Progress

Priorities for Addressing **O**pportunities and **G**aps of Industrial Biotechnology for an efficient use of funding **res**ource**s**

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Deliverable D 5.1: R&D&I needs in six IB value chains

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About PROGRESS

PROGRESS is a coordination and support action for the European Commission and aims to support and accelerate the deployment of Industrial Biotechnology (IB) in the EU industry by identifying high-value opportunities for IB and proposing actions to address them successfully. For that purpose, we will first provide a comprehensive and dependable information base (including modelling and simulation approaches) which allows for plausible estimations on the future of IB in the EU in the short and medium-term. Second, in collaboration with stakeholders we will elaborate a future scenario and a common vision for IB in Europe containing the most promising value chains, related R&D&I needs and necessitated policies for IB in Europe. Based on these steps, we will provide strategic advice for research, industry and policy making regarding potential issues and topics for collaboration, future policy programmes, the required technological infrastructure, capabilities, and economic structures. A main focus will be to identify opportunities for collaboration between EU member states and proposed actions to increase awareness and incentives for those collaborations. For more information see www.progress-bio.eu

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Content

1 Introduction

In the PROGRESS CSA six value chains were selected for further analysis (see Deliverable 2.1).

The selected value chains are:

- Lignocellulosic ethanol
- Bio-based plastics
- Enzymes (with specific reference to laundry and dishwasher applications)
- Production of biopharmaceuticals
- Biotechnologically produced flavours and fragrances
- Microbiomes for food and healthy nutrition

Besides a value chain analysis and the elaboration of scenarios for each of those six fields (see Deliverables 2.2 and 4.1), an analysis of the R&D&I needs for the next years has been conducted by using current literature, expert interviews and results from previous Deliverables. This Deliverable 5.1 summarizes the results of those R&D&I needs for each value chain.

2 R&D&I needs in the value chain "Lignocellulosic ethanol"

Technologies for the production of 2nd generation/lignocellulosic bioethanol are approaching maturity and have been developed for demonstration at commercial scale. However, production volumes are still low, as besides policy issues, the production at commercial scale is not economically feasible or profitable at the currently low oil prices. There is also competition from US and Brazilian bioethanol. As a consequence, R&D&I must primarily be focussed on improving cost-competitiveness of production concepts, giving economic considerations and assessments a key role. A techno-economic roadmap should be elaborated which covers the whole supply chain from feedstock price, transport, storage, conversion to ethanol and by-products, downstream-processing and formulation, to product commercialisation. In this roadmap, the costs and the cost-reduction potential of different options should be assessed and R&D&I performed on those approaches which will be required to achieve cost-competitiveness at realistic market prices. It should also take the interdependence of various steps in the process into account, which means that solutions chosen in early process steps may create or avoid problems in later steps.

From the point of view of consulted experts, addressing the following R&D&I issues should be prioritized:

- achieving complete conversion of sugars in the fermentation stage
- achieving higher ethanol production rates and concentrations in the fermentation stage
- cost optimisation of downstream processing (i.e. separation and concentration of ethanol)
- converting by-products (e.g. lignin, xylose) to higher-value products

Table 1 lists options and approaches for these issues. These options and approaches, however, must be assessed and prioritized with respect to their expected contribution to improving cost-competitiveness, as outlined above.

Table 1: R&D&I needs for lignocellulosic ethanol

Topic	*State-of-Art*	*R&D&I needs*
2nd generation/advanced cellulosic bioethanol: pre-treatment	A number of pre-treatment strategies have been developed to enhance the reactivity of cellulose and to increase the yield of fermentable sugars.	• In addition to improving the cost-effectiveness of the pretreatment steps themselves, the quality of the pretreatment also influences the yield and bioavailability of fermentable sugars and the presence of inhibitory substances which impact the following fermentation and downstream processing steps. – Identification of cost-efficient combinations of different pre-treatment methods (e.g. alkaline pre-treatment followed by steam pretreatment or organosolvent pre-treatment coupled with steam explosion), to improve the biomass digestibility. – achieve higher degrees of hydrolysis of lignocellulosic biopolymers components into sugars, especially higher yields of hemicellulose separation, of cellulose from lignin, and of glucose from cellulose – achieve low concentrations substances which act as inhibitors in the fermentation step • Addressing the following issues may improve the knowledge base for optimisation of pretreatment – Development of tools to investigate the cell wall deconstruction and understand the recalcitrance during the pre-treatment process, expansion of knowledge on cell wall structure and ultrastructure, and the physicochemical changes occurring within the cell wall at the molecular level and the cellular/tissue scale during various pre-treatment technologies – Breeding (with the help of marker-assisted breeding, genetic engineering and genome editing) of genetically modified lignocellulosic plants with altered lignocellulosic structures, rendering lignocellulose less recalcitrant to pre-treatment

Topic	*State-of-Art*	*R&D&I needs*
2nd generation/advanced cellulosic bioethanol: hydrolysis	Enzyme mixtures are applied for the conversion of pretreated lignocellulose to produce fermentable sugars. Yields of fermentable sugars are not yet high enough, and the enzymes are still too expensive.	• Yields of fermentable sugars need to be improved, the formation of inhibitory substances reduced, and costs for enzyme production and use reduced. Biotechnology and process engineering approaches are needed to develop new highly active enzyme mixtures which can be produced at lower cost: – Identification and optimisation of enzymes that can break down different types of polysaccharides to fermentable sugars, have superior activity and can be produced at lower costs. Lytic Polysaccharide Monooxygenases (LPMOs) are examples of recent progress in enzymes which act differently from known hydrolases (i.e. by oxidising on side of the glucosidic bond instead of hydrolysing it). – Optimisation of cost and performance by process engineering.
2nd generation/advanced cellulosic bioethanol: microbial fermentation	*S. cerevisae, E. coli, Zymomonas mobilis and some Clostridia spp* are currently most commonly used for bioethanol production. They have specific strengths and weaknesses with respect to the ability to metabolize pentoses and their tolerance towards high ethanol concentrations and inhibitory substances.	• R&D&I is needed to bring pentose (primarily xylose) fermentation up to the same speed as glucose fermentation for cases where xylose-rich feedstocks, such as agricultural residues or hardwood are to be used, and no alternative use for the pentoses can be found. • As ethanol is toxic, it is essential to improve the tolerance of the production organisms to ethanol, e.g. by systems metabolic engineering and release from end-product inhibition. This is particularly important in case other production organisms than S. cerevisiae with a lower ethanol tolerance are used. • Another option is the improvement of in situ bioethanol separation in order to keep the ethanol concentration in the fermentation broth below inhibitory levels. R&D&I needs are: – reduce the cost of pervaporation, reduce the costs of gas stripping equipment, improve energy efficiency, control foam formation • Inhibiting compounds are most likely present in the hydrolysate medium, e.g. carboxylic acids and various sugar degradation products. This can be addressed by the following options: – avoiding the formation of inhibitory substances by engineering the pretreatment steps – removal of inhibitory substances by engineering cost-efficient separation steps prior to fermentation – Improving the tolerance of the production organism to these compounds.

