China leads in getting a head

BEER, THE WORLD'S MOST POPULAR PROCESSED BEVERAGE, AFTER TEA, IS ALSO THE MOST ANCIENT, WRITES **S ANANTHANARAYANAN**

Fermentation, and the euphoria that comes from drinking its products, was surely discovered by happy accident. The accident must have been of sugary fruit that got bruised in storage. But getting alcohol from grain, which has starch and not sugar, is technology, and represents another level of development.

The discovery, by Jiajing Wang, Li Liu, Terry Ball, Linjie Yu, Yuanqing Li and Fulai Xing, of the Universities of Stanford and Brigham Young and the Zhejian Research Institute of Chemical Industry, Hangzhou, and the Shaanxi Institute of Provincial Archeology, Xi'an, in China, that brewing beer was a regular practice as early as 5,000 years ago, reported in the *Proceedings of the National Academy of Sciences*, is hence remarkable. This places the invention of beer at about the same time as that of the wheel!

A related process of even greater antiquity is the production of rice wine, which is actually a form of beer, as the starting point is the starch in rice, not sugar. The conversion of the starch to sugar in rice wine, however, is natural, with the help of molds and enzymes produced by microbes. This is unlike the case of the usual beer, where production of enzymes in the grain needs to be activated.

Alcohol is the result of the action of yeast on sugar, where the sugar splits into alcohol and carbon dioxide. Wine is traditionally made from grapes that have the yeast on their skin. All that needs to be done, for the simplest wine, is to crush the grapes and cover the juice. But there can be a more copious supply if we start from starch, of which there is plenty in grain. Here, the starch has first to be converted into sugar and then fermented with the help of the external addition of yeast. This process involves germinating the grain for production of the enzymes that first soften the outer layer of the seeds and later convert the starch that lies within.

When the germination, for which the grain is steeped in water, has been started, it is also stopped before the starch is consumed, by drying the grain in warm air, a process that is called *kilning*. The dry grain, which is *malt*, is then coarsely ground or *milled*, to be followed by the process of *mashing*. This process involves running warm water, at carefully controlled temperature, over the malt to promote the formation and extraction of sugars, as the sweet *wort*, which will be fermented. We can see that making beer from grain is a process of some complexity and the outcome of trial and experiment, and unlikely to develop unless there is a social, commercial and critical appreciation of the product.

Ancient beer

Wang and co-workers found evidence of ancient beer making in a site called Mijiaya in Shaanxi district, a plateau in north-west China, in the middle reaches of the Yellow River (Huáng Hé). The basin of this river is considered the cradle of civilisation in China and teems with archaeological finds. Mijiaya falls between Xi'an, known for finds of well organised New Stone Age settlements (6,700-5,600 BC), and Zhengzhou, the centre of the Shang culture (1,600-1,300 BC).

Writing developed during the Shang period and inscriptions on bone and tortoise shell, the so-called *oracle bone inscriptions*, were discovered and deciphered in the early 20th century.

While the inscriptions contain descriptions of the social, economic and administrative conditions of the time, they also contain the first records of beer in China, made by the Shang people with malted grain, with millets and barley or wheat as the main brewing ingredients, the authors of the *PNAS* paper say. Scholars have considered that the tradition may be older, as there was much agriculture in the region during the New Stone Age, *Yangshao* period (5,000-2,900 BC), with records of rice-based fermented beverages in the even more ancient Jiahu site, the authors say. But there has been no evidence of alcohol production in the Yangshao sites so far, they say.

The Maijiaya site, where Wang and colleagues have found new evidence, was discovered in 1923 and excavated from 2004 to 2006. Artifacts that appear to be beer-making equipment were recovered from two pits that were found on excavation and they were carbon dated to 3,400-2,900 BC, or the late Yangshao period. The artifacts consisted of intact, wide mouthed funnels and pieces of wide mouthed pots and amphorae and stoves. The funnels, pots and amphorae appear to be specifically for brewing, filtration and storage, while the stove would have been used for heating and temperature control while mashing.

