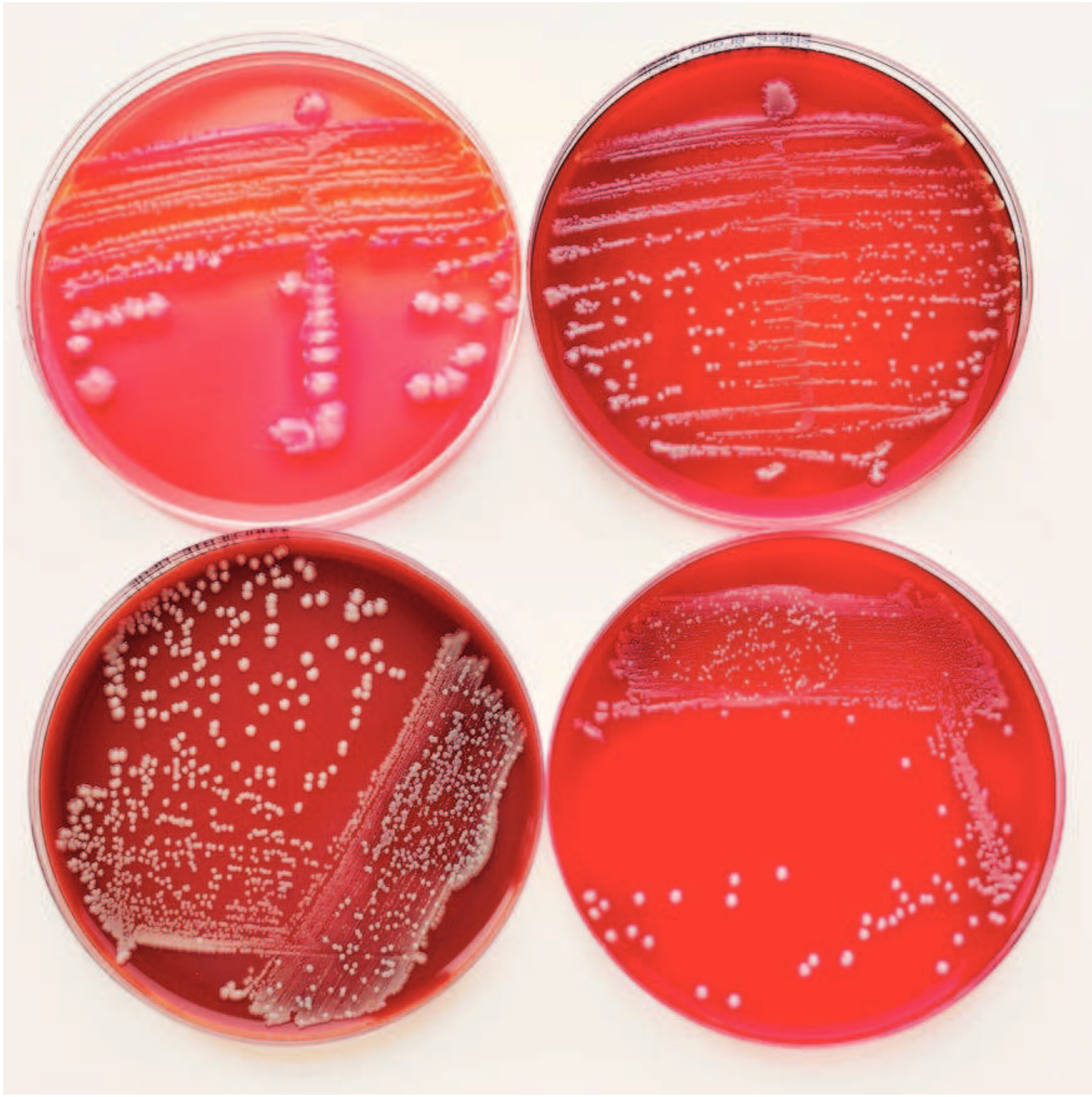

POST-CONTAMINATION SOLUTION

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photo: Jarun Ontakrai, Shutterstock.com

Preservatives | Dr Alexander Thiemann and Dr Jan Jänichen from Dr. Straetmans explain what preservative blend can help to adjust a preservation system once it has been contaminated.

