Brussels, 13 November 2018

COST 094/18

DECISION

Subject: **Memorandum of Understanding for the implementation of the COST Action "Understanding and exploiting the impacts of low pH on micro-organisms" (EuroMicropH) CA18113**

The COST Member Countries and/or the COST Cooperating State will find attached the Memorandum of Understanding for the COST Action Understanding and exploiting the impacts of low pH on micro-organisms approved by the Committee of Senior Officials through written procedure on 13 November 2018.

MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

COST Action CA18113
UNDERSTANDING AND EXPLOITING THE IMPACTS OF LOW PH ON MICRO-ORGANISMS (EuroMicropH)

The COST Member Countries and/or the COST Cooperating State, accepting the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action (the Action), referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any new document amending or replacing them:

a. "Rules for Participation in and Implementation of COST Activities" (COST 132/14 REV2);
b. "COST Action Proposal Submission, Evaluation, Selection and Approval" (COST 133/14 REV);
c. "COST Action Management, Monitoring and Final Assessment" (COST 134/14 REV2);
d. "COST International Cooperation and Specific Organisations Participation" (COST 135/14 REV).

The main aim and objective of the Action is to create a community of scientists working on the impacts of low pH on important micro-organisms, enabling the sharing of new concepts and methods which are being developed, but are not crossing boundaries between different disciplines and sectors including industrial, clinical, and food and drink microbiology. This will be achieved through the specific objectives detailed in the Technical Annex.

The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 68 million in 2018.

The MoU will enter into force once at least seven (7) COST Member Countries and/or COST Cooperating State have accepted it, and the corresponding Management Committee Members have been appointed, as described in the CSO Decision COST 134/14 REV2.

The COST Action will start from the date of the first Management Committee meeting and shall be implemented for a period of four (4) years, unless an extension is approved by the CSO following the procedure described in the CSO Decision COST 134/14 REV2.

TECHNICAL ANNEX

OVERVIEW

Summary

This COST Action is broad in its technical and scientific scope, as its central aim is to bring together people working in quite diverse fields but with a common scientific interest: namely, the understanding and exploitation of the responses of micro-organisms to low pH. These organisms in this context include bacteria, yeasts, and other fungi. This topic is already being studied in considerable depth and has many important practical applications in a number of diverse sectors; however, these sectors traditionally do not communicate well with each other. A new forum for communication will be highly beneficial both for scientific progress and, importantly, for the applied fields in which this topic is important. These include the microbiology of food and drink, many aspects of industrial biotechnology and bio-processing, and clinical and veterinary treatment of infections in a time of increasing antimicrobial resistance. Through a combination of Working Groups, workshops, Short-Term Scientific Missions, and dissemination activities, plus open conferences, this Action will (a) aid increased understanding of the details of how micro-organisms detect and respond to low pH (b) ensure that technical developments being made in one field are rapidly translated into other fields (c) leverage the many different areas of expertise that exist across Action members and (d) ensure, through participation and dissemination, that these developments reach as wide an audience as possible, including pure and applied scientists in the Inclusiveness Target Countries.

Areas of Expertise Relevant for the Action	**Keywords**
● Biological sciences: Microbiology ● Industrial biotechnology: Food microbiology ● Industrial biotechnology: Bioprocessing technologies (industrial processes relying on biological agents to drive the process) ● Clinical medicine: Prevention and treatment of infection by pathogens (e.g. vaccination, antibiotics, fungicide) ● Other engineering and technologies: Food science and technology	● Microbial physiology ● organic and inorganic acids ● Stress responses ● Bioprocessing ● Microbiological safety

Specific Objectives

To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination

● Build links across different relevant disciplines, both in and related to microbiology, so that methods for studying acid stress responses can be shared, developed, and exploited.
● Establish and foster synergies between pure and applied researchers so that developments in understanding of mechanisms of responses of micro-organisms to low pH can be utilised quickly in practical applications.
● Disseminate information about Action activities and deliverables as widely as possible in the relevant research and development communities from academia to industry and health care.
● Gather, record, and disseminate standardised information about characteristics of diverse micro-organisms exposed to acid stress, to identify knowledge and technical gaps.

Capacity Building

● Support and develop knowledge exchange and development of overlapping research agendas and deeper understanding between researchers in both pure and applied fields with an interest in microbial responses to low pH.
● Develop expertise in researchers, particularly ECIs, in new methods and conceptual approaches which have general applicability in the field of microbial responses to low pH.
● Involve younger ECIs and researchers from ITCs in all aspects of the running and development of the Action.

1. S&T EXCELLENCE

1.1. CHALLENGE

1.1.1. DESCRIPTION OF THE CHALLENGE (MAIN AIM)

This COST Action will create a connected community of scientists working on the impacts of low pH (acid) on a range of important micro-organisms. This will enable the sharing of new concepts and methods which are being developed but are not crossing boundaries between different experimental systems, or, in particular, between the sectors of industrial, clinical, and food and drink microbiology. New approaches to tackling pressing needs in these areas will be developed. Removing bottlenecks between these fields has high potential to help in the development of a sustainable bio-based economy, to refine the use of preservatives in food and drink, and to tackle the problem of anti-microbial resistance (AMR), both to antibiotics and antifungals.

Micro-organisms (bacteria, yeasts, and other fungi) are of great importance for the things they do for us, and the things they do to us. Without yeast we would have no bread, beer, or wine; without bacteria most cheeses and yogurts could not be made. Micro-organisms are used on a large scale in industrial processes such as production of food supplements (1.7 million tonnes p.a. of glutamate is produced by a single bacterial species), paper, detergents, industrial enzymes, drugs from insulin to antibiotics, and vaccines. As we move to a bio-economy as discussed in the EU 2015 Action plan “Closing the loop”[1], the importance of micro-organisms will increase. In the future, petrochemicals may be replaced by compounds made from engineered micro-organisms, and new methods for manufacturing high volume and high value chemicals using them will be developed.

However, some micro-organisms have always been a scourge. Everyone knows of their impact on our health through infections, and most are aware of our growing inability to treat infections, caused by the spread of AMR. New antimicrobials and approaches to prevent the spread of infection were part of the 2011 EU Action Plan against AMR[2] and will be a key component of the second Action Plan, launched in 2017. In addition, activities of micro-organisms are responsible for the loss of ~25% of the world’s food. The challenge of feeding a growing population as well as keeping food fresh and safe to eat by preventing microbial growth continues to require innovation on behalf of food scientists, particularly as consumer demand for fewer artificial preservatives increases. Reducing food waste is a key component of the EU’s Circular Economy Package[1].

