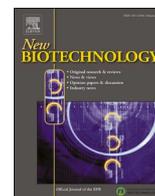


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A COST Action on microbial responses to low pH: Developing links and sharing resources across the academic-industrial divide

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ABSTRACT

We present work of our COST Action on “Understanding and exploiting the impacts of low pH on micro-organisms”. First, we summarise a workshop held at the European Federation of Biotechnology meeting on Microbial Stress Responses (online in 2020) on “Industrial applications of low pH stress on microbial bio-based production”, as an example of an initiative fostering links between pure and applied research. We report the outcomes of a small survey on the challenging topic of developing links between researchers working in academia and industry that show that, while people in different sectors strongly support such links, barriers remain that obstruct this process. We present the thoughts of an expert panel held as part of the workshop above, where people with experience of collaboration between academia and industry shared ideas on how to develop and maintain links. Access to relevant information is essential for research in all sectors, and because of this we have developed, as part of our COST Action goals, two resources for the free use of all researchers with interests in any aspects of microbial responses to low pH. These are (1) a comprehensive database of references in the literature on different aspects of acid stress responses in different bacterial and fungal species, and (2) a database of research expertise across our network. We invite the community of researchers working in this field to take advantage of these resources to identify relevant literature and opportunities for establishing collaborations.

1. Introduction

Since 2009, the European Federation of Biotechnology (EFB) has run a successful series of meetings around the topic of Microbial Stress Responses. The meetings have taken place in Austria (2009), Italy (2012), Spain (2015), Ireland (2018) and online (2020, due to the COVID-19 pandemic). These meetings gathered a vibrant scientific community around this topic, working on different microorganisms and from various perspectives, both in fundamental and applied sciences [1–3]. Following the 2018 meeting, a group of researchers with a shared

interest in the ways in which bacteria and fungi respond to treatment at low pH successfully applied for funding to establish a COST network in this research area. As its name implies, the COST (European Co-operation in Science and Technology) organisation funds research networks that link researchers at all stages of their careers and across diverse sectors, to work together on research problems of mutual interest, recognising that such research proceeds best when it is interdisciplinary, collaborative and inclusive at all levels. COST agreed to fund the network for four years and as a result the COST Action CA18113 on “Understanding and exploiting the impacts of low pH on

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micro-organisms” was launched in April 2019. From the start, it was recognised that the chosen research topic cuts across many areas of both pure and applied microbiology; indeed, this was the major justification used for setting up such a network, as part of the aim of the Action was to help beat down the barriers that often exist between people working on the same topic but in different fields or sectors. Thus, on the one hand, people interested in preservation and the avoidance of spoilage in food and beverage production often use acids, particularly organic acids, as an effective antimicrobial [4]. On the other hand, researchers in biotechnology may be seeking to develop organisms that can tolerate these same low pH conditions for more efficient bioprocesses [5,6]. At the same time, low pH can be an important antimicrobial treatment that has medical and veterinary applications, and the ability of some pathogens to survive low pH barriers can be an important determinant of their pathogenicity [7–11]. It was believed, when establishing the network, that different groups of researchers - one using low pH to try to prevent the growth of a microorganism, the other looking for ways to engineer microorganisms to grow better at low pH – are basically interested in the same underlying processes and can hence benefit from sharing concepts, methods and expertise. This overall idea that underpins the Action is illustrated in Fig. 1A. Several Working Groups (WGs) were therefore established in the Action to cover these different sectors, but also to find ways of getting them to work more closely together. These WGs and the way they are interlinked are shown in Fig. 1B.

1.1. The workshop on industrial applications of low pH stress on microbial bio-based production

At the EFB meeting on Microbial Stress Responses that was held online in November 2020 (organised by the Sapienza University of Rome), Working Group 4 (Biotechnological applications – exploitation of micro-organisms in low pH manufacturing processes) held a workshop on the topic above, which is briefly summarised here. The workshop was generously sponsored by m2p labs.

Two case studies based on experience with yeast cell factories were presented by Paola Branduardi (University of Milano Bicocca, Italy) in the presentation “Yeasts coping with organic acids: lessons and potential solutions”. Microbial diversity was studied to unlock the potential of a yeast, *Zygosaccaromyces bailii*, which is naturally able to withstand organic acid at low pH, to give improved production of lactic acid.