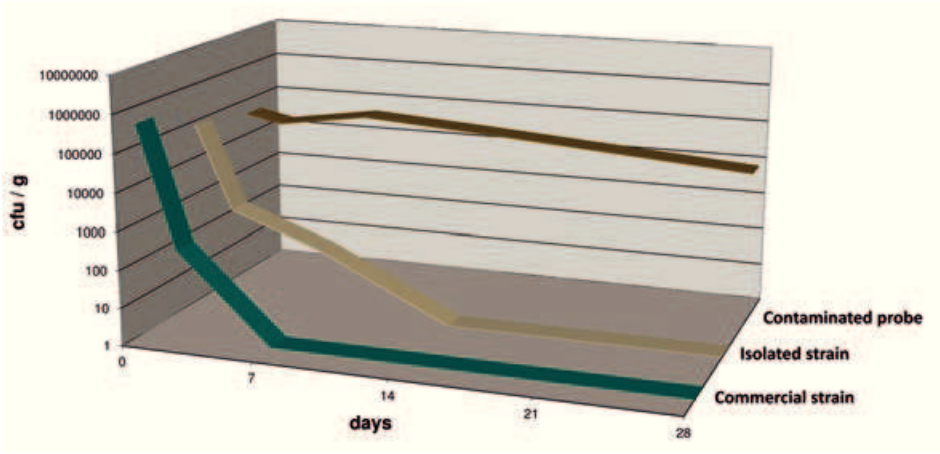

Fig. 1: Resistant house germs (Lactobacillus plantarum) – a case study

Cosmetics, in contrast to most food products, are expected to remain stable and sterile with a shelf life of three years or longer. When the end consumer applies the product regularly, a cosmetic product faces severe and repetitive microbial exposure. However, this microbiological challenge is not solely restricted to the end phase of the product's lifetime, but is also a challenge at the phase of the employed raw materials and throughout the manufacturing process.

According to Article 3 of the Cosmetic Regulation 1223/2009, it is the responsibility of the producer to ensure the product's safety for human health; since some microorganisms in cosmetic products can be pathogenic, this responsibility includes a reliable preservation system. In order to identify a safe preservation system, producers rely on antimicrobial preservation challenge tests. The most common challenge test used for cosmetic products is described in the Ph. Eur. 8 5.1.3.[1] The standard germs associated with the test have been selected based on a risk contamination assessment and the experience on how difficult it is to control their growth in a cosmetic product; these specific germs are, in fact, the cause of the majority of product contaminations. This is why it is assumed that a preservation system able to control these selected microorganisms will also cope well with other potential contaminations.

Failure of preservation systems in spite of challenge testing

Nevertheless, in the course of the past few years, our company has observed a growing number of incidents in which preservation systems failed to protect final cosmetic products sufficiently, despite having passed microbiological challenge testing. The product recall data from the EU's **Rapid Alert System** for non-food consumer products (RAPEX)[2] supported this observation. In many cases, other microorganisms than the standard germs employed in the challenge tests, such as Burkholderia cepacia or Enterobacteriaceae, were identified to be responsible for the reported contaminations.

When confronted with a contaminated product usually the following questions arise:

- What are the potential sources of product contamination?
- Which germs are causing problems for our customers?
- How can we protect cosmetic formulations against these germs?

Sources of contamination

Cosmetic raw materials themselves may carry a microbiological load which later can begin to proliferate in the final cosmetic formulation. A responsible supplier will take suitable measures to prevent all kinds of potential microbial contamination. However, contaminations can still occur, and the risk clearly depends on the type of raw material. As germs rely on the availability of water for their growth and proliferation, the contamination risk in water-free oils is quite low. In contrast, in natural raw materials such as clays, pigments and extracts, it can be quite high. Nevertheless, spores from fungi and bacteria as well as VBNC (viable but non-culturable) bacteria can manage to survive without any water in the oil phase, where they usually stay dormant while waiting for the conditions to change.