Topic	*State-of-Art*	*R&D&I needs*
	Saccharomyces cerevisiae is often chosen for ethanol production due to its high ethanol productivity, high ethanol tolerance and ability of fermenting a wide range of hexose sugars.	• In order to address the above mentioned issues, data-driven and synthetic biology as well as systems metabolic engineering approaches could be followed for different host organisms by introducing pathways for broadening the substrate spectrum (e.g. metabolise xylose), increasing tolerance towards higher temperatures, ethanol and other inhibitors, and for maximizing metabolic flux so that sufficient production rates and complete conversion of substrates can be achieved.
Lignin as co-product	Lignin as a major by-product is currently mainly used as fuel, providing process heat and/or electricity. Cost-competitiveness of the overall process could be improved if higher-value applications for lignin and other by-products could be developed to commercial maturity.	• Improvements in the lignin extraction procedure: lower costs, (higher) lignin quality, depending of its targeted use • R&D&I into various lignin uses, both higher-volume lower-value applications, as well as high-value, low-volume applications; e.g. aromatic building blocks for polymers, composites, coatings, adhesives

3 R&D&I needs in the value chain "Bio-based plastics"

Bio-based plastics is a key value chain for IB as bio-based plastics range from low-cost mass products (drop-ins) to lower-volume-higher-value specialty products, targeted at the Business-to-Business as well as the Business-to-Consumer market. Moreover, it has received significant attention by the public, as there is a rather good understanding of products and applications and a strong interest in environmental issues. As a consequence, the future development of this value chain (with respect to innovations, types of commercialized products, demand, integration into a Circular Economy Concept, contribution to the EU Plastics Strategy etc.) may have a signalling function for the development of IB in general and for other value chains with large-scale products or products with favourable environmental footprint.

Up to now, a limited number of bioplastics have been developed to commercial scale (e.g. PLA) and they are not suitable for all desired application areas. Therefore, R&D&I policy should continue to support the scientific-technological development of bio-based plastics from basic research to near-commercial phases. Specific emphasis should be given to

- market- and application-driven approaches,
- improving the presently limited cost-competitiveness compared to fossil-based products,
- non-food biomass feedstock and scale-up issues,
- intensified cooperation between Green Chemistry, IB, (bio-)process engineering, and material science,
- reducing the environmental footprint of products and processes,
- dealing sustainably with bio-based plastics in their after-use phase,
- attracting novel actors from high-volume markets for traditional fossil-based plastics (e.g. automotive, construction etc.) into value-chain oriented R&D&I projects and information campaigns.

Table 2 summarizes R&D&I needs for bio-based plastics which result from their technological and innovation potentials. Although a strong support by R&D&I policy is very important, it alone will not be sufficient to achieve significant changes in the bio-based plastics market. It should be complemented by demand pull support for bio-based plastics with improved sustainability (e.g. sustainability assessment, labels, public procurement, B2B success stories).

Table 2: R&D&I needs for bio-based plastics

Topic	*State-of-Art*	*R&D&I needs*
Novel bioplastics	Only few novel bioplastics have been developed to commercial scale. They are not suitable for all desired applications. Presently, bioplastics (partly) made building blocks which are not fermentatively produced are economically more important than most fermentatively produced bio-based plastics.	• R&D&I of innovative, novel bio-based plastics with novel properties and respective production processes is needed in order to satisfy the need for novel bio-based plastics with desired properties for novel applications and uses. However, the search for novel bio-based plastics should be market- and application-driven. These bioplastics comprise both biotechnologically manufactured building blocks followed by polymerization, as well as other bio-based plastics, e.g. based on lignin etc. – Market- and application-driven search for promising bio-based plastics, tailor-made bio-based plastics, including the design of novel bioplastics so as to ensure their recyclability. – Identification and characterisation of promising sources (besides corn, wheat, soy) of biomass feedstock to produce bio-based plastics (e.g. waste streams, lignocellulose, plant-based proteins; see also below). – Exploration of a broad spectrum of novel bio-based plastics in order to identify candidates with promising properties and functionalities for the identified market opportunities. This requires intensified cooperation between microbiologists, chemists, (bio-)process engineers, and material scientists. – For novel candidates of bio-based plastics with promising properties and functionalities, green chemistry and/or fermentative production processes have to be developed and optimised, especially with respect to (bio-)catalysts, yield, bio-plastic quality, cost-competitiveness, and sustainability of production (related detailed R&D&I needs see below). This requires intensified cooperation between chemists and/or microbiologists, (bio-) process engineers and material scientists. – Engineering the properties and performance of bio-based plastics, e.g. by blending, functionalisation, nano-particles, additives. – Scale-up of production processes for novel bio-based plastics in order to reach a critical mass for a given bio-based plastic (e.g. in order to achieve economies of scale, address different market segments and applications, etc.)

Topic	*State-of-Art*	*R&D&I needs*
		• Development of new value chains, establishing novel arrangements and collaboration of relevant actors along the value chain in order to bring the novel bio-based plastics to the market and address novel applications.
Novel bio-based plastics: example plant proteins as feedstock	Plant proteins could be used as a source of raw material for bio-based plastic products, possibly biodegradable. Potato and rice have been tested as potential promising sources for bio-based plastic production leading to gluten free food packaging bioplastics.	• New promising sources (besides corn, wheat, soy) of plant based proteins to produce bio-based plastics need to be identified and characterised. • Protein-based bioplastics require R&D&I to improve mechanical and water absorption properties in order to make these materials applicable in various applications, e.g. packaging.
Novel bio-based plastics: example optimisation of PLA production	PLA production is done on commercial scale. However, further optimisation of the process is required in order to reduce production costs and improve yields and product quality (i.e. optical purity). Moreover, commercial processes for PLA from non-food feedstocks (lignocellulose) need to be developed	Different approaches should be followed for optimisation: • Development of large-scale PLA production processes from lignocellulosic feedstocks, specifically addressing scale-up of steam explosion and improving the yields of process steps of lignocellulose conversion to glucose (see below) • If the fermentation process to produce lactic acid is run at the pH optimum of the strain, precise control of the pH level is required and a certain amount of lactate salt is being produced, being both a cost factor and making downstream processing more difficult. In order to reduce the consumption of pH correcting agent, efficient acidophilic production strains with a pH optimum or tolerance near the pKs value of lactic acid (ca. 3.85) should be developed. • Downstream processing needs to be optimised with the aims to reduce production costs, improve yields and product quality (i.e. optical purity). • R&D&I on adding functionality to bio-based plastics (e.g. engineered PLA grades).

Topic	*State-of-Art*	*R&D&I needs*
Biomass: non-food feedstocks	The majority of bio-based plastics is produced industrially from food crops. Due to the food-first principle, there is a need to additionally exploit non-food feedstocks for bio-based mass products. Bio-based plastics based on non-food feedstocks are still mostly in various R&D&I stages. These processes are still technologically complex (and non-profitable)	There is a need for further R&D&I that would expand the technological biomass potential for IB, especially by utilizing non-food crops, both for production of established (e.g. drop-in) or novel bio-based plastics. • Screening for and assessment of novel, still underused non-food feedstocks: e.g. lignocellulose, whole plants or crop plant residues of food crops (e.g. straw), specifically grown non-food crops (e.g. Miscanthus, switchgrass), industrial waste streams (e.g. from food processing, such as whey or from the textile industry), CO_2, municipal waste fractions. • Characterisation of quality of these feedstocks, followed by three complementary approaches: – achieving a constant level of quality independent of growth conditions in biomass production etc. – development of "feedstock-tolerant" green chemistry processes or fermentation processes and the respective downstream processes which can deal with fluctuating quality of input materials with a fluctuating content of (partly unknown) impurities • Concepts for collection, storage and logistics of the relevant feedstock supply • Development of processes for the fractionation of feedstocks into major components, hydrolysis, if needed cost-effective purification or conditioning processes routes to yield substrates without inhibiting or contaminating substances • Development of processes for valorisation of side streams and fractions of the feedstock which are not converted to bio-based plastics building blocks.

