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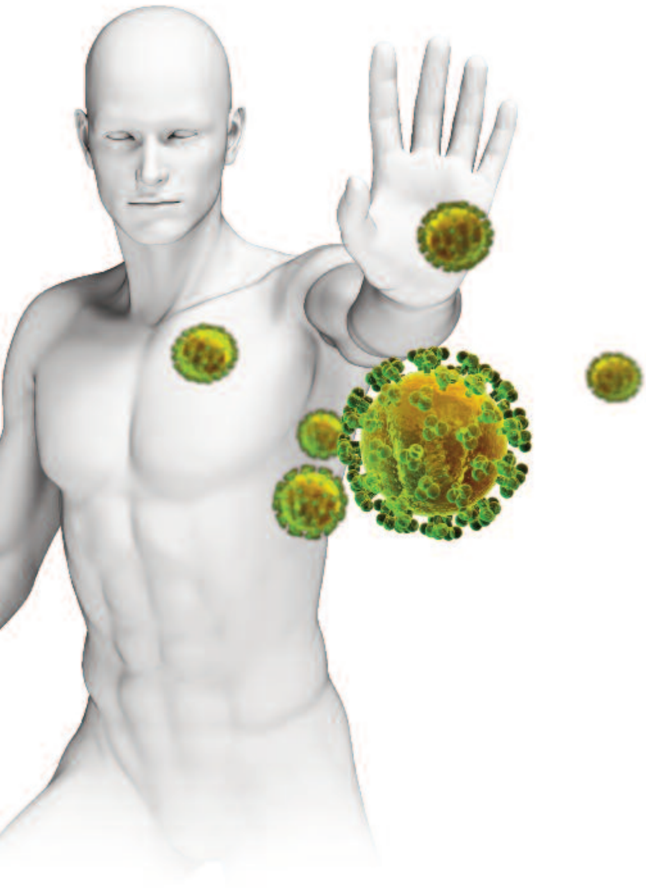

Lactic Acid
Efficient disinfection inspired by nature

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Towards green biocides

Hygiene and disinfection are an indispensable part of our daily life. We use anti-bacterial hand-soaps, surface cleaners and laundry detergents on a day-to-day basis. Biocides protect many products from germ formation and therefore contribute towards making our world safer, cleaner and more liveable. However, established biocides such as polychlorinated phenoxy phenols or isothiazolinones also pose risks such as harmful effects on human health or environmental toxicity. In addition, many conventional actives are synthetic chemicals, while there is also a growing demand for bio-based solutions. Green alternatives, e.g. lactic acid produced by a natural fermentation process, may overcome these drawbacks by combining natural origin, effectivity, safe handling and environmental friendliness.

The goal of this report is to explore the anti-microbial potential of lactic acid and to prove its suitability as a genuine green alternative in the aforementioned sense. Pure lactic acid and its mixtures with surfactants were therefore tested according to standard protocols. The biocidal performance of lactic acid was evaluated by screening its effect on four different germs.

Jungbunzlauer's L(+)-lactic acid is obtained from fermentation based on natural and renewable resources. It is approved by ECOCERT as raw material from natural origin for the use in detergents and personal care. Before presenting and discussing the scientific results, a short overview of the relevant regulations will be outlined.

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Regulatory background

Regulation (EU) No 528/2012, the Biocidal Products Regulation (BPR), in force since September 2013, provides a detailed framework on the use of biocidal products and on putting them on the market [1]. It makes sure that only products approved by this regulation as biocides may enter the EU market. The regulation concerns 22 product types (PT) which can be classified in four main groups: disinfectants (PT 1-5), preservatives (PT 6-13), pest control (PT 14-20) and others (PT 21-22).

Since 1 September 2015, only substances from listed suppliers are allowed to be used and claimed as a biocidal active substance in the final formulation (Article 95 list). This requirement certainly contributes to create a protected market made up of only a few players because the hurdles for registration are quite high (financial and human resources).