Cellular rewiring was also used to improve the robustness of the yeast *Saccharomyces cerevisiae* against acetic acid released from lignocellulosic biomasses, by the modification of a hub element. Such approaches can help to promote an effective and viable implementation of microbial-based processes at industrial level [12,13].

Jana Sedlakova-Kadukova (University of Ss. Cyril and Methodius in Trnava, Slovakia) discussed bioleaching as an effective, environmentally friendly and cost-efficient approach for metal dissolution from various sources using acid-producing micro-organisms. The process is widely used in the low-grade ore processing industry and its importance is growing in the waste treatment sector. Bioleaching offers several advantages, but it suffers from limitations which hamper its wider application for waste processing. Among them, slow leaching rate and metal ion toxicity for bacteria, are the most challenging. The ways in which these limitations can be overcome were discussed [14,15].

Merve Atasoy (formerly at KTH, Royal Institute of Technology, Stockholm, Sweden; presently at Wageningen University and Research, Netherlands) introduced the effects of different pH conditions (pH5, 8, 10 and without adjustment) on volatile fatty acid (VFA) production, acid composition and microbial community profile in the presentation “Mixed culture fermentation for volatile fatty acids production from waste streams under acidogenic conditions”. She showed how in VFAs, which are valuable feedstock for biofuels and bioplastic production, the composition was changed by pH and VFA production was positively affected by the high relative abundance of *Firmicutes*; a negative correlation was seen with the relative abundance of *Chloroflexi* [16,17].

Mustafa Turker (Pakmaya, Turkey) discussed potential opportunities of ameliorations in low pH response in terms of industrial needs and challenges. Low pH, which interferes with pH gradients and proton motive force across cellular membranes, influences many physiological functions and can affect industrial bioprocesses deleteriously. Thus, from the industrial point of view, it is crucial to learn how to cope with acid stress. Although much work exists on elucidating the acquired homeostatic mechanisms in model organisms and lab conditions, translation of this knowledge to improve industrial processes is rather limited. He highlighted industrial demands from an economical and technical perspective, with examples from food, environmental and industrial biotechnology in a presentation titled “Low pH in food and industrial biotechnology: opportunities and potential applications” [18, 19].

Lucian Staicu (University of Warsaw, Poland) in the presentation

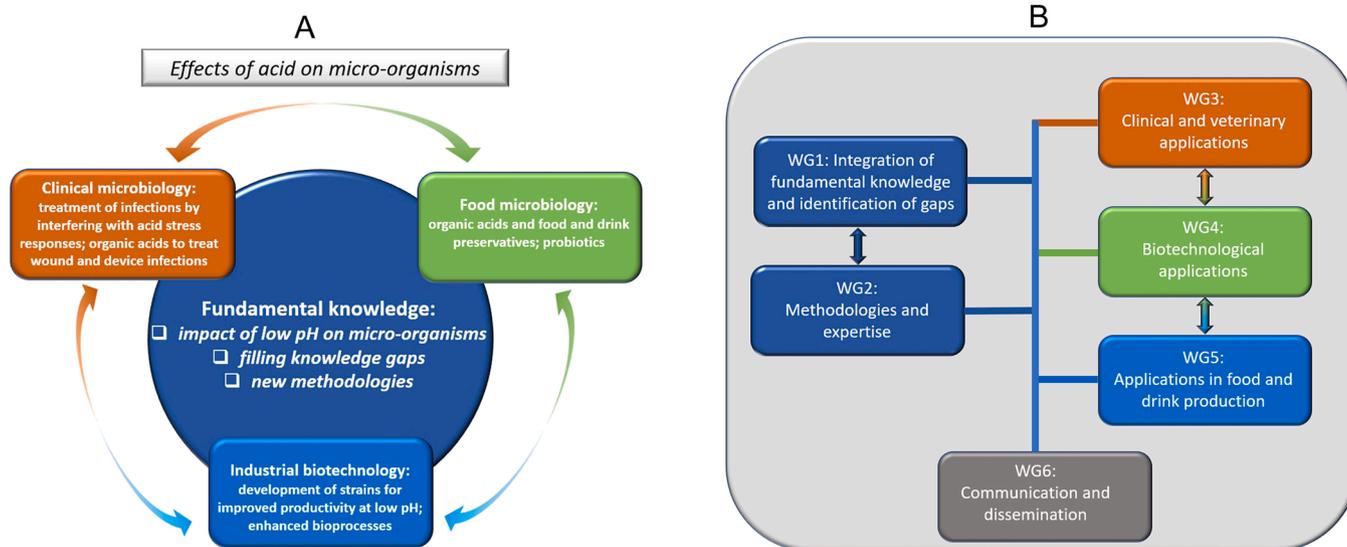


Fig. 1. The structure of COST Action CA18113: Understanding and exploiting the impacts of low pH on micro-organisms. A: Conceptual basis of the Action: Fundamental knowledge on microbial responses to low pH is critical for several different applied areas. B: Areas of activity and relationships of the six working groups in the Action.