Topic	*State-of-Art*	*R&D&I needs*
Biomass example: lignocellulose	Lignocellulose is being investigated as an abundant non-food feedstock for the manufacturing of bio-based plastics. Cost-competitiveness can only be achieved, if all fractions of the biomass feedstock are valorised, preferably following the cascading principle. A major fraction of lignocellulose is lignin which is presently used mainly energetically. Research is underway to valorise lignin as a source of aromatic chemicals. Potential lignin-derived products could be hydrocarbons, phenols, macromolecules and oxidized products.	For wood as the most dense lignocellulosic material, the following challenges have to be addressed: • Safeguarding the supply of sufficient wood feedstock but at the same time protecting forest ecosystems, avoid contributions to climate change (by deforestation) and maintain soil fertility (by avoiding desertification) by implementation of (certified) sustainable forestry practices and making new plantations. • Addressing the following bottlenecks: – Upscaling of current steam explosion installations to the sizes required for large industrial scales – Improving the yields of hemicellulose separation at steam explosion – Improving the yields of separation of cellulose from lignin – Improving the yields of glucose production from cellulose – overcome hurdles posed by the structural heterogeneity of lignin and the presence of impurities • Market- and application-driven search for promising lignin-derived products • Develop processes for lignin-derived products in order to valorise the lignin fraction, e.g. by integration of biotechnology and green chemistry
Biomass example: Cashew nut shell liquid (CNSL)	CNSL, a relatively underused by-product/waste stream of cashew nut production, has not yet been widely used for bio-based plastic production. Phenolic compounds which could be used in resins or composite materials could be derived from CNSL, thus valorising this by-product and contributing to a circular economy.	• Market- and application-driven search for promising CNSL-derived products, e.g. use in paints and surface coatings • Optimise cost-efficient extraction processes of CNSL and its subsequent processing • Improvement of CNSL-derived products for use in paints and surface coatings: improvement of colour range, minimize oxidation, improve adhesion to surfaces. • Synergistic combination of biotechnological and green chemistry process steps.

Topic	*State-of-Art*	*R&D&I needs*
Bioplastics Value Chain: Cooperation	There is a lack of cooperation and knowledge transfer between different actors along the value chain.	• Initiatives to support – the formation of novel actor configurations along the value chain, with a specific focus on sectors/industries, which wouldn't be in contact for their "own/core" business – the exchange of information and knowledge between different actors along the value chain, – the joint development of strategies and R&D&I priorities, shared by different actors along the value chain – R&D&I projects with industry-defined topics and goals.
Bioplastics Value Chain: Sustainability	Bio-based plastics (already in use or under development) have several drawbacks regarding sustainability. Recycling and reuse in the after-use phase have hardly been addressed yet.	• Taking economic, ecologic and social sustainability seriously into consideration already in the concept and design phase of R&D&I projects/processes • Improving the environmental footprint of bio-based plastics and their production process, e.g. by using low-input biomass, use of renewable energy in production of bio-based plastics, increasing yields, valorisation of by-products and side streams, improving the energy and resource efficiency of process steps, improving the water use efficiency by water recycling or reuse, waste reduction, replacing process chemicals by less hazardous ones • Water recycling/reuse for saving process steps, costs and improving downstream processing: a major challenge is to connect mostly water-free chemical reactions with biotechnological process steps in aqueous media. • Improving occupational safety of the production process • R&D&I into safety concerns of (bio-based) plastic products, e.g. additives, nanoparticles. • Development of a holistic system to recycle all plastics, including bio-based plastics after use, ideally to high-value products: Logistic concepts for the collection of used plastic products, separation of plastic waste from other waste fractions, recycling process or biodegradation process, processing of recyclate to high-value products.

Topic	*State-of-Art*	*R&D&I needs*
Bioplastics Value Chain: Logistics	Logistic issues are crucial on all stages of the value chain, especially in the feedstock processing and in the after-use phase	• Logistic concepts and bio-based plastics manufacturing sites must be designed in a way that technical, environmental and economic requirements are simultaneously addressed: Challenges in collection of feedstock lie in the relatively large (agricultural) area for producing the feedstock, its low energy density and high water content and the resulting limited storability of many feedstocks, having implications for the size, number and location of biomass processing plants (on-farm-site small processing plants vs. large integrated biorefineries) • Logistic concepts in the after-use phase of bio-based plastics still need to be developed, aiming at either biodegradation or recycling, and being compatible with existing concepts for fossil-based plastics.

4 R&D&I needs in the value chain "enzymes"

Enzymes are critical for novel products, processes, services, and applications in a broad range of process industry sectors and consumer goods, as they are key enablers for substituting fossil feedstocks by renewable ones, for reducing manufacturing costs, for optimising the environmental performance of industrial production processes. The spectrum of enzymes ranges from low-value-high-volume products to high-value-low-volume products, delivered to other businesses or directly to consumers, with a significant contribution to the added value of final products.

A European strength in the enzyme business is the focus on technological excellence and innovative products and applications. Although public R&D&I policy will be important to keep the European position in the increasing global competition, R&D&I priorities and business strategies of the large leading enzyme companies will be equally important. SMEs as agile and pioneering actors provide substantial input into innovative product pipelines.

Public R&D&I policy should prioritise the broadening of the spectrum of enzymes for use in IB by addressing the following R&D&I topics:

- identification of novel enzymes with a focus on other enzyme classes/reaction types than hydrolases.
- *de novo* design of novel or improved enzymes from scratch, and its application in the design of industrially relevant enzymes with new and robust catalytic functions.
- optimization of enzyme properties for industrial use.
- the optimisation of enzyme production hosts, and the development of alternative enzyme production concepts to industrial scale maturity.
- the further optimisation of enzyme production processes with respect to technical, economic, ecologic and safety parameters. Specific attention should be paid to further automatisation and integration of unit operations, process analytical technologies, and the digitalisation of production.
- Optimisation of enzyme applications and development of novel ones, specific for the respective value chains.
- Emerging approaches (e.g. enzyme production in cell-free systems, complex biocatalytic systems for cell-free metabolic engineering, co-factor regeneration, bioelectrochemical systems).

Table 3 summarizes R&D&I needs for enzymes which result from the technology and innovation potential.