Progress in all these fields needs a better understanding of the impact of low pH, whether this is low external pH, or low internal pH caused by organic acids that cross membranes and dissociate. Micro-organisms all have an optimal pH; as the pH drops they function less well, and at low enough levels they cease to grow or they die. Many industrial processes require a low pH to reduce costly downstream processing, while industrial bioprocesses like the conversion of lignocellulose into biofuel precursors are limited in part by the effects of the acetic acid produced. Ways to engineer better acid tolerance into the production organisms are thus needed. The drive towards natural food preservatives requires better understanding of the effects of organic acids like acetic, benzoic, citric, lactic, and sorbic acid which inhibit growth of spoilage organisms. The ability of these acids as an alternative to antibiotics and antifungals is receiving clinical attention, in the use of organic acids to treat infected wounds, and medicinal honey, a low pH wound dressing.

Exciting developments in understanding the effects of low pH at the mechanistic level are underway, including single cell imaging, systems-level approaches like transcriptomics and metabolomics, and

computer modelling. However, there are major bottlenecks in transferring these methods into applied fields, where the strains and growth conditions used are often different from model organisms in pure research. The applied fields do not exchange methods: clinicians using organic acids to treat infections do not communicate with food microbiologists, and neither talk to industrial microbiologists who may be using microbes to make products at low pH. Yet their interests are two sides of the same coin, either preventing bacterial growth, or assisting it, at low pH, both of which need a deeper understanding of mechanisms. Hence this COST Action, which will link pure research with sectors that can exploit this knowledge, while training and improving the mobility of students and Early Career Investigators (ECIs). The synergies will be far-reaching and will have a positive impact on both applied and fundamental research.

Figure 1 shows the potential to link together different fields while developing research methods and movement of researchers.

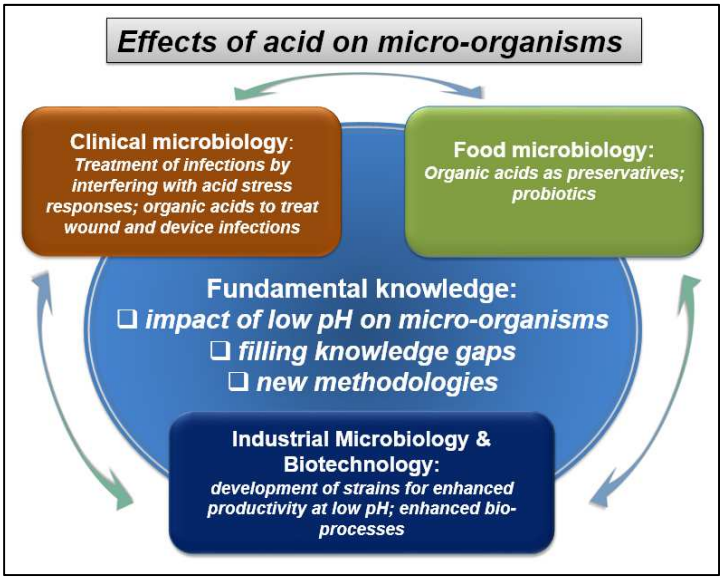

Figure 1: The COST Action will enable movement of people and expertise between different fields studying and applying microbial responses to low pH.

1.1.2. RELEVANCE AND TIMELINESS

This Action arose from three conferences on Microbial Stress Responses, supported by the Federation of European Microbiology Societies and the European Federation of Biotechnology, which were set up to bring together pure and applied microbiologists with interests in stress responses. It was realised that the sectors described above could all derive mutual benefit from greater sharing of expertise, and that novel approaches and methods that are being developed to study low pH responses in model organisms have the potential to be much more widely applied.

The current rapid development of methods relevant to this field makes this application very timely. Systems level methods for integration of large amounts of information at the 'omic level are developing fast. These include standard methods like RNAseq, plus new approaches like Tn-seq[3] (that measures gene fitness on a genome-wide scale) and lab-based evolution with whole genome sequencing. Methods for observing physical processes in individual bacterial cells are becoming more sophisticated, and methods for predictive computational modelling of events at molecular, cellular, and population levels are developing rapidly. Collaboration will enable to link functional genomic and systems level information to an understanding of the events that occur at the cellular and molecular level, and to manipulate these processes. There are ample opportunities for training and cross-disciplinary work, and importantly this will lead to significant advances in the various fields and provide training to students and ECIs.

1.2. OBJECTIVES

The Action overall objective is to create a community of scientists working on the impacts of low pH on important micro-organisms, enabling the sharing of new concepts and methods which are currently being developed, but are not crossing boundaries between different disciplines and sectors including industrial, clinical and veterinary, and food and drink microbiology.

1.2.1. RESEARCH COORDINATION OBJECTIVES

This Action will link disciplines and sectors that do not currently work together, and enable sharing of new techniques, best practice, ideas and concepts. This will be achieved through activities that integrate the diverse fields and ensure the widest possible cross-disciplinary transfer of knowledge and training of researchers, students and ECIs. Activities are designed so that the impact of the Action will continue after it has formally ended, so that researchers outside the current network can become integrated into it, and so that the outputs will be disseminated as widely as possible. The research co-ordination objectives are to:

- Build links across different relevant disciplines, both in and related to microbiology, so that methods for studying acid stress responses can be shared, developed, and exploited.
- Establish and foster synergies between pure and applied researchers so that developments in understanding of mechanisms of responses of micro-organisms to low pH can be utilised quickly in practical applications.
- Disseminate information about Action activities and deliverables as widely as possible in the relevant research and development communities from academia to industry and health care.
- Gather, record, and disseminate standardised information about characteristics of diverse micro-organisms exposed to acid stress, to identify knowledge and technical gaps.

1.2.2. CAPACITY-BUILDING OBJECTIVES

Capacity building will build a group of well-trained young researchers who are competent in diverse methods that can be used in many different areas relevant to the study. To do this, the Action will:

- Support and develop knowledge exchange and development of overlapping research agendas and deeper understanding between researchers in both pure and applied fields with an interest in microbial responses to low pH.
- Develop expertise in researchers, particularly ECIs, in new methods and conceptual approaches which have general applicability in the field of microbial responses to low pH.
- Involve younger ECIs and researchers from ITCs in all aspects of the running and development of the Action.

