

# A clean job: Innovative detergent enzymes



Biotechnology, nanotechnology and other pioneering research fields are indispensable even for the continuous refinement of everyday products like detergents and cleaners. Today's detergents are already high-tech products. Their excellent performance is due in part to special enzymes. Acting as biocatalysts, these enzymes help to remove such tough stains as blood, lipstick and cocoa.

As laundering habits change, consumers expect more and more from the detergents they use. And so the requirements profile for detergent enzymes is becoming tougher than ever before. The hunt is on for tailor-made enzymes that perform even better under extreme conditions than those currently in use

Innovative biotechnological methods are the key to achieving two crucial

The microorganisms that live here produce extracellular enzymes suitable for use in laundry detergents.

In Corporate Enzyme Research, they are constantly on the lookout for laundry detergent enzymes offering superior performance:

Dr. Karl-Heinz Maurer (l.)  
and Dr. Susanne Wieland.

goals – discovering new, even more powerful enzymes and developing more efficient production processes.

To accomplish this, the enzyme specialists at Henkel have entered into cooperations with skilled partners to pursue new development projects. Combining Henkel's breadth of knowledge regarding detergent enzyme requirements and the expertise of selected biotechnology companies is proving successful.

### Buried treasure

New enzymes don't have to be invented. In fact, an almost endless variety of enzymes is produced by millions of different microorganisms. But how do you identify the right ones among the countless others? And how can they be exploited for technical applications? The answers literally only have to be unearthed, because the soil itself is full of microorganisms. These microorganisms constantly produce proteins, including various enzymes. Stored in their genes are the "blueprints" for these proteins.

Mining such treasures is the goal of Henkel's cooperation with the Biotechnology Research and Information Network AG (BRAIN) in Zwingenberg near Darmstadt. Dr. Susanne Wieland of Enzyme Technology at Henkel: "The biotechnologists at BRAIN are capable of taking any soil sample and isolating the genes of microorganisms that contain the blueprints for producing enzymes."

What makes the expertise of the scientists in Zwingenberg so special is that they are able to utilize the genetic information even of microorganisms that cannot be cultivated in the laboratory. There are some two to six million such species, as opposed to the roughly 5,000 cultivable varieties. The researchers isolate the genes for the sought-after enzymes straight from the soil sample, propagate them in the laboratory, and integrate them in microorganisms that are easy to cultivate. These then produce the desired enzymes.

The potential of these still unstudied species is vast. By investigating the entire gene pool of these organisms – the

### Detergent enzymes

Enzymes are high-molecular proteins that accelerate biochemical processes. Subtilisins, one of the most important classes of enzymes, are employed in laundry detergents to break down proteins. Enzymes that break down proteins are generally known as proteases.

Of bacterial origin, subtilisins are named after the best-known original organism, *Bacillus subtilis*. They work best in alkaline washing water. In their active center, all subtilisins contain the amino acid serin, which is instrumental in releasing protein-containing soil from laundry. The enzymes break down blood and other proteins so effectively that they can be rinsed out by the washing water.

### Genome and metagenome

The genome is the totality of the genetic information of a cell. In addition to helical DNA, bacteria usually also contain small, ring-like DNA molecules known as plasmids.

The metagenome is the totality of the genetic material from organisms of a single habitat – from the forest floor, for instance. It also includes organisms that cannot be cultivated in the laboratory.



## Optimal cellulase blends

Henkel is the first laundry detergent manufacturer to employ not just proteases, but also cellulase blends in laundry detergents to achieve optimal performance. Cellulases are enzymes that break down cellulose. In laundry detergents, they work in two areas:

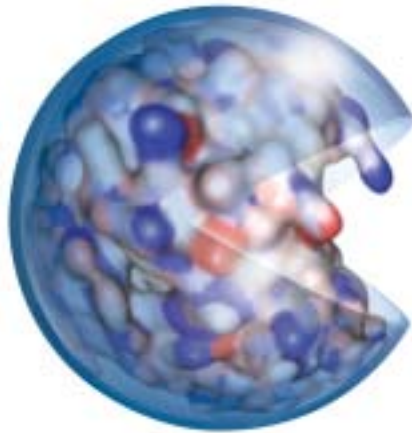
- The first area has to do with the graying of laundry due to deposits of limescale and pigments during the washing process. Cellulases prevent this deposition and hence graying.

- The second area has to do with the formation of undesirable pills, caused by mechanical stressing of the fabric when worn and when washed. These pills make the surface rough and also make once bright colors look pale. Cellulases remove existing pills and prevent the development of new ones.

Up to now, however, no known industrial cellulase has been able to perform well in both areas, i.e. graying inhibition and anti-pilling.

By using novel cellulase blends, Henkel has been able to combine both functions in a laundry detergent for the first time. And this is not all. By blending cellulases selectively, each detergent can be adapted precisely to its particular laundering application.

In its search for suitable cellulases, Henkel has not confined itself solely to the cellulases available to the laundry detergent industry, but has made a point of evaluating enzymes from other industry sectors. The result is two highly effective cellulases that deliver superior performance when blended.



A computer model of a protease: With its mouth – the “active center” – the protease gobbles up dirt from the laundry.

metagenome (see box on previous page) – it is possible to identify enzymes that cannot be detected by classical screening methods. This process is known as metagenome screening.