titled “Metal stress - biomineral remedy” introduced bacteria *Bacillus* sp. Abq, isolated from an aquifer in New Mexico, USA, which was able to precipitate Pb(II) by using cysteine into PbS (galena). He discussed biochemical pathways leading to the biomineral formation and highlighted the importance of the study for PbS recovery from secondary resources, such as low pH Pb-bearing industrial effluents [20,21].

The presentation “Influence of acidic stress on biohydrogen production from biowaste in dark fermentation process” by Adam Cenian (Polish Academy of Science, Poland) focussed on the role of acidic stress in enhancing biohydrogen production. The growing demand for renewable energy sources is increasing the interest in new and sustainable sources of molecular hydrogen. He showed that microbial stressing of mixed microbial communities plays important role in dark fermentation (which results from the action of obligate and facultative anaerobes in the absence of light and oxygen). pH control has a strong positive effect on hydrogen generation, while microaeration supports hydrogen generation and inhibits methane generation [22,23].

1.2. Industrial applications of low pH stress on microbial bio-based production: a survey on the barriers across the academic-industry divide

One of the most important objectives of this COST Action is how to improve links between researchers who are working in pure or more applied areas and to do this the nature of barriers that often limit the extent of collaborative working needs to be well understood. Some are to do with simply not knowing what might be technically possible: many different methods from population modelling to high-throughput molecular approaches are used in this field and it is not easy for individual researchers to be good at all of them, or even to be aware that they all exist or how they might be applied to particular problems. Others are to do with what might be termed research culture: the drivers of activity can be very different between academia (where the focus may be on publication and grant income) and industry (where ultimately it is productivity and profitability that are important), not to forget Contract Research Organisations (where fulfilling contracts is crucial) or government research institutions, which may be more focussed on delivery on national programmes on monitoring and safety.

With the above in mind, CA18113 Working Group 4 conducted a survey to identify more clearly what the major barriers to interactions were and to get a sense of the willingness in different sectors to find ways of overcoming them. In association with this, they then convened a discussion panel as part of the workshop above, with several of the speakers having successfully worked across the academic-industrial divide. In this, the speakers drew on their own experiences in areas such as budgeting, IP, and communication, to discuss factors that are important in developing and maintaining such connections. The results of both of these are summarised below.

For the survey a series of questions, the most relevant of which are listed below, was sent to existing industrial contacts. The aim of this was to identify their sources of information about developments in their field, their willingness to collaborate with academics and the barriers to such collaboration. Although the survey was relatively small (nine respondents representing companies with a range of sizes) the results are nonetheless useful in providing a snapshot of issues that many other people have reported across the academic-industrial divide [24,25], particularly as responses were received from companies of all sizes from micro-companies to multinationals. Details of the individual companies are not given, for confidentiality reasons. Nearly all the questions in the survey allowed free text and some of the key findings are summarised below.

How does your company receive information on technical innovations in your field? – A range of information sources were identified, including academic journals, conferences, and collaborations. A very wide range of academic journals was used, from broad high-end to more specialised ones (examples given of the latter were Journal of Functional Foods, Beneficial Microbes and Journal of Dairy Science). In addition,

companies reported using trade journals and trade shows, patent searches and information from their customers, as well as from analysis of their competitors’ activity. Thus, in addition to publication in academic journals, writing articles for trade journals and attending trade shows are two ways in which academics could raise awareness of their work and build links with relevant industries.

Is your company a member of any professional organization(s) at European or national level? How useful do you find membership to be? – Most (though not all) companies were members of one or more professional body, finding them to be a useful source of information on innovations in their field and a way of building networks. The organisations mentioned were very diverse, including those representing biotechnology (Europabio), enzyme manufacture (Amfep), probiotics (IPA Europe), food safety and nutrition (ILSI Europe), food and feed cultures (EFFCA Europe) and many others. Engaging with such organisations is thus another potential route for academics to build links with industrial partners.

