biological sulphurous "Bioglee" on psoriasis. They have used an organic matrix derived from the microflora metabolism grown in shallow hyper thermal waters possessing high sulphur concentrations. Cyanobacteria and sulfobacteria thrive in this matrix. The effect of this matrix on patients affected by psoriasis was evaluated through the monitoring of typical symptoms such as desquamation, cutaneous erythema and itching. The anti-psoriasic action of the thermal matrix may be ascribed to substances such as carotene, hydro and lipo-soluble vitamins and naturally-occuring phytosterols. In addition, these substances seem to work synergistically with the mineral components of the thermal matrix to attenuate the psoriasis symptoms [14].

A novel field of research about the activity of glycolipids, is their capacity to inhibit the polymerase activity of HIV-1 RT V. Reshef et al. (1997) have isolated diacylated sulfoglycolipids and acylated diglycolipids from several strains of cyanobacteria and have shown that these compounds inhibit HIV-1 RT enzymatic activity at different concentrations [15].

**Balneotherapy**

The "balneotherapy" includes the use of hot baths and natural vapor baths, as well as of various kinds of muds and sand used for thermal applications. Balneotherapy has been used traditionally for the treatment of various rheumatic diseases since ancient times [16]. There are a few randomized controlled studies about this therapy regimen [16,17,18]. The balneotherapy is used particularly in Europe and its costs is partially reimbursed by national health care systems [17].

Thermal waters and muds exert their activities trough physical, physic-chemical and chemical stimulations, in particular mechanical and heat stress. The therapeutic activities are effective both for short and long time. Application of heat to the skin produces effects in the deeper portions of the body inducing muscle relaxation, increasing blood supply due to dilation of the vessels and stimulating metabolic activity, trough reduction of cholesterol (increase of IgG and IgM levels), and ormonal response (β-endorphins, ACTH). The relaxation of muscular tissue results in the relief of pain due to rigidity and spasms of the muscles. The natural minerals and medicinal properties, along with heat, promotes circulation, enhances the immune system, offers total muscle relaxation, pain and stress relief, reduces inflammation by removing algesic metabolites, free radicals and other pro-inflammatory substances from the tissues, improves range of motion, and detoxifies. It has been recently demonstrated an anti-inflammatory effect of mud-bath applications on adjuvant-induced arthritis in rats [18] and in patients affected by spondylitis or IBD (inflammatory bowel disease). There are almost no side effects during and after treatment, and there is a very low risk to the patient's general health and well-being.

Several studies have shown that a cycle of 6-12 mud-balneotherapy in Abano Terme-Montegrotto lead to [19]:

- A decrease of prostaglandins E2 determining osteoporosis;
- A decrease of leukotrienes B4 determining the degradation of articular cartilage;
- A decrease of interleukins 1 and 2 leaders of inflammation;
- A decrease of TGF that stimulates the bone;
- An increase of TGF beta, which stimulates the growth and differentiation of osteoblasts;
- A decrease of TNF in causing joint damage and cellular;
- An increase of insulin grow factor 1 which indicates an increase in bone metabolism.

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