
Archaeology vis-à-vis Microbiology: Discovering the Vistas of Interdisciplinary Research

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Abstract

The analysis of archaeological samples is very vital toward documentation and significance of archaeological remains and its interdisciplinary understanding. However, due to lack of integration between the two disciplines, i.e., archaeology and microbiology, it becomes very difficult to carry out a wholesome and scientifically supported research. Analysis of various samples like mummies, bones, organic archaeological remains, grave goods, etc., found at various archaeological sites has witnessed a new direction of analysis that has led to drastic changes in the formulation of understanding and theories. There are adequate records of such observations obtained from the analysis of samples from excavations and other archaeological remains and artifacts from different parts of the world. Our efforts should now concentrate on doing microbiological analysis of such rare archaeological samples and try to rationalize the need for such integration toward a better scientific and comprehensive approach.

Keywords

Archaeological remains • Microbiology • Next-generation sequencing • Multidisciplinary studies

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14.1 Introduction

The coming together of archaeology and microbiology has revolutionized several aspects of analysis and understanding of archaeological remains, which, in the want of an interdisciplinary research, had remained untouched. Several

new breakthrough discoveries in collaboration with microbiologists have advanced in the field of archaeology. Analysis of several samples from mummies, bones, organic archaeological remains, grave goods, marine archaeological samples, any material that contains bacterial or fungal growth, etc., from various archaeological sites has witnessed a new direction of analysis that has led to drastic changes in the formulation of understanding and theories (Suzuki et al. 2010; Child 1995; Reinhard and Bryant 1992). Ceramic investigation and other archaeological studies of the artifacts from the excavation make up the greater part of most archaeological reports, but microbiological and chemical analysis is an important and singular contribution to the field. Numerous facets of an archaeological site remain untouched and enigmatic without the microbial analysis (Schug et al. 2013). Microbiology has not just been adding to the data of archaeological importance but rectifying and asserting chemical analysis that had sometimes given alarmingly faulty results (The Mission Magazine, Spring 1996). If mummy or bones in a burial environment in examinable conditions are found, the bacterial and fungal isolates could tell details of genetic and dietary habits and help us understand the decomposing bones (Child 1995; Cavka et al. 2010; Antoine et al. 1992 for 1991).

An archaeological artifact cannot be restored to its original, but microbiology can definitely tell the archaeologist that if the artifact is not rescued soon, microbes are likely to eat it up completely. While this discovery was made, microbiologists also discovered a new species of a microbe of a previously known genus (Sánchez-Porro et al. 2010). So, often the process turns out to have mutual benefits and helps both fields grow in their research.

14.2 Microbiological Work in Concurrency to Archaeology

Microbiology has a significant relevance, contribution, and importance to the field of archaeology. *Mycobacterium leprae* was for the first time

detected using whole-genome amplification and polymerase chain reaction of a sample from archaeological skeletal remains in the Far East of Japan (Suzuki et al. 2010). The Vedic literature has the earliest literary mention (c. mid second millennium BC) of the leprosy, while the more accepted evidence of the first millennium from the Indian surgery science *Sushruta Samhita* and *Arthashastra* (a treatise of statecraft, economic policy, and military strategy) clearly states the details about leprosy. Microbiologists took samples from the Chalcolithic archaeological site of Balathal (Robbins et al. 2007), located in the south of Rajasthan, India, to understand better the timing of first infection as well as the pattern of transmission of the disease and its geographic origin, which is believed to have originated from Africa. Evidence from India during 2000 BC indicates the presence of lepromatous leprosy. It is the oldest documented skeletal substantiation that supports the earliest Indian literary evidence of leprosy (Robbins et al. 2009).

The decline and disappearance of the Indus Valley Civilization still suffer inconclusive theories that don't apply unanimously to the entire extent of the civilization homogeneously. Nevertheless microbiology has joined hands with archaeology to confirm and assert the localized causes of decline of the habitation and effects of various infectious diseases and sociobiological methods by analyzing the samples from the mortuary remains (Schug et al. 2013). A sample from the late Indus valley period at Harappa was studied for the paleopathology to determine the outcomes of the climate change and the social as well as economic disturbances that likely led to the decline of the civilization (Fig. 14.1).