How often do employees of your company attend external training/workshops/conferences? How valuable do you find these? All companies agreed that such events were useful in building networks and staying up to date with developments in relevant fields, with attendance at such events occurring anywhere from monthly to annually. With the gradual return to face-to-face events after two years of online meetings, it has probably never been more important for scientists to use them for networking across different disciplines and sectors. COST provides funding to set up and run such events.

Would you seek the assistance of a university/research institute for aid in solving specific issues in areas such as (a) technology/quality/safety (b) technical innovation? Nearly all companies polled answered yes to these questions, irrespective of their size. The desire to collaborate is, however, greater in early stages of product development, at the pre-competitive stage or when new areas are being looked at. Companies were asked to estimate on a scale of 1 (unlikely) to 10 (extremely likely) their willingness to collaborate with academia at different stages of product development and the answers showed a drop moving from technology development (7.9) through problem solving (6.5) to process integration (6.2).

Has your company been involved in any externally funded projects? If yes, under what programme? (e.g. as a partner in an European Training Network, as a partner in a national innovation scheme). How useful has this been to you? Nearly all the companies polled had indeed participated in such projects, funded by a range of national and EU-level bodies and reported that some participation had been beneficial particularly through the building of better networks, as well as improving their own knowledge base and developing the capacity of internal R&D teams.

Could you describe the barriers that restrict cooperation between you and academic partners? The same issues came up repeatedly in responses to this question. Intellectual property rights were reported as a problem, because of different expectations of universities and companies over who should own them. The time needed to develop good links – to train academics so that they understand the business they are working with and navigating unfamiliar university systems – can be a barrier. So too is the familiar and thorny issue of confidentiality. Finally, despite the high level of participation in funded collaborative projects mentioned above, funding was sometimes cited as a barrier.

Some key points from the panel discussion are summarised in Table 1. This table also flags up some of the ways that the panellists suggested can be used to help build bridges between different ways of working and result in more productive interactions.

1.3. Two new resources for supporting further research collaborations in the field of low pH responses in micro-organisms

As stated elsewhere, understanding how microorganisms respond to acid pH is central to their control and successful exploitation [11]. When the COST Action was launched, a particular focus was the breadth of the

Table 1

Needs/requirements typical for academy and industry, and possible ways to bridge the gap between them.

Needs/requirements	Academy	Bridging needs	Industry
Process scale	Small scale	Academic research using larger processes/devices	Large scale
Material	Standardised lab-made feedstock	Companies to share feedstocks with academic researchers; recognise challenge of poor reproducibility	Industrial raw material, e.g. waste products
Language	Scientific	Define terms precisely, ensure that the relevant problems are identified and clearly articulated	Technical
View	Narrow – very focussed	Academics to look at industrial problems and look for practical solutions; industry to define problems in unambiguous terms	Broader view, pragmatic
Outputs	Publications, funded projects with a strong push to publish Open access	Funded students working in industrial environments	Products sold at profit
Time	Looking for deep understanding; may take longer view	Good communication to understand motivations on both sides	Solutions focussed: may be urgent
Funding	Research needs to be funded at full cost	Provision of pro rata bench fees/consumables budgets/resources by companies looking for collaborations	Companies may not appreciate full cost of academic research
IP rights	Patents delay publications and disclosure at conferences	Clearly define expectations at start of collaborations; academia to measure outputs in ways that support links	Tend to keep processes secret

research fields of its members and the extent to which it is hard for researchers (who may be drawn from academia, biotechnology, the food and beverage industry, from clinical and veterinary research and elsewhere) working on their own specialisations to be aware of data, concepts and methods that may be available elsewhere in the field, even though these things could be very valuable to them. With this in mind and with a specific emphasis on building more links between the disparate research topics and industries that members of this Action represent, two new resources were created by the Action for use of the research community: a database of literature in this area and a database of expertise in the Action. Both of these are ongoing projects in the Action, but they have now reached a sufficient level of development that it is now appropriate to share them more widely.