To verify this attractive supposition, the team carried out tests on the residue found on the funnels and pieces of pots and amphorae. This residue contained specks of starch, which could come from grain, and particles of mineral, the *phytoliths*, which are commonly found in remains of decomposed plant material. The starch grains were identified as being almost all from millet and varieties of wheat or barley and partly from tubers, like yam, which are found in the region. While this suggests a recipe for beer with strength, stability and flavour, the grains of starch also showed signs of damage — pitting and being swollen or folded, which are like what is produced during the brewing process. During malting, enzymes start the process of breaking down starch into sugars and cause pits in the starch grains. And then, during mashing, when the grains are in warm water, they swell and lose shape. "Thus, the damaged state of the starch grains in our archaeological sample provides strong evidence for the conclusion that those starch grains are residues from the brewing process," the *PNAS* paper says.

Different plants produce phytoliths of different specific forms. Analysis of the phytoliths, the paper says, clearly indicate that the origin was of grasses and, particularly, varieties of wheat and barley. "The profile of phytoliths corroborates the starch grain assemblage, indicating the presence of broomcorn millet, Job's tears, and barley," the paper says.

Chemical analysis of the residue also showed unmistakable signs of beer brewing activity. A characteristic product of steeping, mashing and fermentation of cereals is Calcium Oxalate, which settles as "beerstone" in vessels that are used in beer-making. While the presence of oxalates lining the vessels suggests use in beer-making, the paper notes that some other plants, like spinach, also contain oxalates. However, the present vessel types are hardly suitable for storing fresh vegetables and such sources are unlikely, the paper says. One more possibility to rule out, the paper says, is that that the artifacts were contaminated by the surrounding soil or after the site had been excavated. The exterior of the vessels and also other fragments and material at the site were, hence, analysed — with negative results.

Source of barley

While it is clear that barley was an ingredient in this beer of Yangshao, there is no evidence that it was cultivated in China during the period. There have been sporadic finds in the Bronze Age, around 2,000 BC, and it is only in the second century BC that barley became important in China, the paper says. The few Bronze Age finds may hence represent cultivation as a rare, exotic food. The *PNAS* paper suggests that the Mijiaya farmers (or brew masters) may have made arrangements for small quantities of barley, and only for making beer, not as an item of food!

The Yangshao period in this region is known for considerable social complexity, the paper says. The prevalence of beer during the time and the group relationships that alcohol enables may have contributed to the great social structure and organisation that the region is known for, it adds.

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SPECIAL CELL DIVISION

THE EARLY STAGES OF MITOSIS COMPRISES TRANSITION BY PHOSPHORYLATING KEY PROTEINS, WRITES **TAPAN KUMAR MAITRA**

The evidence for the existence of a control molecule that triggers the onset of mitosis came from experiments involving frog eggs. Mature eggs develop from precursor cells called oocytes through meiosis — it is a special type of cell division that reduces the chromosome number in half when eggs or sperm are being formed. During egg development, the cell cycle is halted shortly after the start of meiosis, wherein the oocyte waits until it is stimulated by an appropriate hormone. It then completes most phases of meiosis but is arrested during metaphase in the second of two meiotic divisions. It is now a "mature" egg cell that is capable of being fertilised.

