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Eco-systemic Fermentation – Creating Bio Progression

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abstract

WORESANA® and AURAFIRM® ingredients are produced utilising Woresan GmbH organic, eco-systemic fermentation technology and through several mechanisms of action help a number of skin conditions. These ingredients are cereal derived natural peptides using sustainable, organic plant-substrates and a highly controlled, proprietary eco-systemic fermentation process, which produces a variety of differential small peptide fragments with very broad biological activity.
WORESANA® and AURAFIRM® ingredients are clinically proven to maintain skin integrity and positively influence the skin microbiome.

Introduction

In times of steadily growing demand for natural cosmetic ingredients, the strong growth of this market continues. This demand is no longer limited to the raw materials but increasingly covers the method of production. Today's customer is no longer satisfied with the fact that the ingredients of their care products are derived from plants. Aspects such as sustainability, environmentally friendly cultivation of the substrate and their further processing are increasingly coming into the consumer consciousness. In addition, customers expect the effectiveness of their care products not to suffer, when using these eco-friendly ingredients and indeed want improved efficacy.
This trend has resulted in considerable efforts to implement biotechnology solutions from the food industry, which has faced similar customer demands at an earlier stage.
One of the methods to produce these new ingredients is through targeted fermentation.

Fermentation

The technique of targeted denaturation of various raw materials has been valued in the food industry for centuries. Properties such as increased shelf life, nutrient content, bioavailability of nutrients, breakdown of anti-nutrients as well as various sensory changes have made fermentation the method of choice in many different biotechnology applications. Fermentation is also becoming more and more of a focus for manufacturers of cosmetic ingredients.
There is a number of different fermentation methods.
Submerged fermentation techniques, where enzymes and other reactive compounds are produced and submerged in a nutrient broth are the first choice, as this method offers many additional benefits. The species of microorganisms being used is of considerable importance as individual bacterial species deliver very different chemical profiles and also have very specific operating envelopes within their growth conditions. A key choice is whether to use a single microorganism or a mixed multi-culture.

Single Strain Fermentation

The single strain fermentation method is used predominately, as it achieves excellent results in terms of bioavailability together with the creation of new constituents. In most cases, manufacturers use individual lactobacilli cultures or yeast cultures. This is a safe method of production as it provides reliable, predicable and reproducible results. This method does however reduce the possible opportunities that multi-cultures would offer. Therefore, the range of resultant molecules is likely to be reduced. and the overall utilization of the substrate tends to be poor.

Multi-culture Fermentation

Multi-culture fermentations offer several advantages over single-culture as they are likely to include more complex multistep molecular conversions and individual microorganisms will metabolize the pre-digested compounds of another microorganism. The Process requires the manufacturer to identify the optimum starter by trial, and then develop process conditions to ensure that the resultant mixture can successfully competitive against contaminants. It has been seen that compounds made by this mixture of microorganisms often complement each other and work to the exclusion of unwanted microorganisms.
Multi-cultures improve utilization of the substrate which for fermented ingredients remains a complex mixture of carbo-

hydrates, proteins, and fats. Mixed cultures possess a wider range of enzymes and are able to attack a greater variety of natural compounds. Likewise, with proper strain selection they are better able to change or destroy toxic or noxious compounds that may be in the fermentation substrate. The addition of a symbiotic species that supplies the growth factors is a definite advantage in terms of stability.
But this method can be counterproductive in achieving reliable, constant product quality [1–5].

Eco-systemic Fermentation

The core idea is to combine the advantages of a reliable, reproducible and efficient single-strain fermentation with the strengths of better substrate utilization and resulting in a broader component spectrum of a multi-strain fermentation (**Fig. 1**).
The first prerequisite for this type of technique is a substrate-specific multi-culture which is likely to be highly complex and remain batch identical. The development of this type of culture is based on the selection of a number of specific effective cultures, which originate from an existing large culture database held by a fermentation company. Within this database original sourdough cultures have been collected over a very long period (decades) and then exactly preserved through meticulous regeneration, ensuring the profile remains unchanged. The database was initially created in the late 70's as it was recognized at an early stage that these aged multi-cultures were of significant effect and therefore valuable, although they were originally derived from spontaneous changes in the fermentation process. These selected multi cultures have been further diversified, deliberately exposing it to exogenous and endogenous challenges until the required state of stability and complexity had been achieved.
The other main advantage of this fermentation technique is the ability to extensively control environmental operating envelope during the actual production process. The complex operating envelope can only be achieved by specialized pro-