The acid responses literature database developed by Working Group 1. The literature on the impacts of low pH on microorganisms is vast, ranging from empirical observations of how it affects growth through to detailed molecular descriptions of the underlying processes that take place when organisms are treated at low pH. There are many useful reviews on the topic, but no central curated resource existed where literature on different organisms, different acids and different methods could be searched and consulted. Such a resource was therefore created.

This was done by using a range of relevant search terms (e.g. acid stress, low pH response, acid tolerance, acid resistance, intracellular pH, pH homeostasis) to interrogate public search engines (Google Scholar, PubMed) for literature on the topic. Thus far the focus has been on specific groups of microorganisms, using the target genus names as search terms. These groups include the bacterial genera *Listeria*, *Bacillus*,

Streptococcus, *Staphylococcus*, *Lactococcus*, *Lactobacillus*, *Leptospirillum*, *Thiobacillus*, *Acidithiobacillus*, *Escherichia*, and *Salmonella*. Additionally a number of eukaryotic genera including *Saccharomyces*, *Zygosaccharomyces* and *Aspergillus*, as well as the archaeal genus *Sulfolobus*, have been included. The intention is to continue expanding the entries to include as many microbial genera as possible that have been studied with respect to their acid responses. Currently (July 2022) there are 566 entries in the database.

The inclusion criteria allowed for both research articles and review articles to be included if they contained information that was relevant to the topic. Articles were included only if they included some fundamental (i.e. molecular/structural/mechanistic) information on the mechanisms involved in sensing or responding to acid stress. Articles describing the effects of either strong or weak organic acidulants were included. Descriptive articles that merely contained information on the growth of microbes in acidic media were not included in the database because it was deemed that they would not help to advance significantly the understanding of how microbes sense and respond to acid at a fundamental level. Including such descriptive articles would have increased the scope of the database to an unmanageable extent and also limited the overall usefulness of the database as a resource for understanding fundamental aspects of the behaviour of microbes under acid stress. In an attempt to partially quantify the relevance of each entry to the topic (defined as “how microbes sense and respond to acid at a fundamental level”) a score was assigned to each article from 1 to 5, where 5 was highly relevant and 1 was minimally relevant. Articles whose entire focus was on understanding the acid stress response scored 5, while articles where acid stress was only a small element of the study scored 1. These scores can be used to filter the entries in the database in order to retrieve the most relevant articles for a particular genus or acid type.

The process for constructing this data can be summarised as follows (Fig. 2). Articles identified as meeting the inclusion criteria were collected in the freely available bibliographic software Zotero (<https://www.zotero.org/>). The articles were then extracted into a .csv file, which captures all the article-related fields (including authors, journal, date etc.). Eight additional fields were added to the this file (relevance, genus species identifier, strains investigated, medium/matrix used, pH range investigated, acid type used, date added, person who created entry). The data was then uploaded to Firebase (<https://firebase.google.com/>), an app development platform backed by Google. This allowed the searchable database web-app to be developed. The firebase platform makes use of various functionalities that were implemented in this database: a. Firebase authentication [26]: flexible authentication service which can easily be implemented in additional future services connected to this database; b. The Cloud Firestore: a non-SQL database [27] contains inner control steps that verify uniformity between new data uploaded into the database and data already present within the database. In turn, it allows for future scalability of the database and functionalities on top of high speed in performing actions on the data regardless its size; c. Angular – a free and opensource web application framework.

The database is now publicly available via this weblink: <https://costeuromicroph.web.app/>. Anyone can access the database, but for a user to sign up they are required to enter a name, email address and password. Thereafter, login requires the email address and password to access the database. The database can be filtered using any of the fields associated with the entries (e.g. Author, Title, Abstract, Strain name, Relevance, Publication Year, Growth Medium/Matrix, pH range, Acid Type used, Genus). Alternatively it can be searched using a user defined search term. The user can also enter articles using an “Add New Documents” feature. The fields required are clearly defined and users are invited to include details that will provide added value to the database (e.g. strain names, media/matrix type, acid type, etc.). The newly added articles will be tagged for curation by the database administrator and the entries can be further edited by the curator if needed. The success of the database depends on its use by as many interested end users as possible