Table 3: R&D&I needs for enzymes

Topic	*State-of-Art*	*R&D&I needs*
Broadening the spectrum of enzymes in IB	Very few of the enormous abundance of naturally occurring enzymes are used in IB processes. Most of the industrial bioprocesses are based on biotransformations using single enzymes. Mainly hydrolases are used for bulk applications, for speciality enzymes, the spectrum of enzyme classes is broader than the enzymes that are commercialized or in industrial use	• Identification of novel enzymes (see below), de novo design and generation of novel enzymes • Optimization of enzyme properties for industrial use (see below) • Development of novel enzyme production concepts (see below) • Development of novel concepts for enzyme-catalysed processes (see below), e.g. engineering of enzyme cascades/multienzyme reactions, co-factor regeneration, etc.
Identification of novel enzymes	Currently established methods to identify new enzymes are: screening of enzyme producers from natural sources, metagenomics and in silico screening, high throughput screening and de-novo design of tailored enzymes	• Enzyme classes, reaction types: There is a need to expand the number of enzymes for industrial use which catalyse e.g. the formation of C-C bonds, oxidations and reductions, catalyse co-factor dependent reactions and "dream reactions" (e.g. utilisation of CO_2 as feedstock in chemical synthesis) • Further technological improvements of high-throughput screening methods which are either applied for the screening of naturally occurring enzymes or in the process of enzyme engineering: – Developing different screening concepts: 1) cells as reaction compartments, 2) in vitro compartmentalization via synthetic droplets, 3) micro-chambers. – Screening of genomic libraries without cloning step, using cell-free translation, thus overcoming limitations posed by the expression host *E. coli*; further miniaturisation (e.g. microsystems, microfluidics); – Development of novel detection methods, e.g. novel assays for the desired enzyme property, improved assays that mimic “real life” conditions suitable for high-throughput approaches, novel detection systems (i.e. beyond fluorescence) for high throughput screening

Topic	*State-of-Art*	*R&D&I needs*
		– Screening of still "underinvestigated" sources/ecosystems with a higher likelihood of success: e.g. screening of microbiomes, marine sources, or extreme environments – de novo design and generation of enzymes. see below
De novo design and generation of enzymes	There is knowledge on the structure-function and dynamics-function relationships, but not yet sufficient for de novo design of tailored enzymes from scratch	• For *de novo* generation of enzymes the ultimate goal in rational design of industrial enzymes is to *de novo* generate enzymes with new and robust catalytic functions for industrial processes. For that R&D&I is needed to advance knowledge on the structure-function and dynamics-function relationships. • New/improved models to predict structure/functions relationships in order to improve in-silico predictions.
Optimization of enzyme properties	Protein engineering both by random mutation, by evolutionary and rational approaches is well established. The number of targeted alterations that can be introduced with reasonable effort (e.g. number of amino acid exchanges) has risen considerably.	• There is a general need to optimize enzymes for industrial purposes, to enhance their properties, as their application in industrial processes requires properties that do not exist in naturally occurring enzymes. • Properties of interest for engineering enzyme activity are e.g.: broadening tolerance to harsh process conditions (e.g. pH, temperature, chemicals), altering the optimum range of enzyme activity, increasing or decreasing substrate and reaction specificity or selectivity, extension of substrate and reaction range to non-natural substrates and reactions, alteration of kinetic properties (e.g. K_m-value, velocity of the reaction, reduced product inhibition, inducibility/conditional activity), stability under reaction conditions, activity in organic solvents. • Properties of interest for engineering enzyme production are e.g.: optimisation of overexpression in the production host, e.g. by optimising codon usage, folding, protein export, ease of downstream processing (e.g. tags for purification), minimising protein degradation • Properties of interest for enzyme application are: reduced sensitisation potential (e.g. allergic reactions), performance in the target application, stability and robustness during logistics, storage and under reaction conditions • Enzyme engineering applied in the context of/for the purpose of metabolic pathway engineering: protein engineering strategies employing protein scaffolds for

Topic	*State-of-Art*	*R&D&I needs*
		enzyme co-localization or substrate channelling can enable higher pathway efficiency • Enzyme engineering could be further improved if the general lack of structural and mechanistic knowledge about enzymes could be overcome. More specifically, main R&D&I needs include deeper understanding of: substrate/product inhibition, enzyme stability, substrate specificity and enantioselectivity, and the ability to model and simulate these properties in order to support rational approaches in enzyme engineering. • Enzyme engineering with the aim to establish more complex biocatalytic systems and processes, e.g. artificial multi enzyme complexes, reactions cascades, e.g. by co-localising enzymes on scaffolds, enabling substrate channeling etc.
Hosts for enzyme production	Currently, Bacillus subtilis is the most widely used host organism in industrial enzyme production. Alternative concepts (e.g. cell-free protein synthesis) are established at laboratory scale.	• There is a general need for secretory hosts to enable large–scale production and therefore an R&D&I need to establish novel host organisms (e.g. fungi, yeast) with the ability to effectively secret proteins into the medium, by improving tools for engineering the host, e.g. by the ability to introduce or delete genes and to improve the level of protein expression, and by applying systems biology, modelling and simulation. • Development of synthetic biology approaches (e.g. chassis and cassettes, genome reduction), and their application to construct efficient enzyme production hosts. • Developing alternative concepts (e.g. cell-free enzyme production) to industrial scale maturity.
Production process for enzyme production		• There is a general need to further optimise enzyme production processes with respect to biotechnological, economic, ecologic and safety parameters. • Further automatisation and integration of unit operations, process analytical technologies, digitalisation of production.

Topic	*State-of-Art*	*R&D&I needs*
Development of novel enzyme applications, optimisation of enzyme applications		• See also other PROGRESS value chains, all require novel or optimized enzymes for innovative or improved processes and products. • Combination of chemical and enzymatic synthesis • Enzymes and enzyme cocktails for using novel carbon and energy sources, e.g. for waste and valorisation of production side streams, for CO_2 as substrate and for lignocellulose. • Transfer of enzyme production skills to recombinant protein production and engineering, e.g. new protein-based materials (e.g. made from silk protein).
Novel approaches		• Development of enzyme production in cell-free systems for different purposes (e.g screening, research, commercial production). – Development of novel, cell-free reaction compartments for enzymatic reactions – Development of complex biocatalytic systems for cell-free metabolic engineering, e.g. enzyme cascades by choosing or engineering suitable enzyme combinations (matched by their substrate specificity, catalytic activity and reaction conditions), targeted and ordered immobilisation (co-localisation), e.g. on scaffolds or as artificial multi-enzyme-complexes, scale-up to industrially relevant scales.

5 R&D&I needs in the value chain "Production of biopharmaceuticals"

Biopharmaceuticals (or biologics) are a class of protein based drugs (e.g. hormones, antibodies) which can only be manufactured economically with the help of IB. Presently, the EU possesses a strong competitive position in manufacturing these extremely high-value and very low-volume products. Skills in manufacturing biopharmaceuticals significantly affect production costs, profit margins, decisions regarding the location of production facilities and the competitiveness of original molecular entities against biosimilars.

High needs exist for innovation in biopharmaceutical manufacturing, because the current predominant manufacturing paradigm for biopharmaceuticals with large capital intensive production facilities for large volume single products is being complemented or replaced by scalable, small-volume facilities with less capital expenditure that enable flexible multi-product manufacturing of smaller product volumes on demand. Moreover, novel therapeutic principles that are based on genes or cells (advanced therapy medicinal products ATMPs) reach clinical maturity and require the establishment of highly sophisticated manufacturing procedures.