The analysis of a bioarchaeological evidence demonstrates a continuous prevalence and a gradual increase in infectious disease which is seen at a higher risk of infectious diseases spread unevenly among the burial communities. Analogous mortuary disparities indicate a greater vulnerability in the framework of uncertain climatic conditions for the socially and economically marginalized communities at Harappa. Along with the previous evidence for escalating levels of aggression within the society during that

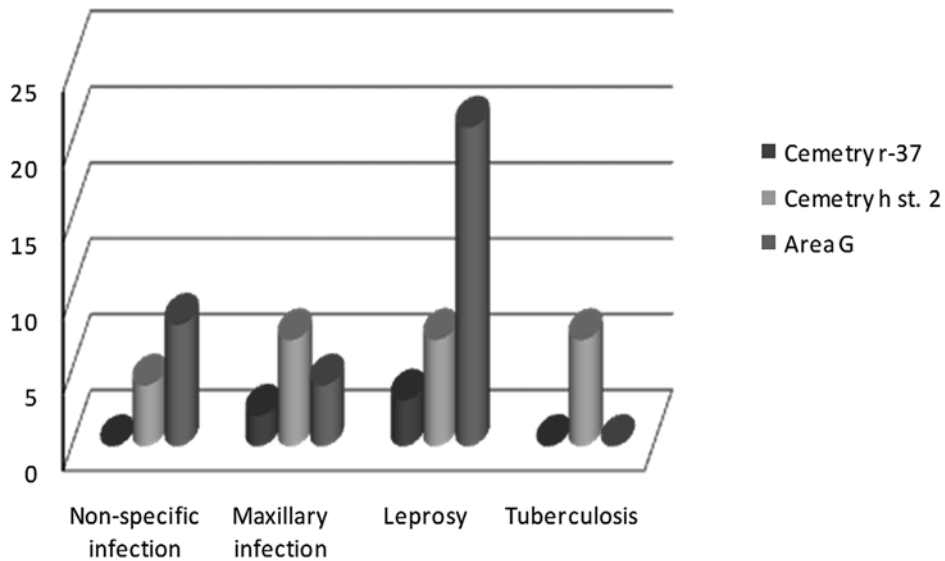


Fig. 14.1 Prevalence of infection and disease in three mortuary assemblages from Harappa (Schug et al. 2013)

time, the statistics sustains an increasing fight for attaining supremacy at the site of Harappa after 2000 B.C. (Schug et al. 2013). Scientists could go as far as to detail the socioeconomic conditions of the settlement from the pathological research, and analysis is indeed a creditable help to the field of archaeology. In fact, some sampling from other sites around or close to Harappa and a multidisciplinary analysis and approach could further strengthen and improve the theory stated. These observations on the relation between the climatic change and the social processes in the protohistoric urban settlements display decisive lessons in terms of susceptibility, diffidence, and the enduring consequences of temporary strategies that could cope with the change in climate (Schug et al. 2013).

A team of multidisciplinary scientists from microbiology, paleopathology, and paleoradiology in collaboration with an anthropologist worked on the samples of a mummy that was kept in the collection of the Zagreb Archaeological Museum, Croatia (Cavka et al. 2010). They documented a report of the outcome of the analysis of the samples of a few genera that belong to the groups of fungi and bacteria and could be considered crucial for carrying out further investigations. These samples were taken specifically

from the oral and the abdominal cavity and the bandages tied around the mummy. Analysis conducted at the laboratory in Zagreb indicated that all of the found organisms belonged principally to the following groups of saprophytic fungi: *Monilia* spp., *Penicillium* spp., *Alternaria* spp., *Aspergillus fumigatus* spp., *Aspergillus nidulans* spp., *Rhizopus* spp., *Chrysosporium* spp., and a few genera of saprophytic bacteria, *Bacillus* spp., etc. (Cavka et al. 2010).

The results of the microorganisms isolated for microbiological analysis clearly demonstrate that they pose no threat to human health. They are not harmful, rarely associated with diseases, and ubiquitous in nature. With this, we cannot only understand the dietary habits of the dead but also its health at the time of the death and sometimes even the cause of the death.

Analysis of few samples from 21 archaeological sites from central Germany provided interesting diachronic insight into the epidemiology of carious defects in teeth of the people settled there over a period of 4,000 years (Nicklisch et al. 2015). The dietary habits and socioeconomic structures of the period were to be studied with the data obtained from a total of 494 individuals with preserved teeth. The results indicated that with the development of the society, meat and

dairy products became more and more important, and the prevalence of caries significantly decreased between two cultural sub-eras. In addition to this, some sex-specific observations were made, which showed that a greater number of women were affected by caries than men. Undoubtedly, dental health reflects both biological and sociocultural and economic structures of the society.

Microbiologists have also tried to understand the decomposition by microorganisms from buried bone samples in archaeological sites (Antoine et al. 1992). A survey of literature suggests that a dead cell releases enzymes in the form of proteases and DNases. This causes an increase in autolytic destruction of the tissues resulting in microbial decomposition at a greater speed. As a result oxic and anoxic environments are created within the rotting flesh and “bone taint” appears within 6 h after the death (Roberts and Mead 1986). Post death, the gut flora affects the abdominal and thorax bones to a greater degree of putrefaction than the long bones and the skull. Firstly the gut flora putrefies the esophagus and diaphragm and then spreads into the thoracic region. Hagihara et al. (1988) have reported that causal organisms for dental caries present in the mouth and the gut feature both demineralization and collagenolytic characteristics, e.g., the yeast *Candida albicans*.