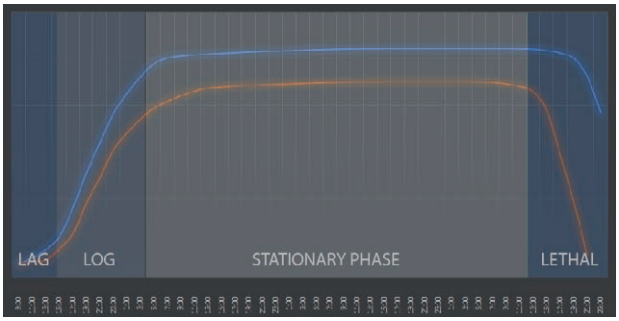

Fig. 1 Comparison of microbial development phases of a microbial multi-strain culture under the influence of 5% rye-based lactobacillus (blue) ferment to untreated microbial multi-strain culture (orange).

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FERMENTED INGREDIENTS

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eco-systemic fermentation

- **WORESANA®** fermented active ingredients for cosmetic and over-the-counter (OTC) products – consisting of amaranth, buckwheat and rye.
- We use natural processes: With our **patented high-tech eco-systemic fermentation**, we focus on promoting the bioavailability of new active ingredients. Scientific monitoring to ensure quality, plus versatile pre-, pro- and post-biotic applications.
- To achieve a harmonious, balanced skin microbiome.

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cess technology. The process is automatically changing certain parameters depending on the multi-culture and substrate used. By doing this the interactive behaviour of the diverse species is kept at a high level, forcing the culture to adjust the production of bio-actives to keep the balance correct within their ecosystem. For each substrate, clear analytical specifications and specific programmable production protocols exist for optimal utilization and effective production of bio-active compounds.

The last significant parameter is the control of the time frames as each individual microbial cycle takes place. Starting with the attachment phase, through the exponential growth phase, through transition to the stationary phase and finally the lethal phase, which is brought about by pasteurization, each phase has a carefully controlled timeframe.

During each phase microorganisms do metabolize certain compounds from the substrate to different metabolites. During the attachment- and exponential growing phase they mainly metabolize easy accessible nutrients to perform reproduction and environmental conditioning e.g. biofilm formation, pH-adjustments and temperature control. Compounds metabolized during this time are so called primary metabolites. The production of secondary metabolites is reasonable in depleting of easy accessible nutrient sources. Because of that microorganisms have to perform enzymatic multistep conversions of polymers to ensure adequate supply of food. To perform those effectively it becomes necessary to interact. Those interactive behaviours can be of commensal, competitive, predative, neutral, amensalism or cooperative nature. They are not exclusively direct but can also be indirect e.g. stimulation/modulation of the immune defence. These interactions are performed by numerous molecules.

Conducting the eco-systemic fermentation in the right manner results in a compound mixture of pre-, pro- and postbiotic activity, accompanied by many additionally tissue nourishing ingredients.

In the following section, the eco-systemic fermentation approach and its benefits are described in more detail. Please, note that the below-stated compounds do represent only a fraction of bio active components involved in these processes.

Prebiotics

Extracellular Polymeric Substances (EPS)

EPS are natural polymers of high molecular weight secreted by microorganisms into their environment. EPS establish the functional and structural integrity of biofilms and are considered the fundamental components that determine the physiochemical properties of a biofilm. The EPS layer acts as a nutrient trap, facilitating bacterial growth. Extracellular polymeric substances (EPS) serve as a biopolymer to protect cells from external environment and serve as energy and carbon sources for food deprived cells.