Against this background, the focus of R&D&I policy should be to support industry in the successful transition from the current to the novel biopharmaceutical manufacturing paradigm. The focus should be on

- continuous manufacturing
- single-use systems
- process analytical technology
- further automation of processes (e.g. industry 4.0)
- development of manufacturing processes of novel biopharmaceutical classes
- novel production organisms and novel production paradigms (e.g. human cell lines, transgenic crop plants and livestock ("pharming"), cell-free production platforms).

Table 4 summarizes R&D&I needs in the production of biopharmaceuticals which result from the technology and innovation potentials.

Table 4: R&D&I needs in the production of biopharmaceuticals

Topic	State-of-Art	R&D&I needs
Novel production paradigms	The current predominant manufacturing paradigm for biopharmaceuticals is characterised by complex and sophisticated 20+ step processes. They are usually carried out in large volume unit operations in cGMP facilities, equipped with stainless steel reactors, large filtration and chromatography skids, as well as associated piping and hardware. These manufacturing facilities are investment capital intensive and have high operating expenses, mainly due to expensive chromatography resins and large buffer volumes. Due to the trends of personalised medicine, orphan drugs and smaller disease paradigms, these production paradigms are no longer needed for most biopharmaceuticals. A number of advanced biomanufacturing technologies have been or are being implemented in various process steps but the integration into holistic novel concepts is still on its way.	• To synergistically combine different technologies into novel holistic manufacturing processes for biopharmaceuticals which allow the manufacturing of several different products of smaller volumes instead of one single product of large volume. These facilities are scalable and small-volume, with less capital expenditure that enables flexible multi-product manufacturing on demand, responding to current trends in the biopharmaceutical market. • R&D&I needs with respect to different technologies and steps in the manufacturing process are described in more detail in the table below. • Nevertheless, further improvements of established production paradigms need to be continued. They comprise – USP: improvements in cell line development and engineering, cell clone selection, media and feed development, cell harvesting, bioprocess development, reactor design and scale up – DSP: general optimization of individual unit operations, further development of non-chromatographic operations (e.g. to develop alternative technologies to Protein A affinity chromatography for MAb purification, i.e. membrane-based procedures, aqueous two-phase extraction (ATPE), precipitation, crystallization or affinity alternatives). – For process development and optimisation, modelling and simulation of unit operation is needed, as well as mini-plant facilities

Topic	State-of-Art	R&D&I needs
Continuous biomanufacturing	Continuous biomanufacturing means that the processed products are continuously/automatically moved to the next step as each unit process is completed. Currently, continuous biomanufacturing is predominantly implemented in upstream processing (USP): with the help of sophisticated single use technology, e.g. perfusion bioreactors. Productivities much larger (e.g. factor 4) than in conventional fed batch culture can be achieved.	• To develop equipment and instrumentation for integration of unit operations so that a continuous flow of material from raw input to finished product can be achieved. • To combine continuous up and downstream manufacturing technologies to enable higher process intensification. • USP: further improvements of perfusion reactors, e.g. reducing the usage of large volumes of medium; reducing the complexity of the process, as it is currently requiring specifically trained personnel • USP: To establish stable cell lines which maintain their high productivity over longer periods, e.g. two to three months. • USP: To reduce microbial contamination risks, especially during long-term operations • DSP: implementation of continuous purification processes and continuous non-chromatographic separation technologies to overcome continuous processing capacity constraints. • Issue of regulatory relevance: how can a "batch" be defined in continuous manufacturing; role and implementation of quality-by-design principles
Process analytical technology (PAT)	At-line and on-line process analytical technologies have been implemented for process monitoring.	• To expand the range of analytical parameters, especially for product purity and product quality (e.g. control of glycoforms) in on-line or at-line monitoring. • Development of novel sensors or improved systems for such parameters. • Development of novel sensors or improved systems that can be used in small scale single-use systems (e.g. development of a real time release testing approach). • To increase process understanding to the extent that closed loop control for feeding can be implemented (the cell culture receives at any time the amount of nutrients it requires). • Development of PAT solutions in down-stream processes unit operations, e.g. on-line, at-line determined product concentration in TFF steps or on-line, at-line determined control of product for appropriate collection of the desired product pool in a chromatography step.

Topic	State-of-Art	R&D&I needs
		• To develop non–invasive accurate, on-line, real-time monitoring instrumentation which enable further automation of processes (e.g. industry 4.0). • Use of synthetic biology to improve the detection of cellular metabolites with biosensors.
Single use systems (SUS)	Viable upstream and downstream SUS processing options exist (especially in mammalian-cell based processes) and there is a trend towards higher use of SUS.	• The performance of single-use systems needs to be optimised further: – USP: To broaden the applications beyond mammalian cell culture processes, increase the SUS suitability for microbial processes, e.g. by increasing the maximum gas transfer rates – Scale up SUS production capacities – Compatibility of single-use equipment solutions from different suppliers needs to be increased by standardisation.
Manufacturing of novel biopharmaceutical classes and advanced therapy medicinal products (ATMPs)	Novel biopharmaceutical classes such as antibody drug conjugates as well as advanced therapies (i.e. gene therapy, cell therapies) are emerging therapeutic paradigms which require specifically developed manufacturing processes. They have the potential to complement and even replace many biopharmaceuticals	• Further R&D&I needed to adapt manufacturing systems to new types of therapeutic molecules, such as antibody drug conjugates (ADCs), and optimize them • Development and optimisation of novel production paradigms for ATMPs
Established production organisms	Transgenic bacteria and mammalian cell lines are the workhorses in biopharmaceutical manufacturing. Alternative production hosts only play a minor role	• To improve established production organisms, especially with respect to the following aspects – Improvements in cell lines to reduce contamination and protein impurities such as host cell proteins. – Improvements of biopharmaceutical quality e.g. desired glycoforms or other desired post-translational modifications. – Production strains adjusted to reactor capability rather than the other way around (e.g. strains or cell lines that cope with the low oxygen transfer capabilities of SU bioreactors).

Topic	State-of-Art	R&D&I needs
Novel production organisms		• To develop new production organisms: R&D&I towards human cell lines, replacement of avian eggs for vaccine manufacturing, and "pharming" of transgenic crop plants, animals • Find solutions for regulatory approval issues of novel production organisms
Cell free production systems/platforms	Cell free systems exist that could be used as potential production systems for nonglycosylated proteins.	• Cell free systems for non-glycosylated proteins need improvements regarding productivity and product quality • Scale up of cell free systems to commercial scale • Expand the range of proteins that can be produced in cell-free production systems, e.g. establish cell free production systems/platforms for glycosylated proteins, for tailored glycosylation, and specifically modified proteins (e.g. with non-natural amino acids).

6 R&D&I needs in the value chain "Biotechnologically produced Flavors and Fragrances"

Flavors and fragrances (F&F) is the name given to a very large group of diverse substances that sensitize the receptor cells of the human olfactory system which mediate the senses smell and taste. F&F are widely used in a broad range of industries and products, such as food and beverage, pharmaceuticals, perfumes and cosmetics, toiletries, tobacco, detergents and household products.

Only a minor share of the plethora of flavors and fragrances which are naturally synthesized by living organisms has already been exploited by industry, mainly by extraction from natural sources or by chemical synthesis. Recently, biotechnological production methods received a technology push by synthetic biology and systems metabolic engineering, so that IB F&F may become competitive in hitherto untapped market segments.