1.3. PROGRESS BEYOND THE STATE-OF-THE-ART AND INNOVATION POTENTIAL

1.3.1. DESCRIPTION OF THE STATE-OF-THE-ART

Many features of microbial responses to acid are universal irrespective of the organism in which they are studied. These include mechanisms to detect the pH change or its consequences, homeostatic mechanisms that modulate internal pH, regulatory mechanisms that alter gene expression to reduce the impacts of the stress, and protection and repair mechanisms that deal with the consequences of acid stress. All of these are currently subject to study, mostly in a few model organisms and strains.

Microbial responses to low pH are studied in a range of ways, depending on the organism, the acid being studied, and on the specific research questions being asked. Questions are asked at the genetic, biochemical, cellular, and whole population level. Academic research tends to focus on linking descriptions of events that take place when cells are exposed to low pH to models of the underlying molecular mechanisms to explain these responses. There are currently no complete descriptions of all these processes available, and it is not possible to build reliable predictive models, based on known

genetic networks, even for model organisms. Variation between organisms, including between strains of the same species, can be considerable. Stress resistant phenotypes arise quite fast in lab-based evolution experiments. Also, even within populations of genetically identical cells, it is known that stochastic variation at the single cell level contributes to the unpredictability of how a whole population will behave when exposed to an acid stress.

Applied studies often use a more empirical approach to measure cell growth or survival or look at the impact of low pH on the outcome of processes such as the yield and quality of product from a bioprocess. In the context of food, synergies between acid and other stresses (e.g., osmotic, temperature) are used to optimise shelf life; this is the so-called "hurdle" approach to food preservation[4]. Many bioprocesses that either require or lead to low pH are mediated by bacterial or yeast species which have been selected over time for their ability to thrive under these conditions, but too little is known about the mechanisms of this to enable the engineering of more resistant properties into strains or transferring the resistance properties to other species.

In health care, the growing need for alternatives to antibiotics and anti-fungals to treat conditions such as persistent wound and burn infections, and growth of bacteria and yeasts that thrive on in-dwelling medical devices, has led to some preliminary work using organic acids as anti-microbials[5], but this work to date is empirical, with no mechanistic under-pinning. The mechanisms of action of weak organic acids (which can pass the membrane and acidify the cytoplasm, a crucial process for their roles as bacteriostatic agents both in the clinic and in food preservation) are poorly understood, as indicated from the fact that their effects cannot be predicted from their pKa alone.

1.3.2. PROGRESS BEYOND THE STATE-OF-THE-ART

The unique network of researchers will, through the effective cross-disciplinary and cross-sectoral collaboration enabled by this COST Action, help to address the most pressing questions in the field and lead to new applications across a range of sectors. The network will involve participants with diverse technical expertise and in sectors that do not currently interact closely, if at all, in order to maximise the potential for new synergies to be found. Significant relevant developments (for example in modelling, in single cell imaging, and in high throughput 'omics methods) are currently taking place in several highly relevant fields in parallel, and the Action will foster cross-fertilisation between these through the meetings, workshops, and in particular the STSMs.

Specifically, this Action will develop and encourage:

- Translation of methods from model laboratory organisms to genuine food spoilage strains, pathogens, and strains used in industrial bioprocesses
- Leveraging areas of expertise in one applied field (e.g. the use of organic acids as food preservatives; lab-based evolution of acid tolerance) into others (e.g. the use of organic acids in clinical practice; development of improved acid tolerant strains for bioprocesses)
- Development and wider application of novel methods of single cell imaging, to more accurately describe events at the cellular level that take place under low pH environments (e.g., changes in internal pH, changes in redox potential across membranes)
- Application of novel whole systems and genomics methods like Tn-seq to organisms of industrial and clinical interest
- Determination of organismal behaviours at the whole population level, particularly where population heterogeneity is relevant, using methods such as flow cytometry
- Modelling of events that take place at membranes, key barriers to strong but not weak acid movement, through greater understanding of the properties of membranes
- Predictive mathematical modelling of population behaviour based on improved understanding of the properties of whole populations and the individual cells within them, including heterologous communities of mixed organisms.

This list is not however exhaustive, as participants in the Action will be strongly encouraged to monitor and report on new approaches in their respective fields at all COST events and through the social media networks the Action will establish. In addition, the Action will use the network to highlight specific problems that different applied sectors want to address, but where they may lack expertise or even be unaware of relevant developments that could help them in this and will leverage its high level of inter- and cross-disciplinary expertise to identify novel approaches and solutions to these. Overall, this may lead to the Technology Readiness Level in the fields affected by the option moving from levels 1 – 6 up to 3 – 8.

1.3.3. INNOVATION IN TACKLING THE CHALLENGE

The COST Action will maximise the chances of successfully tackling the questions outlined above, and others that may emerge during the course of the Action, while building capacity.

- It is specifically designed to break down walls between pure and applied fields (industrial, clinical, and food microbiology), and so to build bridges to research with more impact.
- Members of the network will have expertise in a highly pertinent and wide range of methods, including molecular biology, microbial physiology, membrane biology, high throughput omics methods, fermentation technology, imaging and single cell analysis, biophysics, cytometry, industrial biotechnology, food microbiology, clinical microbiology, and mathematical modelling. They work on the complete range of microbes from Gram negative and Gram positive bacteria to fungi (including studying the interactions between them, for example in mixed infections), and with organisms from laboratory models to pathogenic clinical isolates and highly engineered or adapted industrial strains.
- Knowledge and expertise in these different areas will be combined and awareness of methods and approaches will be raised, through the workshop, conference, STSM, and training activities, the compendium of expertise, and use of open access data repositories.
- The network will draw in interested parties from industrial sectors and direct some of its activities at problems that they specifically identify, as described in the Impact section below.

1.4. ADDED VALUE OF NETWORKING

1.4.1. IN RELATION TO THE CHALLENGE

At the proposal stage network members were working on a range of organisms, asking different questions and using different methods. However, they share a common goal of understanding the impacts of low pH on microbial behaviour, whether this understanding is based in a mechanistic model, a predictive model, or an approach to improve food protection, clinical efficacy of a treatment, or an industrial process. Because of the range of topics covered and questions asked, there is very significant potential for synergy by bringing these people together to exchange ideas, develop new partnerships, and share techniques.

Added value from the Action will be particularly important in four ways.

