Could phytogenic feed additives prevent bacterial resistance?

Antimicrobial resistance (AMR) is a considerable threat to public health worldwide. The related cost to the healthcare systems of EU/EEA countries is estimated to be around €1.1 billion per year. Based on the Antimicrobial Resistance Collaborators' predictive models (2022), an estimated 4.95 million deaths were associated with bacterial AMR in 2019, including an additional 1.27 million deaths directly attributable to bacterial AMR.

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Increasing antimicrobial resistance in pathogenic bacteria has created the need to develop novel therapeutic agents. Sandoval-Motta and Aldana (2016) suggested that by the year 2050, without new antibiotic development and research into the mechanisms of AMR, mortalities from cancer will be surpassed by infections due to AMR pathogens.

How do bacteria develop resistance?

The origin of genes for antibiotic resistance in bacteria is due to a natural process as a mechanism for their protection or generally due to spontaneous mutations in the bacterial chromosome.

Bacteria can show resistance to antibacterial agents through a variety of tools.

Alteration of target sites, active efflux of drugs, structural modification of porins, enzymatic degradations, and the destruction of an antibacterial agent by changing the chemical type are among the strategies bacteria employ to develop intrinsic resistance to antibiotics.

A significant component of the bacteria's ability to avoid antibiotic action is forming protective, resistant biofilms.

Multidrug resistance in bacteria occurs by the accumulation of resistance (R) plasmids, transposons, or genes, with each coding for resistance to a particular agent and/or due to the action of multidrug efflux pumps (EP), which can pump out more than one drug type.

Phytogenics as an alternative

Phytogenics are plant-derived preparations, including herbs, spices, and fruits, as well as preparations like essential oils or oleoresins, containing highly active secondary plant metabolites with a wide variety of activities that can potentially substitute feed antibiotics.

Phytogenics can contain thousands of active constituents. Based on this, it is often assumed that bacteria will have difficulty developing resistance to phytogenics as multiple and potentially synergistic active compounds may be present. Essential oils can penetrate and damage the bacterial cell wall by triggering the coagulation of cytosolic proteins and the efflux of important intracellular compounds.

However, the MIC of essential oil added in the animal feed to exert bactericidal effects are relatively high, so prudence is necessary regarding evaluating susceptibility to the development of bacterial resistance. Also, the economic return on investment might not be given.

Possible cause of bacterial resistance to phytogenics

Since phytogenics may contain multiple antimicrobial constituents and potentially novel mechanisms of action, the question remains whether bacteria can develop resistance.

It has been reported that some bacteria (for example Salmonella enterica) have a natural resistance to phytogenics.

Still, there needs to be a clear understanding of micro-organisms' resistance mechanisms against these

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naturally occurring antimicrobial compounds.

Willing et al. (2020) reported that some studies already show that pathogens like Staphylococcus aureus, Escherichia coli, and Listeria monocytogenes demonstrated to be capable of developing resistance to phytogenics in vitro at a similar rate and level to conventional antibiotics.

Deleting the rpoS gene in E. coli and deleting the sigB gene in L. monocytogenes were associated with decreased resistance to carvacrol.

Bacterial resistance mechanisms towards essential oils include selective membrane permeability, regulated efflux/influx, and chemotaxis-controlled motility.

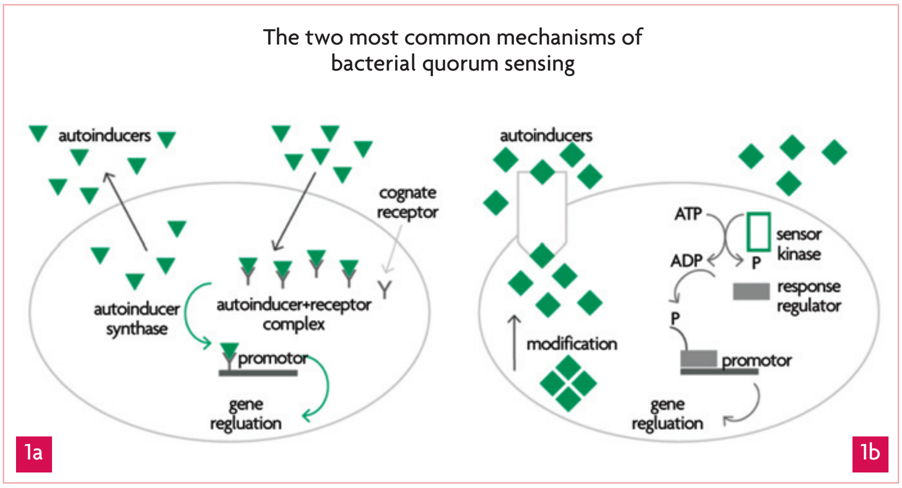

Fig. 1a. Quorum sensing in gram-negative bacteria using N-Acyl-Homoserine-Lactone derivatives as autoinducer molecules. Fig. 1b. Quorum sensing in gram-positive bacteria using Short-Peptide-Molecules as autoinducer molecules.