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Germs in cosmetic manufacturing

The contamination risk is even higher during the manufacturing process and when the product is applied by the end consumer. The most relevant germs in cosmetic manufacturing belong to the Pseudomonas species, because they are spread ubiquitously in nature and are capable of building hard-to-remove biofilms at higher concentrations. That is why Pseudomonas species are often found in process water systems and throughout the whole production chain (mixing vessel, bulk container, tubes, filling line). Other ▶

Figure and tables: Dr. Straetmans

prominent contamination germs inside the manufacturing process comprise Enterobacteriaceae, such as Klebsiella pneumoniae or several Enterobacter species[3]. Enterobacter gergoviae, a facultative pathogenic germ, was found to be the most prominent germ, beside Pseudomonas aeruginosa, in creams analysed in the tropical climate of South Africa[4].

In the use environment of the end consumer, the contamination risk largely depends on the hygienic conditions but also on the packaging type. For instance, the product application using refill packs is like a repeated challenge test. Such conditions are particularly challenging for any cosmetic preservation system.

Microbiological challenge testing – a case study

From time to time we receive microbiologically-contaminated products from our customers, all of which had previously passed challenge testing. In one case, we received a natural shampoo formulation with an unidentified contamination of unknown descent. The alternative preservation system originally implemented in the formulation was **Dermosoft 1388**, a patented multifunctional blend of sodium levulinate and sodium anisate. This formulation had passed the original challenge test with A-criteria. The germ responsible for the contamination via PCR (Polymerase Chain Reaction) turned out to be a Lactobacillus, more precisely L. plantarum. Lactobacillus is a genus of non-pathogenic bacteria, which pose a high contamination risk to final cosmetic products because of their ubiquitous distribution, e.g. in many fermented food products, on fermented and intact plant surfaces and in human saliva and the gastrointestinal tract.

Preservation system		CFU*/ g after 7 days
3 % Dermosoft 1388 (INCI: Glycerin; Aqua; Sodium Levulinate; Sodium Anisate)	+ 1 % Phenoxyethanol / Benzoic Acid	2.0×10^6
	+ 2 % Levulinic Acid / Sorbic Acid	1.1×10^7
	+ 1 % Benzyl Alkohol	3.4×10^4
	+ 0.5 % Caprylyl Glycol	1.5×10^6
	+ 1 % Ethylhexylglycerin	1.9×10^6
	+ 0.5 % Sodium Caproyl / Lauroyl Lactylate	2.5×10^6
	+ 5.0 % Pentylene Glycol	1.5×10^6
	+ 5 % Ethanol	1.6×10^6
	+ 0.3 % Chlorophenesin	2.6×10^6
	+ 0.5 % Salicylic Acid	< 10
	+ 0.35 % Salicylic Acid	50
	+ 0.2 % Salicylic Acid	290

Fig. 2: Reinforced original preservation system with Dermosoft 1388 inside a natural shampoo formulation
CFU: Colony forming unit

CONTAMINATION

Microbiological challenges are not restricted to product application but also occur during the manufacturing process

Preservation systems can fail in spite of products having passed microbiological challenge testing

Only salicylic acid combined with the blend of sodium levulinate and sodium anisate led to a significant microbial reduction after seven days

We then repeated the original challenge test and compared the Lactobacillus isolated from the contaminated product with a commercial strain of L. plantarum and the contaminated product itself. As a result, the Lactobacillus inside the contaminated product turned out to be much more resistant to the preservation system than its isolated counterpart and even more resistant than the commercial strain (Fig. 1).