- First, the different sectors shown above in Figure 1 (which may broadly be referred to as production, prevention, and treatment) overlap significantly in the biological phenomena that underpin their study. However, people who work in them do not read the same journals or attend the same meetings; they are unlikely to recruit students trained in one area to work in another. This significantly reduces the potential for interaction between sectors. This Action will specifically develop such interactions.
- Second, many of the questions posed above will need to be addressed using combinations of techniques. As methods develop and become more technically challenging, the number of people who can cross disciplinary boundaries grows smaller. By focusing on crossing traditional disciplinary boundaries, new scientific insights will emerge.
- Third, it is hugely important for the future of the bioeconomy that students and ECIs do not become over-specialised in their training. A thriving scientific enterprise requires the constant injection of new ideas and methods, and by encouraging training through this Action, it will have added value in the field of microbial responses to low pH and in fields beyond that where these concepts and methods can be applied. For students based in academia this COST Action will provide them with an opportunity to understand the challenges facing industry and help them to shape their research focus as well as improving their future employment prospects.
- Fourth, a key part of the Action is involvement of the Inclusiveness Target Countries (ITCs) and the development of new pan-European collaborations. This will help to bolster and develop scientific expertise in the ITCs, to the benefit of their economies and educational sectors, while making their expertise more widely available to the European community as a whole than is currently the case.

1.4.2. IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

There are three particular areas where efforts in this Action will contribute to important applied developments which are happening at both European and international level, in addition to the general development of high quality European scientific research. A careful search has no evidence for other overlapping COST Actions. There also do not appear to be any similar initiatives in this particular field outside the Europe, so this network genuinely has the potential for global, as well as European, impact.

The first is in the development of a more sustainable economy. This is best exemplified by the circular economy[1], where waste from one process is used as raw material for another, and where the use of organisms (whether natural or engineered) can enable a more efficient production processes than would be possible by chemical means. The use of engineered micro-organisms is key as the traits needed are unlikely to reside uniquely in species that can be isolated from the natural environment. A key trait that will need to be enhanced is resilience to variations in pH, in particular the ability to grow and carry out metabolic processes at low pH. This will enable the enhancement of the i) production of organic acids with greater efficiency, ii) exploitation of enzyme activities acting in the low pH range to generate bulk chemicals in an environment which is less susceptible to growth of contaminating micro-organisms, iii) employment of natural polymers of microbial origin that can easily allow the entrapment of whole cells and proteins in order to carry out their activity in the low pH range (iv) the implementation of biorefineries, which are currently limited by the amounts of acetic acid present in lignocellulosic hydrolysates.

The second is in the area of food safety and food preservation, which continues to be a core challenge to food producers across Europe. Often empirical approaches are taken to food formulation that are expensive and over-engineered. As the demand for minimally processed foods increases there is a growing demand for “natural” preservation regimes that harness naturally occurring compounds, including organic acids produced by microbes as waste fermentation products[6]. This Action will develop understanding of how pathogens and spoilage organisms respond to acid in order to support the rational design of improved food preservation. In the long term this approach will achieve improved food safety and give European food producers an advantage in world food markets through extended shelf life and perception of higher quality.

The third is in combatting the spread of AMR among fungal and bacterial populations. This has been identified as a major economic and social threat in several studies commissioned at national and international level, including by the EU[2]. The use of organic acids as antibacterial is widespread in food and drink manufacture but is poorly developed in clinical applications. In order to make it more robust, more needs to be learned about the mechanisms of growth inhibition and killing of bacteria and fungi by organic acids, and the extent to which pathogens may already possess or may evolve resistance to them. Novel agents that inhibit the development of the acid stress response may also be developed.

2. IMPACT

2.1. EXPECTED IMPACT

2.1.1. SHORT-TERM AND LONG-TERM SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS

Short term impact:

- Increased contact and scientific interactions between distinct areas in pure and applied microbiology, which will facilitate expertise transfer and development of joint projects
- Training of students and ECIs in specific new techniques (single cell methods, modelling, food microbiology, ‘omics methods), to enhance research activity, improve their mobility, research expertise and employability
- Increased awareness of importance of and applications of acid stress in micro-organisms in academic, industrial, and health care circles
- Improved links with ITCs and labs elsewhere in Europe
- Improved open access to data and information on acid responses in micro-organisms, of value to academics, clinicians, and industrialists

Long term impact:

- Improved practices in food microbiology, leading to more efficient methods for food and drink preservation with reduced load of “artificial” chemicals and preservatives
- Improved potential in industrial microbiology to enable catalysis of processes at lowered pH and improved production of chemicals via microbial fermentation at low pH, which will enhance the development of the sustainable bio-economy
- Improved practices in engineering problems (control, scale-up) in fermentation technology, in particular under low pH stress.
- Enhanced understanding and improved practice in medical microbiology including use of organic acids as anti-bacterial agents, with potential in tackling AMR
- Improved understanding of fundamental aspects of microbial stress responses
- Raise the competitiveness and impact of European science internationally
- Integration of ITCs into strong European research networks

2.2. MEASURES TO MAXIMISE IMPACT

2.2.1. PLAN FOR INVOLVING THE MOST RELEVANT STAKEHOLDERS

This COST network addresses an issue which has ramifications in multiple sectors and ensuring the involvement and commitment of the most relevant stakeholders has therefore been a key aim in establishing the network. At the proposal stage it already included representatives from relevant industries (such as Food Microbiology and Industrial Biotechnology), and from government institutes and contract research organisations (CROs) involved in food safety assessment and research, as well as academics from a very wide range of relevant disciplines. Activities such as conferences and workshops to be held as part of the Action will be widely promoted by working with professional bodies such as Federation of European Microbiology Societies, the European Federation of Biotechnology, and the European Technology Platform (ETP) Food for Life. Clinical microbiologists will be engaged through organisations such as the European Burns Association and via the high-profile annual European Congress for Clinical Microbiology and Infectious Diseases. The Action will use extensive personal contact to ensure the outreach to relevant academic and non-academic stakeholders is as efficient and comprehensive as possible. By this multipronged approach the Action will identify and recruit members from relevant industries and other stakeholder bodies to the COST Action. The Action will ensure that both academic and non-academic partners in the network are closely involved in the running of the network activities via the Working Groups described below in 3.1, which will help ensure their commitment to the network. The involvement and commitment of all relevant stakeholders will be specifically monitored by the Action Management Committee.