Quorum sensing strategy

Quorum sensing (QS) mechanisms provide a novel strategy for combating bacterial infections. Most infectious diseases are caused by bacteria that multiply within QS-facilitated biofilms. QS is a chemical communication system that boosts the survival of bacteria.

It plays a critical role in regulating diverse cellular functions in bacteria, including bioluminescence, virulence gene expression, mating, sporulation, and biofilm formation leading to antibiotic resistance.

This involves numerous processes and molecules, such as specific signalling molecules called auto-inducers, that bind to and activate receptors that transduce the QS signal into intracellular second messenger responses similar to ligand-receptor interactions.

The QS system is based on the autoinducers, signal synthase, signal receptor, signal response regulator, and regulated genes. Gram-negative bacteria generally produce acylated homoserine lactone as autoinducers, which are synthesised by a LuxI-type enzyme (signal synthase).

Gram-positive bacteria employ secreted peptides processed from precursors that can be used as autoinducers for QS. QS-controlled behaviours take place only when bacteria reach a specific cell density. The results from several reports suggest that targeting QS could be a new strategy for fighting biofilm infections.

Several methods have been outlined for QS evaluation, such as anti-swarming assay, LasAstaphylolytic assay, pyocyanin assay, flask incubation assay, in silico technique, agar well diffusion assay, and disc diffusion assay to assess the QS inhibitory potential of bioactive metabolites.

Quorum quenching by phytogenics

The quorum quenching (the inhibition of QS) approach can interrupt or prevent microbial communication instead of eliminating the micro-organism. Quorum quenching (QQ) refers to all processes involved in the disturbance of QS.

The mechanism of QQ is to bind to the relevant signal receptor and control gene expression in microbial metabolism by interrupting the signal molecules produced and secreted by the micro-organisms.

This invariably blocks the signal molecules from the receptors, and the signalling mechanism cannot regulate the resultant cumulative behaviour.

Erhabor et al. (2019) studied 46 plant species (indigenous and naturalised) with potential against microbial biofilms, and QS. 31 compounds were identified with antibacterial and QQ potential. Of these 31 compounds, 16 were assessed against biofilm formation, while eight were evaluated for their ability to disrupt cell-to-cell communication of the tested organisms.

In a study by Axmann et al. (2021) supported by Delacon, the effects of garlic oil, cinnamaldehyde, carvacrol, thymol, and thyme oil on growth and biofilm formation of Escherichia coli and Salmonella serotypes of field isolates and type strains were investigated.

Cinnamaldehyde was the most effective in reducing E. coli crystal violet (CV) staining-biofilm formation at the sub-MIC level, followed by carvacrol, thymol, and thyme, then garlic oil.

CV-biofilm formation of salmonella serotypes at the sub-MIC level was clearly reduced with cinnamaldehyde, carvacrol, thymol, and thyme oil. No reduction of CV-biofilm formation was observed with garlic oil.

This study demonstrates the potent antibacterial activity of cinnamaldehyde, carvacrol, thymol, and thyme oil.

The similar response of field isolates and type strains to these phytogenics suggests a general effect within the bacterial species tested. Essential oils' QQ potential might substantially reduce the virulence and pathogenicity of drug-resistant bacteria in vivo.

Conclusion

Alternative and complementary approaches are needed to combat harmful foodborne bacteria (like salmonella, E. coli, L. monocytogenes, etc.).

Phytogenics may offer one solution to the problem. The synergistic effect of phytogenics, in combination with other environmentally challenging applications (improved farm management), may be a safer and more effective approach to address growing concerns of bacterial resistance.

The classification of combination effects within complex mixtures and the identification of contributing compounds remains a challenging task. Despite all this, the need for new, effective, less expensive, and safer antimicrobials has become paramount for overcoming the above mentioned challenges, particularly antimicrobial resistance. ■

References are available from the author on request