It has been suggested that the first phase of decomposition is carried out by microbial flora of the interred corpse, while in the second phase soil microorganisms are also involved. The soft tissues of the abdomen are mainly decomposed by anaerobic oxidation, which produces organic acids. These organic acids formed demineralize the surrounding bone. Some observed this to be the case; however, other studies indicate the reverse (Child 1995).

Analysis of microbes contained in the soil from archaeological sites in Italy and Belize was conducted by picking up the soil samples from the pottery of both sites. The samples were cultured for aerobic and anaerobic bacteria, acid-fast bacilli, fungi, and actinomycetes. This study was a challenge for the clinical microbiology. The organisms that were isolated were quite similar to

those that might be found in contemporary soil specimens from equivalent geographical areas, though a more diverse and/or exotic microbial flora was expected owing to the presumed ages of the soil samples. The microscopy of the microbial flora and the results of the cultures of soil from the two archaeological sites did not differ much, except that the flora from the Lubaantun site, Belize, displayed more complex structure (Southern 2008).

In another report the archaeologists explored the great legendary “unsinkable ship” Titanic. They isolated the bacterium called *Halomonas titanicae* from the samples of the rusticles collected during the Akademik Keldysh (1991) expedition. Ballard (1989) and MacInnis (1992) have described “rusticles” as bioconcretious structures made up of rust, which look like icicles. These pieces of rusticles were sampled from the hull with the help of the articulated arm of the Mir-2 submersible. In this study, *Halomonas titanicae* strain BH1T was isolated from the rusticles using streak plate method on Bacto marine agar 2216 medium (Difco). The pure culture was subsequently preserved in lyophilized form till gene-sequencing program was initiated (Kaur 2004; Wells and Mann 1997). Microbiologists carried out a polyphasic taxonomic study and found the characteristics of the strain in detail. Analysis of the sample revealed the discovery of a new strain of the genus *Halomonas*. Overall, these studies prove that the abovementioned strain BH1T is showing phylogenetical similarity to various different species of the genus *Halomonas*, though they have essential differences in their phenotypic and chemotaxonomic features. However, DNA–DNA hybridization studies established it as a distinct species. Backed by this data, it was observed by scientists and microbiologists that a novel strain BH1T represents a distinctively separate and a new species of the genus *Halomonas*, named *Halomonas titanicae* sp. nov. (Sánchez-Porro et al. 2010). They realized that this isolated bacterium discovered from the rust samples of the RMS Titanic appears to accelerate the disintegration of the wreck of Titanic.

14.3 The Avenues and Challenges for Cross-Disciplinary Research

Another major problem stood in front of scientists when they carbon-date certain artifacts, and one of the most astonishing ones was in the case of the Shroud of Turin, precisely the burial cloth of Jesus of Nazareth. The radiocarbon dates showed its antiquity to a maximum of thirteenth to fourteenth century CE when it was realized that what had been radiocarbon-dated was in fact a jumble of linen and bacteria and fungi and bioplastic coating that had the occasion to grow on the fibers for centuries. The linen itself had not yet been dated. Often the conventional methods of cleansing a sample fail to remove bacteria and fungi. For example, biogenic varnishes were discovered on an ancient Mayan carved jade called “Itzamna Tun.” The scientists had to decontaminate the sample of the plastic-like coating that is a by-product of bacteria and fungi (The Mission Magazine, Spring 1996 issue).

Archaeological remains from the burial environment have often yielded good samples for understanding dietary habits of people. In the nineteenth century, it was discovered that coprolites are the most noteworthy and remarkable dietary remains recoverable from archaeological contexts. Harshberger (1896) discovered for the first time the potential importance of human coprolite and concluded that bones and seeds found in ancient feces could very well offer clues to ancient diet. Later, Netolitsky (1911, 1912) studied the coprolites from Egyptian mummies and identified a number of important dietary components in it. Either dead bacteria can directly be identified in coprolites or else live bacteria have to be cultured for analytical examination. Two types of bacteria species are present in coprolite, viz., cystic and noncystic anaerobic bacteria. Endospore-containing bacteria, for instance, *Clostridium*, can be isolated and characterized from coprolites. However, noncystic disease-causing bacteria, viz., *Salmonella* and *Shigella*, are vulnerable to decay in the postdepositional surroundings and are difficult to be characterized (Reinhard and Bryant 1992). In addition, to these bacteria, there are

reports of well-preserved hyphal segments and fungal spores in coprolites. These evidences help to understand the coprolite preservation strategies and also offer dietary data. Interpreting these remains is obstructed by the lack of taxonomical identity for specific class of fungi (Reinhard 1985a, b, c). Majority of research activities on coprolite have enlarged to include the study of many different disciplines along with microbiology. Nevertheless, its results and interpretations still remain a challenge for the scientists.