In order to further support the production of F&F by IB, R&D&I policy should focus on support for

- the formation of strong networks between European actors from academia, SMEs and key players in the F&F industry
- the strategic identification of top F&F candidates for R&D&I funding, e.g. substance families with a broad spectrum of diverse F&F and different uses which are also attractive from a business and market perspective
- a broad scope of technology solutions, from synthetic biology to non-GMO approaches
- the establishment of universal platforms of substances, production organisms and enzymes, of toolboxes and engineering strategies that can be applied in natural product research, thus speeding up the R&D&I process for biotechnologically produced F&F
- achieving industrially relevant titers, yields and production rates for F&F,
- making a greater diversity of F&F available to industry, also novel ones not found in nature,
- reducing the environmental footprint of F&F production processes
- research into consumers' perception and acceptance of F&F produced by extensively engineered organisms, and respective regulatory options.

Table 5 summarizes R&D&I needs in the production of F&F which result from the technology and innovation potentials.

Table 5: R&D&I needs for biotechnologically produced flavors and fragrances (F&F)

Topic	State-of-Art	R&D&I needs
Strategic focus of R&D&I efforts	F&F are mainly developed on a case-by case basis. F&F ingredients have to meet the taste specifications of the food or beverage in which they will be incorporated, have to meet national regulations and must cater to - often regional - consumer preferences. Technology experts may lack this knowledge and may focus on F&F and issues which do not make sense from a market perspective.	• Synergistically bring together profound knowledge of technological potentials and of market perspectives for the identification of top F&F candidates for R&D&I • Identify substance families with a broad spectrum of diverse F&F and different uses (e.g. terpenoids) • Identify novel uses and applications beyond the F&F sector for specific compounds or substance families
Identification of novel F&F	Compound libraries and sample collections can be screened for new flavor and fragrance compounds. A major challenge is to produce enough products for further characterisation, as the expression levels or concentrations of the target compounds are extremely low	• Expand the libraries and collections, expecially by underinvestigated sources (e.g. unculturable organisms, extreme environments) • Develop analytical techniques further which are employed to evaluate the aromatic profile (e.g. GC-MS, "electronic nose"), also automated, miniaturized, high-throughput methods • Establish precursor-providing platforms which provides sufficient precursors for testing and characterising novel F&F (and biosynthetic elements, see below)

Identification of novel biosynthetic pathways and enzymes (= biosynthetic elements)	Organism collections and gene databases can be screened for new genes involved in the biosynthesis of F&F. Relevant genes are often organised as biosynthetic gene clusters (BCGs), which encode the enzymes, regulatory elements and transporters that are necessary to produce, process and export a given metabolite. Significant efforts in genome mining for natural product biosynthesis (not restricted to F&F) have yielded several hundreds of novel molecules in the past decade.	• *in silico* screening of genome sequences of mostly unexplored microorganisms (e.g. unculturable organisms, extremophiles) • Further development and use of computational tools in the field of natural product research (e.g. identification of BCGs, annotation of functions based on DNA sequence information, prediction of target compound structures from DNA sequence information of key enzymes) • Develop good practice to narrow down the immense genomic diversity to a limited number of biosynthetic pathways which is feasible to be evaluated. For this purpose, algorithmic approaches for the identification, classification, dereplication and prioritization of biosynthetic gene clusters (BGCs) in genomes and metagenomes are required. Moreover, there is a need to further develop high-throughput and automated procedures, and combinations of bioinformatics and mass spectroscopy • Develop and apply bioinformatic tools which link genomic data on enzymes and pathways to data from the screening of compound libraries or to data from proteomic and metabolomic analyses • Establish precursor-providing platforms which provides sufficient precursors for testing and characterising novel F&F and biosynthetic elements • Feed newly discovered biosynthetic elements and their characteristics into repositories and databases in order to build a resource of a large diversity of biosynthetic elements that can easily be accessed for further targeted engineering

Construction of F&F overproducing strains, suitable for industrial production	As F&F are usually produced by organisms only in minor amounts, substantial engineering of heterologously expressed genetic constructs is required to reach industrially relevant production rates, yields and titers (appr. more than 1g/L). Often, more than 20 genes have to be altered. Therefore, systems metabolic engineering has to be applied. However, classical in vivo metabolic and host engineering is too resource- and time-consuming. Targets of engineering are central metabolic pathways to ensure sufficient F&F precursor supply, reduce flux to competing metabolic pathways, enhance flux to the target metabolic pathway, balance supply of energy and reducing equivalents (ATP, NAD(P)H), establish an appropriate regulation of this system, and address the potential toxicity of overproduced F&F precursors or target compounds.	• Develop a profound understanding of metabolic pathways, required biosynthetic elements and underlying mechanisms, by quantitative characterisation of the required elements (e.g. kinetics, regulation etc.), and by building and refining in silico models of the pathway • Identification (supported by bioinformatic tools) of the best performing biosynthetic elements (e.g. enzymes), ideally from large databases or repositories/collections (see above), and assembly into a functional biosynthetic pathway • Establishment of reconstituted biosynthetic pathways in vitro, • Proteomics and/or metabolomics analyses of in vitro reconstituted biosynthetic pathways with the purpose to better understand rate-limiting steps and to guide further pathway engineering • Broaden the amount of available bioparts (e.g. promotors of different strengths, ribosomal binding sites, regulatory elements) to be easily accessed and used in generating gene and pathway variants, e.g. made available through repositories • Improve and apply combinatorial approaches for generating large numbers of pathway variants and test them in vitro, ideally in high throughput manner for the best performing variants • Improve methods for the assembly of large multi-gene operons (e.g. bacterial artificial chromosomes, BAC) and their integration into the production host genome (e.g. by developing and using faster and more robust genome editing techniques, by providing integration cassettes that facilitate unlimited sequential integration of genetic elements) • Further optimisation of the genes/functional pathways finally introduced into engineered production hosts (chassis) that are most suitable for production, addressing the issues of sufficient F&F precursor supply, reduced flux to competing metabolic pathways, enhanced flux to the target metabolic pathway, balanced supply of energy and reducing equivalents (ATP, NAD(P)H), appropriate regulation of this system • If relevant for the target compound, toxicity of overproduced F&F precursors or target compounds must to be addressed. Further R&D&I is needed for strategies such as – compartmentalization of the pathway, e.g. in peroxisomes in yeast and proteinaceous micro-compartments in bacteria