AHAs

Strains of lactobacilli can produce α-hydroxy acids (AHA's) to exhibit pH-adjustments and antibacterial activity against most dermal pathogenic bacteria. AHAs are widely used as exfoliators and are effective on desquamation of the skin. In addition, AHAs can improve the stratum corneum barrier function and enhance the production of ceramides by keratinocytes.

Ectoin

Ectoin is a natural substance which is produced by bacteria to protect against extreme conditions. It has cell-protecting, anti-inflammatory, nourishing and membrane-stabilizing properties. It is an effective long-term moisturizer that prevents dehydration of the epidermis. It can also be used at relatively low concentrations as a whitening agent because of its inhibitory effect on melanin synthesis. Ectoin also alleviates skin inflammation and is currently recommended for the treatment of moderate atopic dermatitis. In addition, ectoin strongly absorbs ultraviolet (UV) radiation and protects DNA from breaking down in some cell types.

Phenolic Compounds

Polyphenols are secondary metabolites with antioxidant, anti-inflammatory and antimicrobial activity. Phenolic acids are key class of polyphenols. They exhibit a variety of functions including growth, development, and defense mechanisms. They are precursors of other significant bioactive molecules regularly used for therapeutic purposes and cosmetics.

Bacteriocins

Bacteriocins are proteinaceous or peptidic toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). Research suggests that bacteriocins contribute to the modulation of the skin microflora, skin lipids and the immune system, leading to the preservation of natural skin homeostasis. Bacteriocins may represent an opportunity to cure infections caused by multi-resistant bacteria. They are currently used against Propionibacterium acnes and as immune modulators.

Short-Chain Fatty Acid

Lactobacilli and bifidobacteria are not only protective in many ways, they are also immunologically active. There is evidence that commensal microbes affect the immune system via expansion of regulatory T cells (Tregs) on the skin. This is mediated via short-chain fatty acids, bacterial metabolites generated during fiber fermentation. They suppress excessive inflammatory responses by stimulating the formation of regulatory T cells. Regulatory T cells form the messenger Interleukin 10, which has a balancing effect on the various immunological processes.

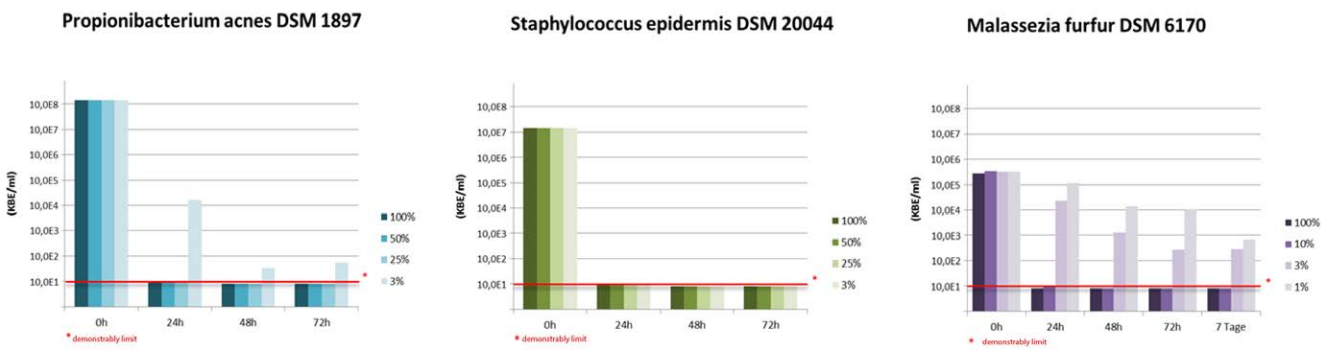

Fig. 2 Antimicrobial Activity

Lipoteichoic Acids and Peptidoglycan

Lipoteichoic acid (LTA) and Peptidoglycan (PG) are structural components of the cell walls of Gram-positive bacteria and play a vital role in the growth and physiology of the bacteria. Upon topical application, LTA and PG have been found to stimulate skin defense against microbial threats via induction of toll-like-receptors (TLR). Activation of TLR triggers the release of soluble actives such as the antimicrobial peptides which assist in the maintenance of the sterility in the dermis.