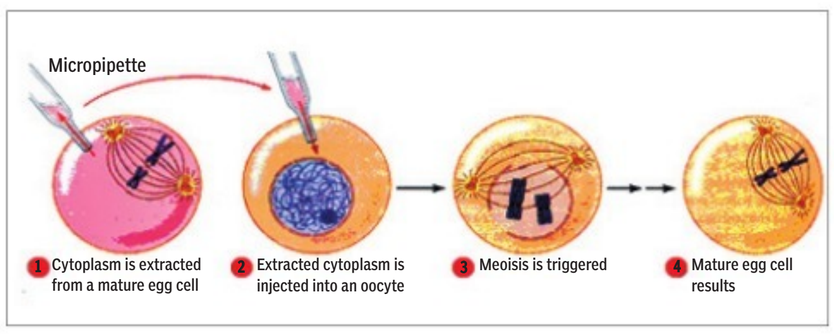
A crucial experiment demonstrated that if cytoplasm taken from a mature egg cell is injected into the cytoplasm of an immature oocyte that is awaiting hormonal stimulation, the latter will immediately proceed through meiosis. The researchers therefore hypothesised that a cytoplasmic chemical, which they named maturation-promoting factor, induces this oocyte "maturation". Subsequent experiments demonstrated that in addition to inducing meiosis, MPF also triggers mitosis when injected into fertilised frog eggs. Comparable molecules were soon detected in the cytoplasms of a broad range of dividing cell types, including yeasts, marine invertebrates, and mammals.

Biochemical studies of such mitosis-inducing molecules revealed that they consist of two sub-units — a Cdk and a cyclin. In other words, MPF is a mitotic Cdk-cyclin complex. Moreover, the mitotic Cdk portion of this complex is almost identical to the protein produced by the yeast cdc2 gene. In yeast cells with a defective or missing cdc2 gene, the human gene coding for mitotic Cdk can substitute perfectly well, even though the last ancestor common to yeasts and humans probably lived about a billion years ago!

Having established that MPF is a mitotic Cdk-cyclin that triggers the onset of mitosis in a broad spectrum of cell types, the question arose as to how mitotic Cdk-cyclin is controlled so that it only functions at the proper moment — that is, at the end of G2. The answer is not to be found in the availability of mitotic Cdk itself because its concentration remains relatively constant throughout the cell cycle. However, mitotic Cdk is only active as a protein kinase when it is bound to mitotic cyclin, and mitotic cyclin is not always present in adequate amounts. Instead, the concentration of mitotic cyclin gradually increases during Gl, S, and G2, eventually reacheing a critical threshold at the end of G2 that permits it to activate mitotic Cdk and thereby trigger the onset of mitosis.

In addition to requiring mitotic cyclin, the activation of mitotic Cdk also involves phosphorylation and dephosphorylation of the Cdk molecule — the binding of mitotic cyclin to mitotic Cdk yields a Cdk-cyclin complex that is initially inactive (step ©). To trigger mitosis, the complex requires the addition of an activating phosphate group to a particular amino acid in the Cdk molecule. Before this phosphate is added, however, inhibiting kinases phosphorylate the Cdk molecule at two other locations causing the active site to be blocked (step @). The activating phosphate group is then added by a specific *activating* kinase. The last step in the activation sequence is the removal of the inhibiting phosphates by a specific phosphatase enzyme (step ©). Once the phosphatase begins removing the inhibiting phosphates, a positive feedback loop is set up — the activated mitotic Cdk generated by this reaction stimulates the phosphatase thereby causing the activation process to proceed more rapidly.

After mitotic Cdk-cyclin has been activated through the preceding steps, its protein kinase activity triggers the onset of mitosis whose early events include chromosome condensation, assembly of the mitotic spindle and nuclear envelope breakdown. In the case of nuclear envelope breakdown, the mitotic Cdk-cyclin phosphorylates (and stimulates other kinases to phosphorylate) the lamin proteins of the nuclear lamina to which the inner nuclear membrane is attached. Phosphorylation causes the lamins to depolymerise, resulting in a breakdown of the nuclear lamina and destabilisation of the nuclear envelope. The integrity of the nuclear envelope is further disrupted by phosphorylation of envelope-associated proteins, and the membranes of the envelope are soon torn apart. Finally, phosphorylation of microtubule-associated proteins by mitotic Cdk-cyclin is thought to facilitate assembly of the mitotic spindle.