At the same time, some conventional anti-microbial actives may disappear from the market because they do not fulfil the regulation's criteria. For example, the well-known active Triclosan has not been approved by the Biocidal Products Committee (BPC) for use in PT1 products (human hygiene) [2]. This in turn is further triggering the demand for safe and sustainable alternatives and opening doors for organic acids such as L(+)-lactic acid. In fact the BPC has already adopted a positive opinion on the approval of lactic acid in PT1 [3].

For the use of lactic acid in PTs 2 (non-food-contact surface disinfection), 3 (veterinary hygiene), 4 (food-contact surface disinfection) and 6 (in-can preservation) first decisions are expected in the course of 2017.

Table 1: Generic concentration limits and labelling requirements for lactic acid in mixtures according to Regulation (EC) No 1272/2008 (CLP)

pH	Categorisation	Labelling
< 2	c ≥ 1% Skin, eye corrosive	
> 2	c ≥ 3% Eye corrosive	
	1% ≤ c < 3% Eye irritant	
	c < 1%	none

Another relevant regulation is Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP). Originally only applied to pure substances, since 1 June 2015 it also applies to mixtures[4]. As a consequence, final formulations and consumer products have to be labelled according to the specified criteria (e.g. pH of the mixture and concentration of the active substance). This Regulation also applies to the use of biocides. Table 1 shows the generic concentration limits for lactic acid: hazardous labelling is required from 1% unless evidence to the contrary is provided by suitable tests.

In the following chapter we will demonstrate that low (label-free or label-friendly) use levels do not necessarily represent an obstacle to the use of green biocides. Furthermore, common anti-microbial actives are often associated with additional detrimental effects to human health (e.g. sensitising the skin) or the environment (e.g. aqua toxicity, bio-accumulation) even at small concentrations. Here, lactic acid provides a valuable, safe and eco-friendly alternative.

Anti-bacterial performance of Lactic Acid

The increasing demand for disinfectants together with concerns about their origin is obvious in the personal and home care market. We therefore analysed the performance of bio-based lactic acid, pure and combined with different surfactants, based on established EN challenge tests. First we carried out screenings with tests based on the EN 1040 protocol to assess general efficacy. Here, a suspension of bacteria is added to the disinfectant solution and the mixture is allowed to react for five minutes at 20°C.

Subsequently, the bactericidal potential is immediately neutralised by the dilution-neutralisation method and the extent of bacteria reduction is measured. In order to pass this basic disinfection test, at least a 99.999% or log 5 reduction of the gram positive and gram negative bacteria has to be achieved. Biocidal performance was investigated on the four common germs *Pseudomonas aeruginosa* (ATC 15442/DSM 939), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536) and *Enterococcus hirae* (ATCC 10541).

Table 2 shows the effect of the pure substances in the modified EN 1040 test. As expected the surfactant has no impact on the bacteria. Lactic acid at moderate concentrations provides good effectiveness against gram negative bacteria. With gram positive bacteria efficacy is lower, most probably due to their specific cell membrane structure. Finally, higher concentrations succeed in achieving a log 5 reduction of *S. aureus* cultures. However, such highly concentrated solutions might not be viewed favourably by consumers as they would require “corrosive” labelling and might potentially pose risks to users and treated surfaces.

Table 2: Performance of pure substances in aqueous solution in a modified EN 1040 test (five minutes contact time); n.d. = not determined; colour represents pass (green; reduction > log 5) or fail (red) of the test

Log reduction (EN 1040)	*P. aeruginosa*	*E. coli*	*S. aureus*	*E. hirae*
1.5% SLS	< 5	< 5	n. d.	n. d.
3.0% SLES	< 5	< 5	n. d.	n. d.
1.0% Lactic Acid	> 5	< 5	n. d.	n. d.
5.0% Lactic Acid	> 5	> 5	< 5	< 5
10% Lactic Acid	> 5	> 5	> 5	< 5