2.2.2. DISSEMINATION AND/OR EXPLOITATION PLAN

Effective dissemination of the activities and findings of the Action will be implemented through the work plan for WG6, in accordance with COST guidelines and recommendations. Reviews for the scientific literature, plus articles in professional and trade journals, will provide a route for disseminating news and research outcomes, and the contribution of the COST network to these will always be clearly identified. All research papers will be Gold open access. WG6 will run the website that will contain all details of the Action, and host feeds on outlets including LinkedIn, ResearchGate, and Twitter. The Action will work with the press offices of participating institutions to prepare releases about significant events and outcomes from the Action, ensuring its activities are brought to the attention of the widest possible audience. The main dissemination events will be the opening and closing conferences which will enable relevant stakeholders and scientists to become aware of the Action at its outset, and to contribute to its subsequent progress, and to learn about the outcomes of the Action at its close. Network members will also be making regular presentations at other conferences and workshops where the activities of the COST Action will be showcased. To ensure the relevance of the network across Europe and to help maximise effective dissemination and impact, the Action will work with the European Food Safety Authority (EFSA), the European Medicines Authority (EMA) and the European Centre for Disease Prevention and Control (ECDC), and the EC Expert Group for Bio-based Products. By using their links into the areas of food safety, infection control, and industrial biotechnology respectively, the Action will improve the access to the communities of scientists and industrialists to whom this Action will be relevant.

Because of the broad and cross-disciplinary nature of the network, and the involvement of industries, government institutes, and CROs, it is anticipated that there will be ample opportunities for rapid

exploitation of the opportunities arising from the network. These will of course be the responsibilities of individual stakeholders and network members, but the Action will create opportunities to maximise their likelihood through the programme of conferences, workshops, and STSMs, where the Action will use structured activities (e.g., speed dating and industry-led fora) to break down discipline barriers. Progress in exploitation will be monitored through the activities of WG6 (see below).

2.3. POTENTIAL FOR INNOVATION VERSUS RISK LEVEL

2.3.1. POTENTIAL FOR SCIENTIFIC, TECHNOLOGICAL AND/OR SOCIOECONOMIC INNOVATION BREAKTHROUGHS

Historically, scientific innovation has almost always been the outcome of bringing diverse fields together, as the combination of new concepts and novel methods opens new routes to innovation. The Action has been deliberately structured so that the potential for new interactions is maximised. This therefore leads to confidence that new approaches to problems and bottlenecks in several different pure and applied areas of microbiology are likely to arise from the Action, and the potential for breakthroughs in one or more of these areas is high. Breakthroughs may come about at conceptual, methodological, or applied levels: any of these will be beneficial to participants in the Action. Examples of areas which are current bottlenecks in the field where breakthroughs may result include accurate imaging of the internal environments of microbial cells under acid stress; rapid identification of the potential for resistance to new treatments to evolve; translation of work on laboratory organisms to organisms of food, industrial, or clinical relevance; more effective computational modelling of events associated with low pH stress; and impacts of low pH on microbial communities as well as on individual species. Risks may arise through (a) technical obstacles to progress (b) organisational difficulties (c) financial issues. Detailed risk management plans for these areas are presented in 3.1.4 below. Briefly, (a) is hard to predict by its nature, but the involvement of many expert scientists with a high level of technical competence and high commitment to the network objectives, plus extensive networking, will minimise it. (b) and (c) will be minimised by the organisational structure described in Section 3, with the Management Committee overseeing progress and dealing promptly with any problems that may arise.

3. IMPLEMENTATION

3.1. DESCRIPTION OF THE WORK PLAN

3.1.1. DESCRIPTION OF WORKING GROUPS

The aims are to maximise the benefit of the cross-disciplinary nature of the network, to balance the research co-ordination and capacity building objectives, and to provide the best opportunity for developing research links, collaborations, and programmes that will move the field forward in the diverse areas described in 1.4.2. To do this the Action has been devised with a structure of six Working Groups, linked as shown in Figure 2. WG1 and WG2 will focus on integration of knowledge and expertise across the diverse fields of work; WG3, WG4 and WG5 will focus on applied areas but with strong representation from across all disciplines, and WG6 will work on maximising the inclusivity and dissemination of the Action. For each WG, a committee will be elected composed of a WG Leader (WGL), a Vice Leader (WGVL) and 3 members of the network.

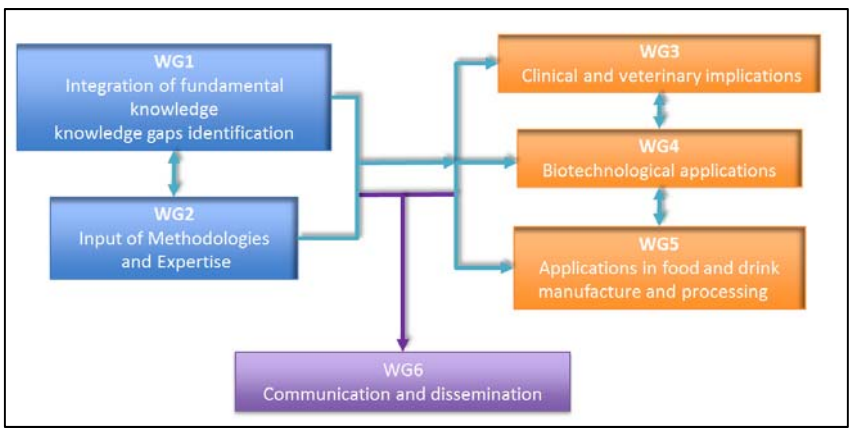

Figure 2: Links between the proposed Working Groups in this Action

Working Group 1: Integration of fundamental knowledge on impacts of low pH on micro-organisms and identification of knowledge and technical gaps

Rationale: To fully understand, influence, and manipulate the ways in which micro-organisms are affected by acid, whether it is to grow, to cease growth, or to be killed, requires an understanding of the categories of physical, chemical and biological events that result at low pH, and how the organism meets or fails to meet them. Comparative studies that list these challenges and evaluate what is known about how responses of different micro-organisms, particularly for organisms with important applications (industrial organisms, food spoilage organisms, pathogens), are very rare. This WG will integrate studies on different organisms in a holistic fashion across different categories. This information, made available via open access, will be a unique resource. It will flag up the areas where experimental detail is lacking or in conflict, and where methodological advances are required, stimulating further collaboration in ways that will benefit the applied topics.

Objective: to produce comprehensive, comparative, and fully referenced, descriptions of the key physical and biological changes that have been determined to occur in a group of model organisms upon acid exposure, and to identify and tackle knowledge gaps and areas where data from different sources may conflict. Organisms chosen will be ones that are well characterised and have specific properties, uses, or importance (e.g., resistance to low pH, pathogenesis, industrial application). These will include *Escherichia coli* (the best characterised organism, which has several inducible acid-resistance systems[7]), *Helicobacter pylori* (a well-studied acid resistant stomach pathogen[8]), *Brucella* spp. (causative agent of Brucellosis, a world-wide zoonosis, with inducible acid tolerance[9]), *Lactobacillus* spp. (acid tolerant, causes food spoilage but also used in some fermented foods[10]), *Saccharomyces cerevisiae* (the well-studied bakers and brewers years[11]), *Candida albicans* (a well-studied yeast pathogen able to tolerate a wide pH range for growth[12]), and *Kluyveromyces marxianus*, an acid tolerant yeast with multiple industrial uses[13].