In nature, hormones act on frog oocytes to trigger meiosis and development into mature frog eggs, which are arrested (until fertilisation) in metaphase of the second meiotic division. The experiment shown here, performed by Y Masui and CL Markert in 1971, established the existence of a substance involved in this process; they called it Maturation-Promoting Factor (MPF). In their experiment, they used a micropipette to remove cytoplasm from a mature egg cell, arrested in metaphase of the second meiotic division, and to inject it into an immature oocyte. The oocyte then proceeded through meiosis and became a mature egg cell. This experimental procedure could now be used as an assay for the detection and eventual isolation of MPF. The hormones that trigger oocyte maturation in the frog were presumed to act by stimulating the synthesis or activation of MPF. MPF is now known to be a mitotic Cdk-cyclin.

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A biblical mystery

PHILISTINES ARE A DEROGATORY FIGURE OF SPEECH AND ONE OF THE BIBLE'S MOST IMPORTANT GROUPS OF PEOPLE BUT WE MIGHT HAVE GOT THEM ENTIRELY WRONG AS A NEW DISCOVERY IN ISRAEL SUGGESTS. **ANDREW GRIFFIN** REPORTS

One of the Bible's deepest and most important mysteries may be about to be solved. Archaeologists appear to have found a cemetery belonging to the Philistines for the first time ever, along with the remains of 200 people who were buried there. And together they might help shed light on one the Bible's most mysterious people. The scientists have said that the members of the Biblical nation didn't appear to be "philistines" — finding the people buried alongside jewellery and perfumed oil. They will now conduct further tests that could shed yet more light on the maligned people.

Those discoveries might be enough to make us rethink today's use of the word philistine, which tends to refer to uncultured people who don't know enough about the arts. "The Philistines have had some bad press but this will dispel a lot of myths," said Lawrence Stager, an architect who has led the expedition that found the cemetery since 1985.

Until now, most of our understanding of the Philistines came from the things that they have left behind. But now for the first time we have found their remains. "After decades of studying what Philistines left behind, we have finally come face to face with the people themselves," said Daniel M Master, professor of archaeology at Wheaton College and one of the leaders of the excavation. "With this discovery we are close to unlocking the secrets of their origins."

In the Bible, the Philistines are depicted as the ancient Israelites' archenemy, a foreign people who migrated from lands to the west and settled in five main cities in Philistia, in today's southern Israel and the Gaza Strip. The most famous Philistine was Goliath, the fearsome warrior who was slain by a young King David. The Philistines' legacy lives on in the name Palestine — the term the Romans gave to the region in the 2nd century, and which is used today by Palestinians.

The discovery was finally unveiled on Sunday at the close of a 30-year excavation by the Leon Levy Expedition — a team of archaeologists from Harvard University, Boston College, Wheaton College in Illinois and Troy University in Alabama. The archaeologists kept the discovery a secret for three years until the end of their dig because of a unique hazard of archaeology in modern-day Israel — they did not want to attract ultra-Orthodox Jewish protesters according to Masters. "We had to bite our tongues for a long time," he said.

In the past, the ultra-Orthodox have staged demonstrations at excavations where human remains are found, arguing that the remains could be Jewish and that disturbing them would violate a religious prohibition. Stager's team dug down about three metres (10 feet) to uncover the cemetery, which they found to have been used centuries later as a Roman vineyard.

Scholars have debated where exactly in the Aegean region the Philistines came from — mainland Greece, the islands of Crete or Cyprus, or even Anatolia, in modern-day Turkey.

The excavation of the cemetery has also shed light on Philistine burial practices. They buried their dead with perfume bottles, placed near the face. Near the legs were jars that likely held oil, wine or food. In some cases, archaeologists found the dead were buried wearing necklaces, bracelets, earrings, and even toe rings. Some were buried with their weapons.

"This is how Philistines treated their dead, and it's the code book to decoding everything," said archaeologist Adam Aja, a participant in the dig.

THE INDEPENDENT

PLUS POINTS

Named & shamed

A recent report says developed countries top the list of nations that have pirated Ecuador's genetic resources and lists the USA, Germany, the Netherlands, Australia and South Korea as the five countries that requested most patents for products derived from its endemic resources.