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In a next step we tested the biocidal activity of lactic acid combined with different surfactants. Most anti-microbial products contain surfactants, often combining an anionic with a non-ionic surfactant. Typical anionic surfactants include sodium lauryl sulphate (SLS) or sodium lauryl ether sulphate (SLES); common non-ionic surfactants are, e.g., ethoxylated fatty alcohols (Pareth) or alkyl polyglycosides (APG). Another important class of surfactants are the zwitter-ionic betaines (for instance cocamidopropyl betaine, CAPB), which are often used in liquid soaps. The results of our screening, again following the above presented modified EN 1040 protocol, are displayed in table 3. Lactic acid shows a similar performance with both SLS and SLES: all tested bacteria are efficiently reduced. Both anionic surfactants strongly support the activity of lactic acid, permitting the desired disinfection claim. The picture for non-ionic surfactants is more differentiated. While the lactic acid/APG (C8-16) combination is effective against all tested bacteria, the mixture with Pareth (C12-14 Pareth-7) works best against the gram-negative bacteria species. A similar observation is made for the betaine/lactic acid mixture, where gram-positive bacteria are less efficiently attacked.

Evidently some surfactants, in particular the anionic surfactants, are more efficient in boosting lactic acid performance than others. It seems that their interaction with the phospholipids forming the bilayer of the bacterial cell walls is somewhat more pronounced. This interaction might reduce surface tension more effectively, destabilising the cellular membrane and facilitating the entry of lactic acid into the cell. Corresponding indications are provided by the literature cited [5-8]. Regardless of the possible mechanism, it is highly remarkable that synergistic effects are not limited to a specific surfactant or a particular class of surfactants. Antibacterial efficiency of lactic acid can be demonstrated in both anionic and non-ionic surfactant-based cleaner formulations.

Table 3: Performance of lactic acid/surfactant combinations in a modified EN 1040 test (five minutes contact time); colour represents pass (green; reduction > log 5) or fail (red; actual reduction in log-units) of the test

Log reduction (EN 1040)	***P. aeruginosa***	***E. coli***	***S. aureus***	***E. hirae***
2.5% Lactic Acid + 3.0% SLES	> 5	> 5	> 5	> 5
2.5% Lactic Acid + 1.5% SLS	> 5	> 5	> 5	> 5
2.9% Lactic Acid + 3.0% APG	> 5	> 5	> 5	> 5
2.9% Lactic Acid + 3.0% Pareth	> 5	> 5	> 5	4.3
2.9% Lactic Acid + 3.0% CAPB	> 5	> 5	4.0	4.5

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In order to get closer to a realistic cleaning situation, we performed additional tests with a modified EN 1040 protocol (shorter contact time) and with the more challenging EN 1276 and EN 13697 protocols (Table 4). Here, we focused on combinations of lactic acid with SLES.

The contact times of disinfectant cleaners in classical household applications are typically shorter than five minutes. But even when we reduced the contact time to 30 seconds the biocidal performance of our test solutions remained strong. The same applied when we decreased the dosage of lactic acid significantly, to 0.6%: the modified EN 1040 test was passed without restriction.

Continuing with the more demanding test protocols, we performed a test using a modified EN 1276 protocol. Here, anti-microbial efficacy is assessed under clean, 0.3 g/L bovine serum albumin (BSA), or dirty, 3.0 g/L BSA, conditions. Products passing this test can be applied as sanitisers for food applications. Apart from the presence of bovine albumin, this suspension test was carried out analogously to EN 1040 as described above. Again, the lactic acid/SLES combinations (including one label free combination with less than 1% lactic acid) successfully passed the test protocol, underlining the promising potential of lactic acid for the disinfection industry.