Tasks and Activities: WG1.1: decide the critical variables that need to be looked at in order to categorise existing research data. WG1.2: gather, record, and integrate that information from all available sources. WG1.3: identify clear knowledge gaps and communicate these. WG1.4: make the data and its interpretation available as widely as possible in comprehensive and accessible form; in this task the activities will link up with those of WG6.

WG1.1 will agree on areas for study (e.g. ionic fluxes that follow pH change, membrane potential changes, measurement of cytoplasmic pH, 'omics data, identification of key genes through random or genome–wide screens, impact of pH on cell multiplication at the population and single cell level, etc.). Standardising this across organisms will facilitate comparisons between organisms and identification of knowledge gaps. WG1.2 will be achieved by WG members working to collate the available data in a pre-agreed format. WG1.3 and WG1.4 will consist of three types of activity. First, after collation the data will be deposited in the EU OpenAIRE data repository Zenodo, set out in a standard format and linked to the relevant original information sources. Second, a series of reviews on the current comparative state of knowledge for the organisms concerned will be written and submitted, targeting to widely read journals. Third, a series of shorter articles will be produced for more applied (including trade and clinical) journals, to act as an information source in their own right, and also as a pointer to the data on Zenodo, to reach a wider audience.

Milestones: M1.1: Initial meeting, decision about scope of work, agreement on standard formats, and initiation of gathering and integration of data. M1.2. Completion of data gathering. M1.3. Articles on selected areas submitted to applied journals. M1.4: Submission of reviews to high impact journals.

Deliverables: D1.1: White paper published on plans and invitation to other researchers inside and outside network to contribute. D1.2: Completed data set on Zenodo. D1.3: Publication of articles including reviews in professional and scientific literature on comparative analysis and concept/method gaps.

Working Group 2: Methodologies and expertise in the study of micro-organisms at low pH

Rationale: Many techniques are used in qualitative and quantitative studies on the effects of acid on micro-organisms, and no one lab is expert in all of them. Network members will possess a high level of expertise in many methods which are applicable to research on microbial responses to low pH, with considerable potential for research synergy. For this field to advance, it must involve the use of different

methods both to triangulate results from different approaches and to provide complete descriptions of physical and biological events. There is thus a need to raise awareness of the types of methods that are available in the different disciplines and what they can deliver, and to provide training in their use and application. Where methods are hard to transfer (for example because of the need for highly specialised equipment), opportunities for collaboration and laboratory placements need to be developed. This Working Group will address these issues.

Objectives: (1) plan and construct a comprehensive and sustainable open access database of the research expertise of all participants in the network, cross-referenced to their publications and to the information generated by WG1; (2) examine needs and opportunities for new technical advances (3) develop and implement training activities particularly for students and ECIs in specific methods.

Tasks and Activities: WG2.1: gather comprehensive information on members' expertise, methods not covered by the network, methods which are needed but not yet developed, and methods needed in industrial sectors; WG2.2: expand network by invitation to address WG2.1; WG2.3: oversee training s in key methodologies.

The Working Group will initially meet to design and implement an online survey of members' expertise, and to identify key areas that are not covered but are relevant to the Action, by cross-referencing with WG1. These will be brought into the Action by invitation. The group will look at questions that cannot currently be answered because of the lack of suitable methods. Companies will help identify potential methods that they would find most useful. The group will alert network members to the outcomes of these queries, to kick-start novel research areas. On the basis of responses, and activities in other WGs, suggestions for training activities will be solicited from network members, to raise awareness and boost expertise, particularly among students and ECIs. Network members will offer training in many areas including mathematical modelling, dynamic imaging of intracellular pH, analysis of population heterogeneity, use of 'omics methods to characterise key genes in stress responses, physiology measurements under bioreactor conditions, and lignocellulose biotechnology. Training (see GANTT chart) will be hands-on, lab or workshop based; events are expected to last from 3 to 5 days. A key feature of training schools is that they will be designed as much as possible to address issues across all applied sectors, as identified by WG1. Co-ordinators of training schools will be asked to condense key points into short lectures that can be added to the Action's YouTube channel for wider dissemination (see WG6). The availability of a compendium of methods will lead to more proposals for collaborative STSMs, and this Working Group will support and monitor these.

Milestones: M2.1: Complete survey of network members expertise M2.2: Map output of M2.1 onto key areas as they are identified by WG1.2. M2.3: Solicit and co-ordinate training activities in key areas from members of other Working Groups.

Deliverables: D2.1 publication on Action website of network members' areas of expertise.

Note: Working Groups 3 – 5 will apply ongoing work from WG1 and WG2 to specific applied areas. Their composition (which will be drawn from across the network) and the use of cross-disciplinary workshops will ensure high levels of cross-disciplinary activity.

Working Group 3: Clinical applications of microbial responses to low pH

Rationale: Microbial infections are a serious source of global morbidity and mortality and increasing AMR to antibiotics and antifungals is a severe health risk. Antibiotics and antifungals are poorly effective against biofilm growth, such as in wounds and on medical devices like catheters; these thus pose a risk particularly to ageing populations. A small amount of clinical use of organic acids (principally acetic and citric) against bacteria and fungi is being reported in the medical literature. The low pH of the vaginal epithelium, established by the native lactic acid-producing microflora, helps prevent colonisation by pathogenic fungi, and the use of lactobacilli as probiotics against thrush is starting to receive attention[14,15]. However, although organic acids as agents to reduce microbial spoilage in food have a long history, this work has barely translated into clinical usage. Understanding microbial responses to low pH is also relevant to tacking organisms that have to traverse human or animal GI tracts where exposure to varying pH and organic acid concentrations occurs, such as in Brucellosis, so there are also potential applications in animal husbandry and veterinary medicine.

Objectives: (1) evaluate the potential of organic acids as anti-microbial and anti-fungal agents through transfer of existing methods from food microbiology to clinical pathogens (2) develop new approaches

to studying the cellular consequences of acid (including organic acid) stress, from individual cells to populations and biofilms. An area of particular relevance to both WG3 and WG4 is the use of new methods for investigating the states of individual cells in populations.