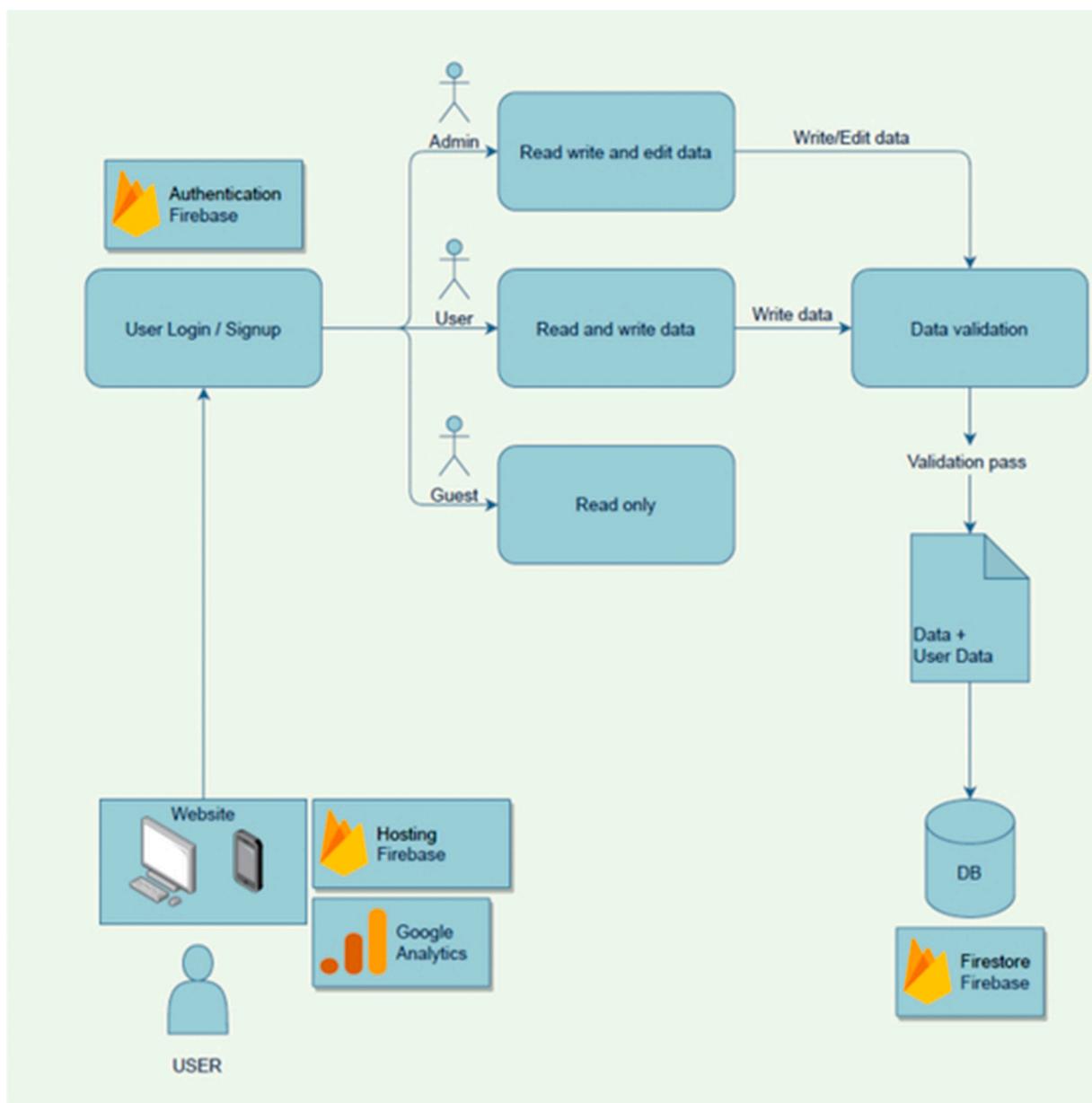


Fig. 2. Flow chart of operation of the Firestore database.

and on the expansion of the entries to cover as many microbial species as possible.

Compendium of expertise in COST Action CA18113 researchers developed by Working Group 2 This COST Action currently has nearly 200 members, the majority drawn from countries in Europe. Members work at universities, companies, research institutes and contract research organisations, working with a wide range of methods on many different organisms and tackling many different research questions. One of the ultimate objectives of the Action is to collectively identify gaps, whether conceptual or methodological, in the broad areas of research of interest to Action participants. It became clear early on that in order to do this, more detail was needed about what expertise could be found in the Action and where it was located. This knowledge could be of value not only to people in the Action, but also to other researchers who are not members of the Action but are looking to develop collaborations, whether short scale (of the type that can be supported through the Short Term Scientific Missions that the Action can fund) through to building consortia that could bid for large grants in the new Horizon Europe program. For this reason, a detailed survey of expertise in the Action was

undertaken and the output of this has been turned into a public resource on the Action website.