14.4 The Success Stories and Applying Next-Generation Sequencing

Next-generation sequencing (NGS) allows us quite easily to see if we are dealing with authentic ancient human DNA. The technique of NGS is metagenomic advancement in which, before sequencing, the DNA fragments in a sample are amplified, together with little samples of ancient DNA that are not possible by traditional DNA isolation methods. This offers an extensive overview of the genetic material of sample under study and recommends an extra precise data of the accurate ratio of prehistoric DNA to modern DNA. The advantage of the speed of next-generation platforms enables us to sequence a sample repeatedly, making it easier to verify the patterns of damage that distinguish ancient DNA from recent DNA (Cossins 2013).

The new NGS findings have not gone unchallenged, however. Some researchers have questioned Pusch’s suggestion that the proportion of human DNA in samples from the Egyptian heads is comparable to that from permafrost-preserved specimens—such as a 4000-year-old Greenlandic mummy known as the Saqqaq man—subjected to NGS (Khairat et al. 2013).

This technique is specially being applied to the samples of mummified bodies as the mummification process protects DNA by rapidly desiccating the tissue, removing the water required for hydrolytic depurination and deamination, two of the primary mechanisms by which DNA is broken down.

The comparison to the ice-preserved specimens is misleading because the vast majority of the sequences obtained from the Egyptian samples were unidentifiable. For instance, in one of the Egyptian samples put forward as comparable, the researchers could not identify 97.5 % of the sequences, so they used the 2.5 % they could, of which 96 % were human. For comparison, 75 % of the sequences for Saqqaq man could be identified, 99.7 % of which were human (Khairat et al. 2013).

Even so, the latest study is a first step in the right direction and that whole-genome sequencing of ancient Egyptian mummies will likely become commonplace in the near future—that is, so long as sufficient funds are available and researchers can get their hands on specimens.

It could also bring about a new era of “molecular archaeology” in which mysteries concerning the ancestral origins of a certain ancient race, and how they lived and died, can finally be solved.

14.5 Microbes Are the Natural Data Bank of Archaeologists

Microbiologists have tried to determine paleoclimatic conditions through viable paleosol microorganisms, which could also help date the archaeological stratas.

The paleosol microorganisms were cultured by scientists from a complete chronosequence of the archaeological site of Hell Gap, Wyoming, USA (Grund et al. 2014), on an experimental basis to see if the colonies contain information regarding the climatic conditions of that region. They even went to the extent to do a relative dating of the archaeological site by looking at the change in the density of the microorganisms present in the soil sample. Studies of pathogens in archaeological contexts and microorganisms in ceramic residues are widespread, though not very frequent. But this is altogether a revolutionary idea, which could supplement the paleobotanic soil and phytolith analysis results. The results of the samples from Hell Gap didn't meet greatly the expectations, but the thorough study of the results helped understand the other external factors involved that could make the analysis'

results go wayward (Grund et al. 2014). Those factors could be kept in mind while undertaking such a study again elsewhere. This experimental study has opened up new horizons for bridging collaborative efforts of microbiology and archaeology to get a comprehensive scientific study of archaeological data.

14.6 Microbes Determine Settlement Pattern

The analysis of soils and cultural layers at an archaeological site in Russia showed that repeated addition of various organic materials increases a microbial biomass and enzymatic activity (Chernysheva et al. 2015). This occurs in particular with urease activity, which increases in soil because of continuous input of compost, manure, and other organic residues and income of ureolytic bacteria along with organic fertilizers. The urease activity was observed in the cultural layers of the site in that area with ruined wall remains. The activity was almost twice higher than the area without wall remains. These results showed that the structures in the settlement were used as cattle pens. So it was inferred that comparison of urease activity, scattered potsherds, and phosphorus content in soil could help demarcate the boundaries of ancient manured lands. These determining parameters could be useful in revealing the sites of cattle keeping, areas of ancient arable lands, and other clues to the settlement pattern of a site.

14.7 Conclusion

Archaeology is a field of multidisciplinary contributions, which has much scope and potential for research. There's much contribution asked of microbiology in the field of archaeology. Archaeologists depend mostly on manual analysis of the samples of the excavations; instead, even the fundamental analysis of the soil, bone, and other samples in microbiological labs would surface manifold inferences that can be drawn to get a more integral understanding of an archaeo-

logical site. Our efforts should now concentrate on doing microbiological analysis of such rare archaeological samples and try to rationalize the need for such integration toward a better scientific and comprehensive approach.

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