Tasks and Activities: WG3.1: from the work of WG1 and WG2, and by working with stakeholders across disciplinary and sector boundaries, identify areas where method transfer will impact on clinical usage of organic acids; WG3.2: survey new methods of investigating individual cell states (e.g. flow cytometry, ratiometric pH-responsive dyes, microfluidics platforms), identify relevant challenges (e.g., heterogenous nature of biofilms, linkage of physical and biological parameters) WG3.3: identify and facilitate opportunities for STSMs to develop these. WG 3.4: evaluate and publicise outcomes of training visits.

This WG will host a cross-disciplinary focused workshop together with WG2 and WG5 in year 1 as part of the first open conference, bringing together food, clinical, and veterinary microbiologists, with presentations on new relevant technologies, to specifically identify approaches that may be applied in both WGs. The WG will then, organise and subsequently evaluate a series of STSMs to transfer expertise in these methods.

Milestones: M3.1: cross-disciplinary workshop with WG2 and WG5; M3.2: Online summary of novel methods for single cell study; M3.3 organisation of STSMs; M3.4: Evaluation and dissemination of STSM outputs.

Deliverables: D3.1: Online summary on Action web site of novel methods for single cell study; D3.2: Production of short methods papers based on STSMs and output of workshops.

Working Group 4: Biotechnological applications – exploitation of micro-organisms in low pH manufacturing processes

Rationale: As fossil fuel reserves decline, the search is on for sustainable and environmentally-friendly processes that can be used to replace them in fuel and polymer production. Microbial fermentation of sugar- and starch -containing raw materials, and lignocellulosic streams, will play a key role in this shift. Susceptibility of microbial cell factories is in this context of major importance. Organic acids are an important group of polymer precursors and their production is preferred at low pH. However, lignocellulosic streams contain large amounts of acetic acid, which is inhibitory for many micro-organisms[16]. It is essential for the further development of bioprocesses to understand this inhibition, to enable rational engineering of micro-organisms for low pH bioprocesses. Research on environmental stress and survival strategies of micro-organisms is, however, quite fragmented. Better interaction between groups working on different types of cell factories has the potential to accelerate biotechnological innovation.WG4 will combine groups and knowledge from basic understanding of microbes and application up to industrial level.

Objectives: (1) Exploit knowledge of microbial responses to low pH collated by WG1 to enhance the robustness of micro-organisms used in biotechnology. (2) Identify gaps in knowledge (including in engineering and implementation) with special regard to industrial microbiology. Propose and promote initiatives to fill these gaps. (3) Link researchers in the field of low pH resistance with researchers in the field of industrial microbiology, particularly through helping to foster industry-academic partnerships.

Tasks and Activities: WG4.1: Compare mechanisms and identify key areas where method and knowledge transfer can have a positive impact on micro-organisms involved in biofuels and biopolymers production; WG4.2: Identify and facilitate opportunities for training and research by STSMs and training schools, to promote strategies to exploit new ways of approaching low pH tolerance. WG4.4: Evaluate and disseminate outcomes from WG4.3 activities.

This WG will host a cross-disciplinary focused workshop in year 1 at the first open conference, to bring together researchers in the field of biofuels and biopolymers production with those who work on the effects of low pH on microorganisms, to specifically identify approaches that may be applied in the field of industrial microbiology. The WG will then organise and implement a series of STSMs and propose training schools to WG2. With support from WG6, the results will be made available widely through the appropriate channels.

Milestones: M4.1: Cross-disciplinary workshop on acid tolerance in bioprocess organisms; M4.2: STSMs for areas relevant to industrial bioprocesses; M4.3: Reporting outcomes of STSMs.

Deliverables: D4.1: Report from workshop, circulated to relevant industry and policy bodies.

Working Group 5: Applications in food and drink manufacture and processing

Rationale: Many bacteria and fungi are found in raw food ingredients. To ensure microbiological safety and shelf life of finished foods and beverages, the food industry uses processing and preservation methods to limit survival and growth of pathogenic and spoilage organisms. These "hurdles" include reduced water activity (osmotic stress), reduced temperature (cold stress) and low pH through the addition of weak carboxylic acids (acid stress)[4]. Despite the efficacy of acids at preventing the outgrowth of important microbial pathogens, some survive and compromise food safety or cause highly wasteful food spoilage. In addition, European consumers increasingly desire minimally processed "natural" foods. Thus, there is a need for effective food preservation systems that can rely on naturally occurring compounds, such as weak organic acids. However, the inhibitory modes-of-action of these acids are not well understood. Different microbes use different mechanisms to overcome these stresses, and evidence is accumulating that even identical cells can respond differently to the same conditions, increasing the challenge of effective preservation[17]. Moreover, spoilage micro-organisms in foods often behave differently from laboratory strains. Using novel methods to deepen our understanding of inhibition caused by weak acids, and how food-borne microorganisms respond to them, is central to developing new, more effective preservation regimes.

Objectives: 1. review the limitations in the understanding of acid-based preservation systems that are currently in place and assess the potential of the novel methods explored by in WG2 to develop improved methods; 2. support training and STSMs to introduce new methods into the food preservation field. 3. refine current predictive models and propose and explore routes for new modelling techniques that incorporate data from the application of novel methods, to enhance food safety and integrity.

Tasks and Activities: WG5.1: With input from the relevant food industries, define the current limitations with acid-based preservation systems and produce a list of areas where technical advances are needed to overcome them. WG5.2: in a cross disciplinary workshop, map the output of WG2 onto the list from WG5.1, to define areas where training and method development will be useful. WG5.3: write articles to summarise the output of this workshop, to raise awareness of network activities WG5.4: Organise training schools and promote STSMs in lab methods and predictive modelling to transfer the relevant methods to the food preservative field.

Milestones: M5.1. Workshop to define limitations of current methods, and to link desired advances to the novel methods being considered in WG2. M5.2. Preparation of articles and reports on workshop activities. M5.3. Training schools and STSMs to facilitate technology and methods transfer.

Deliverables: D5.1. Articles summarising workshop activities, for publication in food preservation trade journals, for report to ETP "Food for Life", and for wide dissemination via Action website. D5.2. Publication of articles in scientific literature and presentations at conferences describing applications of novel techniques and their application in predictive models to problems in food preservation. D5.3. Practical implementation of methods in relevant industrial sectors.

Working Group 6: Communication, dissemination

Rationale: The success of the Action depends equally on high quality work being done while it is taking place, and on reaching the right targets for maximum impact, so that advances developed during the Action are communicated to the right people during the Action and after its completion. This Working Group will be given the overall task of ensuring that the second point is met in full.