The objective of the survey was to map the expertise of the COST Action members with respect to their activity. This was in line with the over-arching objectives of the COST Action which are “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”. The data gathered by the survey is expected to facilitate communication between Action members and stakeholders towards initiating collaborations and exchange of knowledge. Furthermore, the hope is that the data can be used to facilitate the identification of gaps in methods and technology that are needed to advance the field of “low pH microbiology”.

The conduct of the survey included four stages:

- 1) Preparation: in this stage the survey was compiled. As the aim of the survey was to find out exact expertise of the people in the field, a set

of survey question-answer combinations were composed and developed based on personal feedback on the EuromicroPH website from Action members. Grouping the keywords and categorizing them into different question topics facilitated the development of the survey, which was then fine-tuned following feedback from the Action's Core Group. The survey had 6 main parts, as follows. 1: personal details; 2: questions related to Field of activity; 3: questions related to methods and technologies; 4: questions related to objects of interest; 5: questions related to action, mechanism and behaviour; and 6: additional background information (freeform text). Questions in Section 2 to 5 were multiple-choice selections, with additional options for adding freeform text. The survey was built in Google forms and was circulated by email.

2) Active survey phase: information about the online survey was sent to all members of the action. Active support was provided to participants during the survey when needed. The survey was circulated for

two months, during which reminders were sent twice. Overall 85 responses (over 50 % of Action members) were received.

- 3) The data were then downloaded from Google forms as an Excel file. Preliminary data curation was performed manually to remove corrupted or duplicated records. Then, the data was gathered, cleaned, curated, consolidated and processed using Python script to facilitate further analysis and to be able to communicate back the data to the action members as raw data. The resulting dataset included 79 records.
- 4) Data Visualization: For better communicating the results of the survey back to the community, selected data from the dataset was visualized using the online freeware visualization tool Tableau software (<https://www.tableau.com> [28]).

Eventually an interactive dashboard that enables visualization of the survey data was launched (example outputs are shown in Fig. 3). The