So-called "biopirates" in these countries, it said, were responsible for 113 of the 128 applications or patents identified and the countries named did not request authorisation from Ecuador to access the genetic resources used in these patents,

according to René Ramírez, chief of the department of higher education, science, technology and innovation, at a presentation of the report on 23 June. Patenting products based on Ecuador's endemic resources without the state's permission was "abusive, illegitimate and illegal", the report said.

The country would file applications to annul these patents, said Hernán Núñez, executive director of the Ecuadorian Institute of Intellectual Property, which co-authored the report with Ramírez's department.

Among the most pirated species, the study mentioned the Galápagos tomatillo (*Solanum cheesmaniae*), the Ecuadorian squash (*Cucurbita ecuadorensis*) and Darwin's cotton (*Gossypium darwinii*), prized for their pest-resistant properties. The list also included Galápagos seaweed (*Ochrophyta*), used to treat skin diseases, arthritis and obesity.

Biologist Luis Coloma, director of the Jambatu Amphibian Research Centre said the eport's diagnosis was valuable, but warned that fighting biopiracy depended not only on Ecuador, but also on the will and legislation of other countries.

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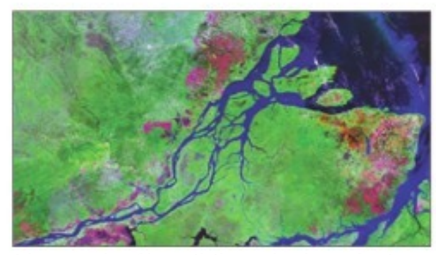
New coral reefs

In 2012, Fabiano Thompson boarded the US Navy research vessel *Atlantis* and set out into the Atlantic Ocean with colleagues aiming for a patch of water off the coast of Brazil about 80 to 180 km from the mouth of the Amazon river. Their mission was to find a previously unexplored reef system located in the unlikeliest of places.

"If you look at textbooks, they say that reefs do not form at large river mouths such as the Amazon," says Thompson, a microbiologist at the Federal University of Rio de Janeiro in Brazil. "We're talking about a river that is exporting over 300,000 cubic metres of water per second into the ocean." Large volumes of muddy freshwater—according to accepted wisdom—disrupt the preferred habitats of marine reef-building organisms, including corals and coralline algae such as rhodoliths.

But Thompson, along with Carlos Rezende of the State University of Northern Rio de Janeiro and colleagues in Brazil and the USA., had reason to believe the textbooks were wrong. "We had two leads to base our research on," Thompson says. "The first was a study published in 1977, describing the presence of fish and sponges at the mouth of the Amazon river. The second was a publication from 1999… reporting the presence of corals."

During the 2012 expedition, and a recent trip, the team deployed a barrage of equipment, including a multi-beam echosounder to map the ocean floor — 30 metres to 120 metres down — coring machines to remove samples and trawl nets to collect larger animals. Earlier this year, they reported their findings: a 9,500 square kilometre carbonate reef system, spread along a 50-km by 1,000-km corridor parallel to the coast. The reef comprises mostly rhodoliths and sponges, the team found, and supports a bustling community of fish and crustaceans, all thriving beneath the murky plume of the Amazon river.



A large coral reef beneath the murky plume of water flowing from the Amazon into the Atlantic Ocean.

Despite the inevitable astonishment that met this discovery, the fact that the Amazon reef has remained "hidden from view" until now is perhaps not so surprising, says marine biologist J Murray Roberts of Heriot Watt University in the UK. "We know very little about what's at the bottom of the ocean, even in areas that are relatively well studied like the Amazon river basin," he says.

Until the 1990s, it was generally accepted that reefs — particularly those built by corals — were restricted to shallow warm water with high light penetration. Indeed, such reefs, which Roberts refers to as "the ones we see on our holidays", have traditionally been the main focus of research. But perspectives have been evolving as technology has taken science deeper into the world's oceans.

THE SCIENTIST