Objectives: (1) develop and maintain a list of contacts in academia, research institutes, industry, clinical practice, professional organisation, and policy bodies, who are kept informed of the Action and of all open events that it organises; the list to be kept in accordance with European data protection legislation. (2) develop and implement a comprehensive dissemination strategy to ensure information arising from the Action is made available for maximum impact.

Tasks and Activities: WG6.1: assemble a continuously updated database that lists contacts with their details and reasons for interest in the work and the outputs of the Action. This will be kept fully secure and comply fully with data protection legislation. WG6.2: devise and implement a dissemination

strategy, with the help of institutional press offices. A key component will be a dedicated web site for the COST action, together with a full publication and social media strategy.

Milestones: M6.1. Production of the contacts database M6.2. Development of the Action web site, YouTube channel, and social media presence M6.3. Full implementation and tracking the effectiveness of the dissemination strategy

Deliverables: D6.1. A contacts database will be held by the Action in a secure part of the web site, and regularly (at least twice annually) updated by WG members. D6.2. The web site which will be an online mechanism for co-ordination of the Action, and a "public face" to disseminate information about network activities as widely as possible. It will feature information about the Action including a description of past and pending activities and outputs of all the WGs, links to participating institutions, and calls for STSM proposals.

3.1.2. GANTT DIAGRAM

		year 1				year 2				year 3				year 4			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
WG1	WG1.1			M1.1													
	WG1.2						S		S		M1.2						
	WG1.3									X				MG1.3			
	WG1.4																M1.4
WG2	WG2.1					M2.1											
	WG2.2									X		M2.2					
	WG2.3								T		T		T		T		M2.3
WG3	WG3.1					FW	M3.1										
	WG3.2							M3.2									
	WG3.3							S			S		S	M3.3			
	WG3.4																M3.4
WG4	WG4.1					M4.1											
	WG4.2					FW			S			S		S	M4.2		
	WG4.3																M4.3
WG5	WG5.1						M5.1										
	WG5.2					FW	M5.2										
	WG5.3									M5.3							
	WG5.4							S			S		S		S		M5.4
WG6	WG6.1					M6.1											
	WG6.2								M6.2								
	WG6.3																M6.4
WG meetings		X				X				X				X			X
open conference						X											X
m'gement committee		X				X				X				X			
Key:	X = meeting		S = STSM		T = training school		FW = focussed worksho			M = milestone			yellow = period of activity				

3.1.3. PERT CHART (OPTIONAL)

3.1.4. RISK AND CONTINGENCY PLANS

Risks may arise from technical, organisational, or financial issues.

- **Technical risks** imply difficulties in transferring methods between different fields, and from model organisms to less well-characterised ones, or unanticipated problems in developing novel approaches. These may also have an impact on the ability of the network to exploit findings into new applied fields.
- Likelihood – medium to low
- Contingency plan - The network has a very wide range of technical expertise plus much experience in running research projects, and the regular meetings will provide opportunity for people to share ideas and approaches in overcoming technical hurdles. The Leader for each WG will have responsibility for monitoring technical difficulties and will be able to call on assistance of any other WG if required. Finding that a particular method does not translate well to a different area would be a disappointment but would be an important finding to report, and one role of the Action will be to ensure that the wider community is as aware of technical

challenges as they are of successes; this will help to stimulate further research while avoiding unproductive duplication of effort.

- **Organisational risks** mean the impact of unforeseen consequences on events organised by the network that could lead to an inability to deliver a planned event. These could include illness, absence for parental leave, or other responsibilities meaning individuals have to relinquish or are unable to take up roles in WGs. They could also include disagreements over strategies for individual Working Groups to follow.
- These by their nature are hard to predict, so they are assigned a likelihood of medium.
- Contingency plans - WG Leaders will be responsible for monitoring progress in planned events and in STSMs and workshops, and organisers of these will be required to deliver short summaries shortly after event for the Action website, as well as writing longer pieces for dissemination. WG Leaders will alert the Management Committee to any problems with delivery, and wherever possible alternative equivalent events will be organised. The level of expertise and commitment in the network, and the presence of Vice Leaders for each Working Group means that it should always be possible to find individuals able and willing to take on roles within the WGs. Any disputes within WGs will be settled using COST-approved procedures as agreed in the Memorandum of Understanding.

- **Financial risks** include over-spending of budgets or insufficient funds being available for planned events.
- Likelihood - low
- Contingency plans – This risk will be minimised by careful setting and scrutiny of the annual budgets and progress of the WGs by the Management Committee. Organisers will always be told the details of COST Action rules, and to maximise cost effectiveness will be encouraged when possible to leverage COST funding with additional resources e.g. from their own institutions.

3.2. MANAGEMENT STRUCTURES AND PROCEDURES

The Action will be managed in line with official COST procedures. The Management Committee (MC) will be convened on an annual basis to oversee the progress of the Action, plan the annual budget allocation and work programme in line with the needs of the Action, and ensure the Working Groups are co-ordinated. In accordance with COST procedures the MC will consist of representatives from the participating countries. Chair and Vice Chair of the MC will be elected by majority voting. The MC and the WGs will meet annually, and meetings will also be linked to conferences and Focused Workshops when possible to keep travel costs down. A Steering Committee consisting of the Chair, Vice-Chair, and WG Leaders will look after day-to-day management of the Action and will work together online and by telephone to co-ordinate activities.

Leaders and Vice Leaders for each of the six WGs will be chosen by majority vote, with ECIs, members from ITCs, and females being strongly encouraged to stand in accordance with COST Policy on inclusiveness. They will take responsibility for implementing the proposed actions of each WGs, for modifying them as necessary as the Action proceeds, and for monitoring milestones and deliverables. They will handle IP issues in accordance with COST rules, involving and informing the MC if any issues arise. Posts will be rotated between Leaders and Vice Leaders as the Action proceeds, maximising training development opportunities for the more junior participants.

3.3. NETWORK AS A WHOLE

In assembling this network, there has been awareness of the very cross-disciplinary nature of the work that needs to be done, and the need to bring people on board from a range of backgrounds with complementary and relevant expertise. In doing this the Action has sought out partners from universities, CROs, and industry, and as the issues being addressed apply across Europe, made the geographical spread as wide as possible without losing the coherence of the group. The network is planned to have a good balance and significant relevant expertise in the group, including between prokaryotic and eukaryotic, single cell and population, molecular and physiological, and in particular between pure and applied research. Several industrial partners expressed a keen interest in joining the network. The network has a good critical mass and excellent breadth of relevant expertise for success of the Action.

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