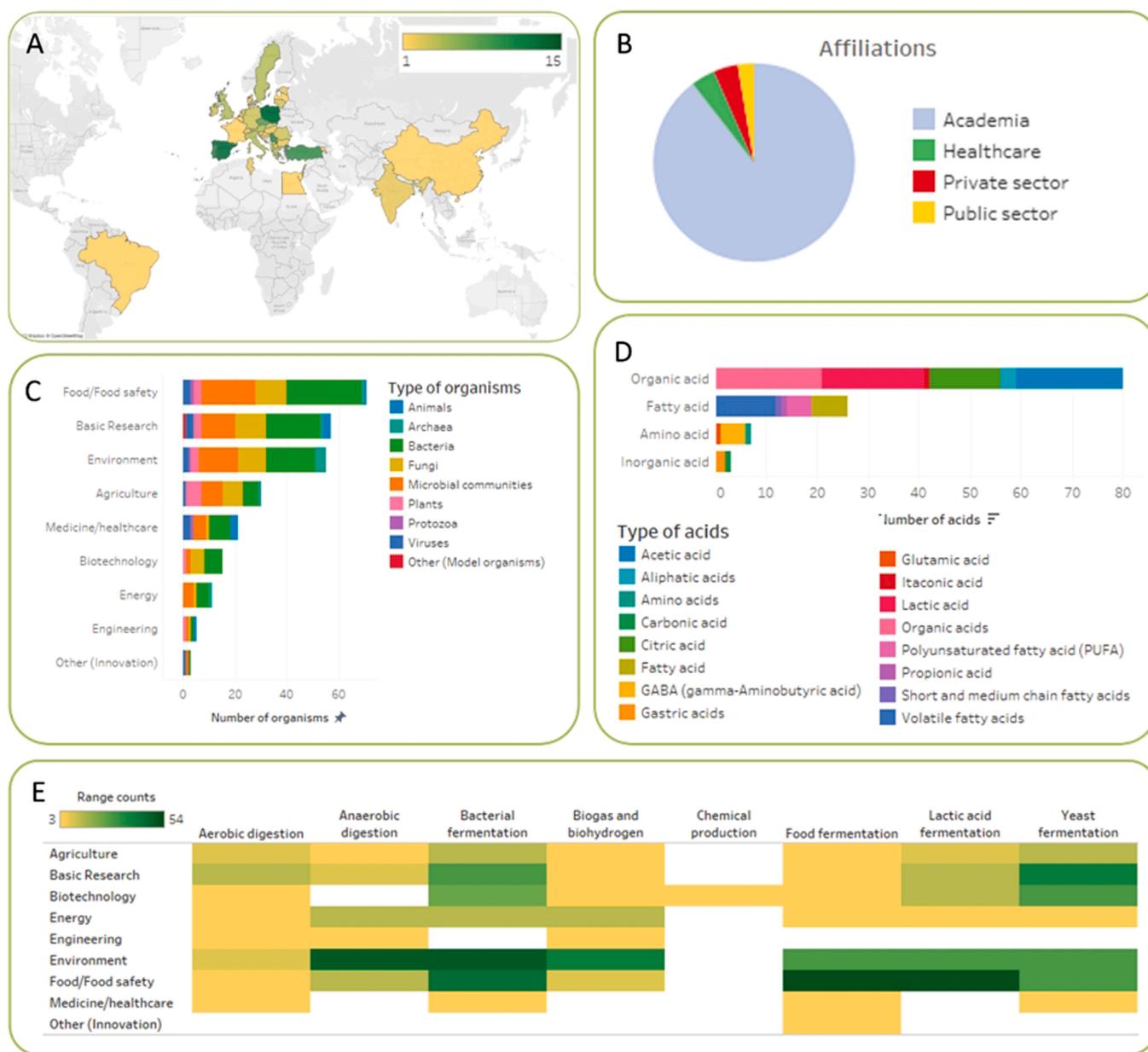


Fig. 3. EuromicroPH network with key characteristics and fields of interest, as presented on the Action website <https://euromicroph.eu/network/>. The network is spread all over Europe with collaborators worldwide (A), and is dominated by academic researchers (B). Main focus fields of network members are basic research, food safety and environmental research (C). Organic acids are the most popular area of study (D). Further expertise in the Action network, particularly in chemical production where there are relatively low levels of activity, continues to be sought (E).

dashboard includes the distribution of EuroMicroPH's network, affiliations, activities, environment parameter, acid of interest, type of fermentation and relation with industry. These results are presented as a Tableau visualization webpage and can be accessed online here: <https://euromicroph.eu/network/>, or on the "Network" tab of the web-site, euromicroph.eu.

The visualization also gives some insights related to the network. For instance, EuroMicroPH's network is spread across Europe, with particular strength in Poland, Serbia, Turkey, Portugal and Spain (Fig. 3A). Many people in the network come from academia (Fig. 3B). The commonest area of research or activity is in Food/ Food Safety (Fig. 3C), mostly dealing with acidic pH particularly with organic acids, reflecting their importance as preservatives (Fig. 3D). There are some gaps in the fermentation field, especially in chemical production (Fig. 3E). Moreover, EuroMicroPH's network has partners in industry, mostly in dealing with bioreactors, food safety and microbiological control. All of these data can be found in the link and can be filtered directly by clicking the figure. There are also some further filters that are available on the top of the dashboard. These filters can also be used to find specific expertise in the field. For example, the filter could be used to find whether EuroMicroPH's network has an expert that works in energy, dealing with microbial communities with fatty acids as the focus of their research. All of the data visualization can be downloaded as a PDF through the button at the bottom of the dashboard.

2. Conclusion

This COST Action on understanding and exploiting microbial responses to low pH involves many scientists across Europe and beyond with a very diverse range of affiliations, expertise, and interests, but with a shared commitment to the topic of the Action. The Action has made resources available to foster collaborations and deepen understanding in this field, and it continues to fund workshops, training schools, laboratory exchanges, and conference visits for researchers from countries with less well-developed science infrastructures. Details can all be found on the Action website <https://euromicroph.eu>. The Action, which will end on October 31, 2023, will help to build bridges between different scientific communities, and its participants look forward to more research progress in this field in the years to come for the benefit of increased knowledge/technological innovation, human health and the environment.

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Conflict of interest

The authors declare no conflicts of interest

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