Probiotics

Microorganisms

Lactobacilli and bifidobacteria can inhibit pathogen attachment to epidermal cells of the skin, even in non-viable state by blocking attachment surface and attracting bacteria of the same or similar species (**Fig. 2**). Furthermore, data indicates that heat-killed bacteria, their fractions or purified components that have key probiotic effects, with advantages versus live probiotics (mainly their safety profile).

Sphingomyelinase

Sphingomyelinase (SMase) is an enzyme that generates a family of ceramides and phosphorylcholine from glucosylceramide and sphingomyelin precursors for the development of extracellular lipid bilayers in the stratum corneum. SMase activity has been demonstrated to be important for skin barrier function.

Diacetyl

Strains of lactobacilli and bifidobacteria can produce diacetyl suggesting its potential to exhibit dermal antimicrobial activities, with greater sensitivity against Gram-negative bacteria and fungi as compared to Gram-positive bacteria [6, 7].

Postbiotics

Amino Acids

Amino acids are the building blocks that form polypeptides and ultimately proteins. Consequently, they are fundamental components of our bodies and vital for physiological functions such as protein synthesis, tissue repair and nutrient absorption. Microorganisms must synthesize amino acids in order to grow, develop and perform all routine metabolic functions.

Microbes have a remarkable capacity to build amino acid frameworks that are not incorporated into proteins. It has been estimated that about 500 naturally occurring amino acids have been identified to date, leaving the 20 proteinogenic amino acids as the 4% minority. While some of the nonproteinogenic amino acids are utilized as intermediates in primary metabolic pathways, most of the unheralded 96% majority serve as building blocks for small bioactive peptide scaffolds. They may represent an underutilized inventory of building blocks for protein engineers, medicinal chemists, and materials scientists (**Fig. 3**) [8].

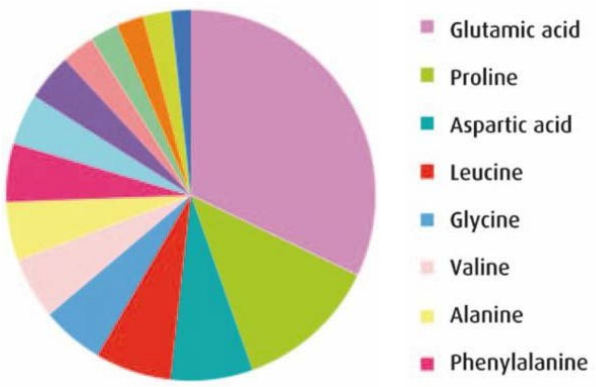

Fig. 3 Lactobacillus Rye Flour Ferment/Filtrate Amino Acid Composition (%)

Bioactive Peptides

There is an expanding focus on bioactive peptides because of their health benefits, and they have been considered as the new generation of biologically active regulators. Peptides are involved in the modulation of cell proliferation, cell migration, inflammation, angiogenesis, melanogenesis, and protein synthesis and regulation. Its high bioavailability and heat stable nature allow its use as cosmetic ingredient.

Summary

The eco-systemic fermentation takes into account the fact that until today science does not have a rough comprehensive understanding of the complex interaction between skin, microorganisms, immune system and further participants. Therefore, we do not attempt to mimic their solutions but use natures vast head start in terms of experience and development. Through integration of natural processes into technical systems, eco-systemic fermentation is capable to produce highly effective, safe, and innovative skincare ingredients. Additionally, this technique does fulfil customer demand regarding ecology, ethics, energy saving, material saving and renewable sourcing. Thereby offering comprehensive overall sustainability. The ingredients do affect the interplay between microbial, mechanical, thermal, physical and immunological functions of the skin by using compounds and formulations developed and produced by nature.

In addition, manufacturers of cosmetic products receive ingredients that can be beneficially used in numerous applications with most different purposes and forms. They are easy to formulate, have a short INCI despite many different components, perfect for relaunches, lowering the preservative content, do have a long shelf life.
Eco-systemic solution equals natures solution.

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