		– establishment of in-vitro biosynthetic systems on a production scale (see below) – engineering of uptake and efflux systems for the toxic compounds
Optimisation of (key) enzymes involved in F&F synthesis	Metabolic engineering in de novo synthesis of F&F or biotransformation/bioconversion usually requires the optimisation of individual enzymes with respect to their substrate and reaction specificity and selectivity, kinetic properties, and other production-relevant characteristics (e.g. long-term stability). Bioinformatic tools to guide optimisation as well as strategies of (semi-)rational design, (sub-)domain swapping and other combinatorial approaches, and evolutionary approaches have been developed.	Depending on the substances of specific interest, certain enzyme groups are of key importance and may be one of the bottlenecks to be addressed, e.g. key enzymes are terpene cyclases for terpenoids, carboxylate reductases (CARs) for the production of aldehydes, or chain-tailoring enzymes for linear, medium-chain (C8–C12) hydrocarbons. R&D&I needs are the application of the existing approaches and strategies for enzyme engineering to enzymes involved in F&F and tailoring the approaches to specific requirements: • For *in silico* screening and genome mining, the refinement and further development of bioinformatic tools is required, e.g. tools for the identification of gene clusters and the prediction of specific enzymes, assessing the novelty of the detected clusters and genes by comparing the predicted genes with different cluster and compound databases. A more standardized procedure for genome mining for natural products and the corresponding enzymes would be desirable • Identification and establishing genetic parts of sufficient diversity and with the required properties for the engineering of target enzymes • Broadening the knowledge of structure-function relationships, elucidate the enzyme reaction mechanism • Application of established enzyme optimisation strategies in order to alter the substrate specificity of key enzymes in a synthetic pathway – high specificity for industrial-scale production of the target compound of higher purity – broad specificity (= promiscuous) for generating product diversity, e.g. for the creation of natural product libraries with many structurally diverse molecules • Develop applications for the engineered enzymes, improve yields in de novo biosynthesis and integrate enzyme into reaction cascades in in vitro systems

Process engineering for de novo biosynthesis	On laboratory scale, optimisation is often still done in Erlenmeyer flasks. However, state of the art process design and equipment (bioreactors, regulation of important parameters) should be routinely employed.	• Process design and engineering in order to reduce the toxicity of F&F intermediates and target compounds, e.g. by feeding strategies, or in situ product recovery • Process design and engineering in order to overcome low solubility and volatility, e.g. by feeding strategies and biphasic systems • Exploring the potentials of solid state fermentation, as it may have higher yields than submerged fermentation • Optimisation of the environmental performance of the production process by applying the principles of Green Chemistry, especially by reducing energy, replacing organic solvents by alternative solvents (e.g. supercritical fluids (e.g. CO_2),pressurised liquids, ionic liquids), reducing hazardous substances, minimizing water demand and waste water production
Process engineering for biotransformation, bioconversion and synthetic biochemistry	Industrially relevant complex biomolecules (e.g. monoterpenes) can be produced in vitro directly from glucose. ATP and Acetyl-CoA are provided by glycolysis. High titers, yields and production over several days can be achieved. It is the method of choice for producing (semi)toxic chemical compounds, for the optimization of individual enzyme steps or their combinations, and for the production of chemically diverse compound libraries, especially when optimizing the production of high-value chemicals in a high-throughput manner. For industrial scale production, production rates are still too low and costs too high.	• Long-term productivity of the systems must be achieved, e.g. by further optimization of reaction conditions as well as in vitro evolution of enzyme stability and activity, and especially by the development of novel systems for regenerating ATP and NAD(P)H • Further development so that more complex reactions can be performed in vitro • Reducing the enzyme cost, e.g. by more stable enzymes which can be used longer, (= increase total turnover number), by recycling of enzymes • Development of inexpensive purification methods • Explore the exchange of enzymes in the system in order to diversify the products

7 R&D&I needs in the value chain "Microbiomes for healthy food and nutrition"

This value chain focusses on human microbiota engineering in nutrition, with the aim of maintaining health and preventing disease, via food and food ingredients and in over-the-counter pills or supplements.

This value chain represents an emerging, science- and technology driven field, in which only few novel services, mainly microbiome profiling analyses coupled with dietary advice, have been commercialized yet. The majority of activities take place in R&D. R&D&I policy should play a significant role by continuing R&D&I funding on the EU and member state level. Specific attention should be paid to the following aspects:

- Shifting the focus of basic research from studying microbiota composition to elucidating microbiota function and mechanisms
- Widening the scope from microbiota-centred research to studying also host-microbiota interactions and host-microbiota-environment interactions (lifestyle, nutrition)
- Complementing descriptive, exploratory, shot-gun approaches by hypothesis- and knowledge-driven approaches, informed by the growing insight into underlying functions and mechanisms.
- Supporting the further development of methods and technologies, in order to elucidate, test and validate proposed mechanisms of action on the molecular level.
- Continuing the support for microbiome research resources (e.g. inventories, catalogues and "reference microbiomes", well annotated clinical repositories with deep phenotype)
- Intensifying interdisciplinarity by overcoming disciplinary silos, and supporting cooperation of academia, and relevant industries (e.g. industrial biotechnology, food, pharma and ICT and data industries).
- Supporting translational research in order to establish evidence-based interventions, which aim at modulating the human gut microbiota, based on mechanistic insight, requiring
- Initiating R&D into manufacturing, formulation, storage and consumption of food-based health interventions in order to ensure safety and quality.
- Investigating implications of microbiome research results for health claims, product labelling and communication of health effects.

Table 6 summarizes R&D&I needs for Microbiomes for healthy food and nutrition, which result from the technology and innovation potentials.

Table 6: R&D&I needs for microbiomes for healthy food and nutrition

Topic	*State-of-Art*	*R&D&I needs*
Microbiome composition	The presence and composition of microbial communities in the human host has been studied and described in different age, ethnic and geographical groups and associations with different health and disease states have been established	Further refinement of the analysis of the structural composition of microbiomes is required by further developing the methods applied, e.g. with respect to • Spatial resolution. Methods for molecular cartography comprise e.g. imaging mass spectrometry (IMS), fluorescently tagged bacteria, transparent model host organisms • Quantitative determination of genus, species, strains • Quantitative determination of metabolites, e.g. by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) • Establishment of R&D resources, such as inventories, catalogues and "reference microbiomes" for better annotation and assignment of functions to genes, transcripts, metabolites, and organisms, such as well annotated clinical repositories with deep phenotype
From microbiome composition to microbiota functions and mechanisms	Associations of microbiome composition and shotgun approaches with different health and disease states have generated many new hypotheses about causes, and many modes of action have been postulated. However, the molecular mechanisms related to health and disease in host-microbe and microbe-microbe interactions still remains a large knowledge gap.	Need for improvement of methodologies, with the goal to apply these methods to the elucidation of mechanisms of action • Establishment of R&D resources, such as comprehensive catalogues of genes, metabolites, synthetic pathways, and their characterization in order to reduce the number of unknown genes, metabolites etc • Overcome silos in -omics technologies, integrate different -omics technologies, including shotgun metagenomics, metatranscriptomics,and metabolomics, and specifically find solutions to the challenges – choice of appropriate statistical methods and tests – differentiating signal from noise, e. g. by tracking changes in perturbed systems via accurate quantification – identification of genes and metabolites and their annotation, assignment of functions to these genes and metabolites, identification of the origin of metabolites (whether from host, microbiota, or environment)