Table 4: Performance of lactic acid/surfactant combinations in modified, increasingly demanding EN tests; colour represents pass (green; reduction > log 5 for EN 1040 and EN 1276, > log 4 for EN 13697) or fail (red; actual reduction in log-units) of the test

Log reduction	*P. aeruginosa*	*E. coli*	*S. aureus*	*E. hirae*
EN 1040, 30 s contact time				
2.9% Lactic Acid + 3.0% SLES	> 5	> 5	> 5	> 5
EN 1276, 5 min contact time, 0.3 and 3 g/L BSA				
2.9% Lactic Acid + 3.0% SLES	> 5	> 5	> 5	> 5
0.9% Lactic Acid + 0.9% SLES	> 5	> 5	> 5	> 5
EN 13697, 5 min contact time, 0.3 g/L BSA				
2.9% Lactic Acid + 3.0% SLES + 3.0% Pareth	> 4	> 4	> 4	> 4
EN 13697, 5 min contact time 3 g/L BSA				
2.9% Lactic Acid + 3.0% SLES + 3.0% Pareth	> 4	> 4	> 4	3.1
5.9% Lactic Acid + 6.0% SLES + 6.0% Pareth	> 4	> 4	> 4	> 4

Finally, we conducted the EN 13697 test, which simulates real-life application conditions to the utmost extent. Bacteria and BSA as the soiling additive are dried on a non-porous surface; the test solution is applied and removed/neutralised without mechanical force. The required minimum log reduction to pass this test is log 4. Here, we chose a lactic acid/SLES/Pareth mixture to have a typical anionic/non-ionic surfactant system as present in many cleaner formulations.

The outcome of the test was extremely promising. At a low concentration of BSA all bacteria were reduced by more than 4 log units. Switching to the higher soiling concentration the test was passed for all but one bacteria species. *Enterococcus hirae* turns out to be the most resistant germ, limiting the biocidal efficacy of the test solution to a log reduction of 3.1. However, when the concentration of lactic acid is raised to 5.9% even this bacterium is sufficiently attacked and full bacterial disinfection achieved.

It follows that with the label-friendly concentration of 2.9% lactic acid (irritant labelling), anti-bacterial claims to 99.9% reduction of bacteria are well supported. Full bacterial disinfection with 99.99% reduction is possible for specific species, or generally if higher concentrations are used. Future studies will address this point further and explore whether combinations of lactic acid with other boosters/surfactant systems could be more efficient at lower dosage levels. Nevertheless, the finding that lactic acid – a green and safe ingredient – shows such strong anti-microbial performance at relatively low concentrations is already remarkable.

Conclusion

We investigated the biocidal performance of lactic acid and its mixtures with different surfactants according to established EN standard protocols. Four common germs were selected for evaluation of the anti-bacterial properties of the subset solutions. Initially we found that, used alone, lactic acid is only effective at concentrations higher than 5%. However – and this is the second, major result of our investigations – a range of anionic and non-ionic surfactants drastically boosts the biocidal performance of lactic acid. This favourable synergy allows for the development of label-friendly yet efficient consumer products at low lactic acid concentrations. Passing to higher dosage comprehensive disinfection claims are supported, providing attractive biocide solutions for industrial or institutional users.

In summary, we have demonstrated that lactic acid is an efficient biocide when combined with a suitable surfactant. Fermentation-derived lactic acid is a bio-based product that meets market and consumer demands for green, safe and sustainable solutions. Even at relatively low concentrations it is effective yet harmless and readily biodegradable.

Furthermore, Jungbunzlauer's L(+)-lactic acid is Art. 95 listed and ECOCERT-approved as a raw material for use in detergents and personal care products. A claim of disinfectant power combined with safety and naturalness no longer has to be a contradiction.

Literature

[1] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products

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About Jungbunzlauer

Jungbunzlauer is one of the world's leading producers of biodegradable ingredients of natural origin. We enable our customers to manufacture healthier, safer, tastier and more sustainable products. Due to continuous investments, state-of-the-art manufacturing processes and comprehensive quality management, we are able to assure outstanding product quality. Our mission "From nature to ingredients®" commits us to the protection of people and their environment.

Jungbunzlauer lactic acid for biocide applications is produced by fermentation of natural, renewable resources and is therefore a good alternative to synthetic chemicals used as antibacterial actives. It is efficient in removing bacteria when combined with surfactant and it is compatible with most other components in common formulations. Jungbunzlauer lactic acid is available as aqueous solution at different concentrations.

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