So it seemed that the bacterial resistance, emerging in response to the selective pressure of the preservation system, lessened in isolation and subsequent cultivation of Lactobacillus. One reason for this decrease in resistance might be that the preservation system was suddenly removed, causing a loss of selective pressure. As a result, the resistant bacteria in the Lactobacillus population lost their former cultivation advantage and were gradually replaced by other Lactobacillus bacteria inside the population lacking the burden of a resistance mechanism, which can often be energy demanding.

This is why in case of an unexpected contamination of their product, producers should investigate and adjust their preservation systems within the contaminated product matrix itself, if possible.

Finding a solution

25 years ago **Kathon CG** (Methylchloroisothiazolinone/Methylisothiazolinone), formaldehyde or methyldibromo glutaronitrile would have been chosen to adjust a preservation system after a contamination. Today, however, methyldibromo glutaronitrile is banned from the list of allowed preservatives (Annex V of the Cosmetic Regulation 1223/2009), and formaldehyde and methylisothiazolinone are highly restricted in their use concentration and suffer from a bad reputation among the general public. Therefore, the development of alternatives is required.

	Commercial strains	Surfactant formulation pH 5.3	Emulsion pH 5.3	
			-	+ 0.3 % Glyceryl Caprylate
		CFU / g		
Commercial strains	*Enterobacter gergoviae*	< 10 after 2 days	< 10 after 14 days	< 10 after 2 days
	Burkholderia cepacia	< 10 after 2 days	< 10 after 2 days	< 10 after 2 days
	Klebsiella pneumoniae	< 10 after 2 days	< 10 after 7 days	< 10 after 2 days
	Bacillus cereus	< 10 after 2 days	< 10 after 2 days	< 10 after 2 days
	Penicillum spp.	< 10 after 2 days	< 10 after 7 days	< 10 after 2 days
	Raoultella ornitholytica	< 10 after 2 days	< 10 after 7 days	< 10 after 2 days
	Gluconacetobacter liquefaciens	< 10 after 2 days	< 10 after 7 days	< 10 after 2 days
	Lactobacillus plantarum	< 10 after 2 days	< 10^3 after 28 days	< 10 after 2 days
Isolated strains	*Raoultella ornitholytica*	< 10 after 2 days	< 10 after 7 days	< 10 after 2 days
	Gluconacetobacter liquefaciens	< 10 after 7 days	Re-growth after 28 days	< 10 after 2 days
	Lactobacillus plantarum	< 10 after 7 days	4200 after 28 days	< 10 after 7 days

Fig. 3: Verstatil Synacid in emulsions and surfactant-based formulations tested against well-known problem-causing microorganisms

This is why we tested a variety of potential preservatives and antimicrobial multifunctionals among organic acids, wetting agents and aromatic alcohols in a series of challenge tests within the original Lactobacillus-contaminated shampoo formulation containing the original alternative preservation system. As a result, out of all the tested substances, only salicylic acid combined with the blend of sodium levulinate and sodium anisate led to a significant microbial reduction after seven days (Fig. 2).

Based on these results, we came up with an enhanced preservative blend designed to cope with future microbiological contamination issues of our customers. This blend, **Verstatil Synacid**, is an effective combination of sodium levulinate, sodium anisate and sodium salicylate at ideal concentrations. It combines the outstanding performance of salicylic acid against resistant germs with the broad antimicrobial performance of the combination of sodium levulinate and sodium anisate.

In addition, the clear, light yellow liquid of this blend makes handling easier than when using solid pure salicylic acid powder. The blend proved to be highly effective in surfactant-based product concepts, not only against the standard germs from the Ph. Eur. challenge test but also against a variety of well-known problem-causing microorganisms (Fig. 3). In emulsions, a synergistic combination with a surface active antimicrobial agent, such as **Dermosoft GMCY** (INCI: Glyceryl Caprylate) is recommended and improved the performance even further. □

The reference list as well as additional product information and formulations can be found on the Internet – see download panel

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Adjusting preservation after contamination