Topic	*State-of-Art*	*R&D&I needs*
	Technologies which are able to help define the function of a system comprise omic technologies (metagenomics, metatranscriptomics, metaproteomics, metabolomics) and related bioinformatic tools, the ability to culture diverse intestinal microbes, to genetically manipulate bacteria so that the effects of gain or loss of particular functions can be evaluated, in vitro models, and animal models such as gnotobiotic mice for establishing causality. However, the function of many genes and metabolites is still unknown, the properties of many bacteria are poorly understood, especially if they evade cultivation or if tools are lacking to genetically manipulate them.	• Identify, characterize and use attractive model organisms and model systems to study various aspects of microbiota functions. Combinations of in vitro, ex vivo, and in vivo models should be used. Model systems to test hypotheses of microbiota functions should cover a wider range in complexity, such as organoids and bioreactors (e.g. artificial gut) • improve culture methods for until now unculturable microorganisms and for defined mixed cultures • overcome the limited applicability of tools for genetic engineering and genome editing of microbiota members (e.g. probiotic strains) with the aims to introduce subtle genome edits without the need for antibiotic selection and to make the methods less challenging and time-consuming Apply the above mentioned methods and different approaches for the elucidation, testing and validation of proposed mechanisms of actions on the molecular level • Approaches should combine -omic technologies with classical bacterial genetics, bacterial physiology, protein engineering, and biochemical characterization • Approaches should dissect the function of each bacterium alone and in concert with complex bacterial communities in well characterized systems • Approaches should explore the relevant mechanisms alone and in concert (if there are more than 1 mechanism) Apply the above mentioned methods and different approaches to targets, to functions and to mechanisms of specific interest, for example to the mechanisms underlying • the beneficial effects of probiotics (e.g. (temporary) alteration of the microbiota composition, regulation of the epithelial barrier function, modulation of immune responses, interaction with the gut-brain barrier). Research could address probiotic effector molecules, such as specific pili, S-layer proteins, exopolysaccharides, muropeptides, as well as more widely produced metabolites such as tryptophan-related and histamine-related metabolites, CpG-rich DNA, and various enzymes such as lactase and bile salt hydrolases • the chemical communication in host-microbe interactions mediated by specialized metabolites (SMs), by which microbial communities can influence the health of their host (e.g. bile acids, short chain fatty acids etc) • the colonization of the gut by beneficial or undesired microorganisms

Topic	*State-of-Art*	*R&D&I needs*
Hypothesis- und knowledge-driven approaches	Due to the limited knowledge of the modes of action of microbiota in total or with respect to individual members of the microbial community, rational, hypotheses-driven approaches are difficult to pursue.	Apply the growing knowledge to design hypothesis- and knowledge-driven approaches, e.g. • Rational, reproducible probiotic strain selection, based on knowledge of the underlying mechanisms by which probiotics elicit their effects • development of tailored probiotics with increased stress tolerance, or enhanced metabolic activity • validation by in vitro assays, animal models, and genetic manipulation of bacteria (e.g. loss/gain-of-function experiments)
Taxonomic distribution of mechanisms	There is evidence that some mechanisms of action of microbiota are confined to specific strains whereas other mechanisms are shared by wider taxonomic groups.	Once mechanisms have been eludicated, there is a need to study the taxonomic distribution of mechanisms in order to identify shared mechanisms of taxonomic groups. This knowledge could be used, e.g. • for the rational selection of probiotic strains or strains with the targeted property (e.g. ability to synthetize beneficial molecules or specialized metabolites) • for metaanalyses of clinical trials by pooling of data from trials in which the intervention is based on the same mechanism (but may apply different strains) • for the further development of the EFSA health claims approval procedures.
Host-microbiota interaction and mechanisms	The analysis of microbiota structure and function remains incomplete if the specific host is not taken into account. However, host-microbe interactions have not yet been studied intensively.	There is a need to include also the host into the analysis of functions and mechanisms, as described above. R&D needs in studying host-microbe-interactions comprise e.g. • Studying in model systems the chemistry and mechanisms of host-microbe communication • Expansion to other host-microbe systems to investigate whether there are conserved mechanisms in different bacteria • Design and test targeted interventions into the host-microbe communication
Exploring the host-microbiota-environment interdependence	The analysis of microbiota structure, function and host-microbiota remains incomplete if the specific environment (e.g. lifestyle, diet) is not taken into account. There is still a lack of approaches which integrate all these issues.	There is a need to include also the environment into the analysis of functions and mechanisms, as described above. R&D needs comprise e.g. • Improvement and validation of tools to monitor diet and lifestyle with respect to accuracy, reproducibility, reliability, usability • Integration of lifestyle, nutrition, and environmental data into the analysis

Topic	*State-of-Art*	*R&D&I needs*
Development of novel interventions precisely targeting the microbiota	Mechanistic insight into microbial drivers of maintenance of health or in disease phenotypes is essential for translation to novel interventions. Different approaches have been proposed: • Additive approaches (e.g. probiotics, ranging from single strains via genetically engineered strains and defined mixed cultures to undefined microbial mixtures) • Subtractive approaches (e.g. engineered bacteriophages, antibiotics) • Modulatory approaches (e.g. prebiotics, selective non-lethal small molecules that target defined (and causal) microbial or host pathways Most studied probiotic organisms to date are several Lactobacillus strains and bifidobacteria. Only very few examples of microbiome-targeted small molecules are known.	The proposed approaches and strategies aimed at modulating the gut microbiota should be explored. An ecological perspective grounded in theory should be applied to design, predict and interpret the impact of microbiome-modulating strategies. R&D needs comprise • Screening for beneficial bacteria, molecules and functions • Characterisation of the mechanism of function • Validation of function in in vitro tests • Validation of function in animal model • Validation of function by genetically engineered strains • Clinical trials • Integration of lifestyle, nutrition, and environmental data into the analysis
Substantiation of health effects and health claims	There is still a substantial lack of evidence for the causation of microbiomes and disease. For example, in most cases of probiotics, it is not yet confirmed whether the known probiotic effector molecules are the actual drivers of the clinical effects observed in human trials.	• Properly designed clinical trials in human subjects are needed • Further research is needed to confirm the link between a given mechanism and clinical benefit and to establish associations between the presence of specific mechanisms and clinical benefits more broadly than up to now. • The clinical study design has to be based on comprehension of mechanism of host-microbe interaction or microbe function, and the trial should be performed with dedicated isogenic knock-out or knock-in mutants of the probiotic microorganisms or with proper formulated isolated bio-active compounds

Topic	*State-of-Art*	*R&D&I needs*
	It is known that many factors influence the clinical effects, e.g. the potency of the probiotic strain itself, but also dose, viability, formulation, targeted pathogen, targeted host response, targeted host site, prevention or treatment set-up. Difficulties of measuring certain biomarkers, combination effects, time frames for the probiotic activities (seconds, minutes, hours, weeks) need to be taken into account	• Clinical trials should also be used to explain inter-individual variation in responses to the interventions
Ensuring safety, quality and claimed health benefit of novel microbiota targeting interventions	On EU level, a health claim approval system is implemented at EFSA in order to ensure that the claimed effects are evidence-based and consumers are properly informed. Safety and quality of the food-based health interventions has to be ensured by the manufacturer.	• There is a need to investigate implications of microbiome research for health claims, product labelling and communication of health effects, as a basis for adapting the EFSA health claims system to the state of the art in science and technology. • R&D is required for the manufacturing, formulation, storage and consumption of food-based health interventions in order to ensure safety and quality, e.g. with respect to the effective dose and to standardization.
Food surveillance system	Research is underway to establish a food surveillance systems along the entire food value chain which is based on microbiome sequencing.	R&D needs comprise • Improvement of sensitivity, specificity and reliablity/reproducibility of pathogen identification • Broadening of the scope of pathogens that can be identified by generating sequence data of more food pathogens • Validation for different foods and locations • Development of a web-based platform to store, process, and analyze the data and to quickly generate easy-to-read food safety reports • Investigate the implications for regulation, guidelines and surveillance practice