Henkel’s enzyme specialists gave BRAIN’s scientists the task of searching for novel proteases. The results are exciting. They already have totally new proteases at their disposal. And the search continues. The current goal of this cooperation is the development of optimized proteases for low washing temperatures. With new, more effective enzymes, Henkel hopes to gain a substantial edge over the competition.

### Cut and shuffle

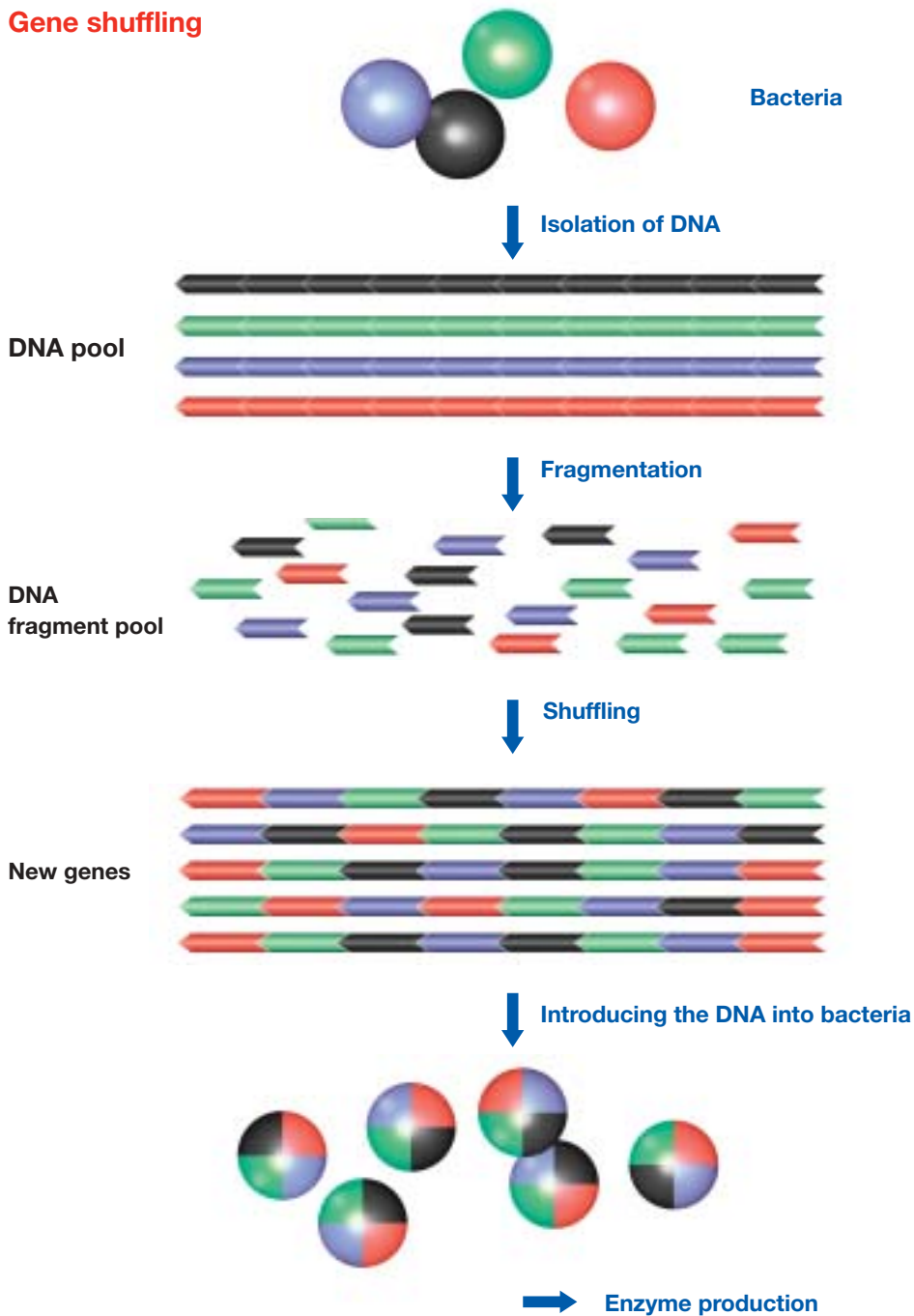
In their search for unknown proteases, Henkel’s enzyme technologists have used another method drawn from molecular biology, known as “gene shuffling” (see

opposite page). The Protéus company in Nîmes in southern France specializes in this process.

In simplified terms, gene shuffling means fragmenting genes, the blueprints for known enzymes, and recombining them in a random sequence. The resulting new genes produce novel enzymes that perform better or worse than the original genes. Artificial diversity is thus created.

Protéus has shuffled the genes of selected enzymes and established a good basis for further work. This pool was used by Corporate Research and Product Development in the Laundry & Home Care business sector. After long and intensive cooperation, they produced new proteases with improved characteristics. The best of them are currently being tested for their suitability in practical applications.

## Gene shuffling



## Gene shuffling

The fragmentation and reassembly of genes (DNA) permits the rapid creation of new gene sequences. The point of departure is several variants of a gene. These gene variants are broken down enzymatically into small parts. The fragments are gathered together and shuffled like a deck of cards, then recombined to form different gene sequences.

The fragments can be “glued together” again with a variety of molecular-biological methods. By combining the fragments in different ways, it is possible to create many new gene variants. These are then introduced into host organisms, e.g. bacteria, and propagated.



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