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 - [Our History](#)
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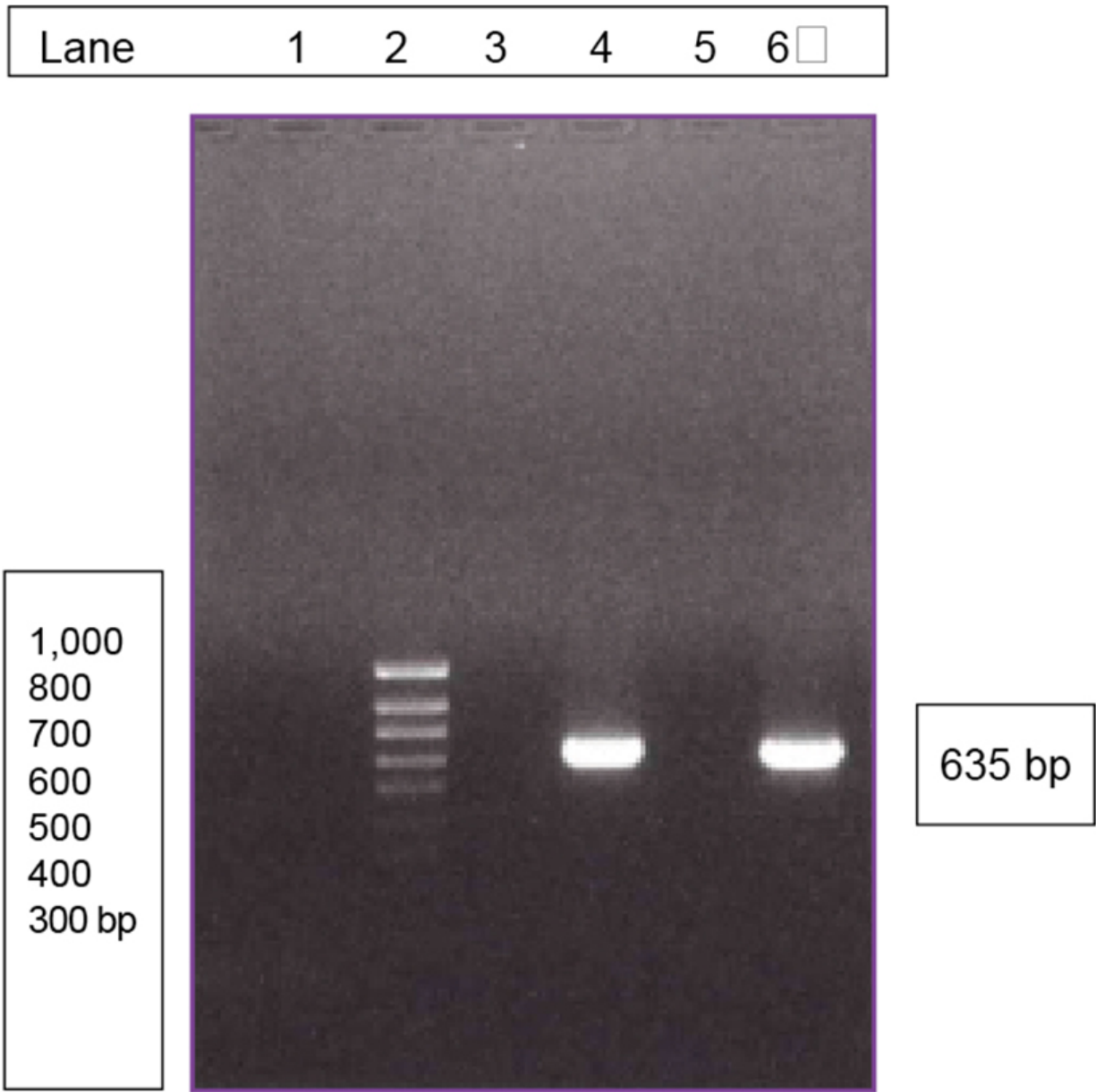
Reviewing the safety of aquaculture probiotics



1 April 2006 O. Decamp D.J.W. Moriarty, Ph.D.



Large variability in the selection procedures



Commercial assays for toxin genes can verify that probiotic *Bacillus* strains do not produce toxins. This test indicated that two *Bacillus* strains from INVE (lanes 3 and 5) did not carry the investigated emetic toxin genes (positive control strains on lanes 4 and 6).

The application of beneficial bacterial probiotics has been considered a valid alternative to the prophylactic use of antibiotics for the control of bacterial pathogens in aquaculture. Numerous microorganisms are used as probiotics in the field of aquaculture, but true probiotics are microbes that occur naturally in shrimp or fish, and their environments. Through competition with bacterial pathogens for nutrients and/or inhibition of their growth, probiotics alter the composition of the microbial communities in rearing environments and improve the shrimp or fish health.

Issues

The origins of the microorganisms sold as probiotics range from terrestrial or aquatic animals to soil, wastewater, and even fermented products. Furthermore, there is large variability in the selection procedures applied. Some strains are initially selected for their performance in aquaculture environments, whereas others are selected for their performance in completely different environments.

This had led to a confusing situation where aquaculturists are simultaneously offered probiotic bacteria developed and produced with proof of performance and safety, and other microorganisms that, although safe to humans, may never have been tested in aquaculture. Some “probiotics” could even be potentially pathogenic to humans and/or aquaculture species.

Probiotic strain safety

As part of research and development, the performance of probiotics and their safety to target organisms are obviously investigated. However, others issues have to be considered when assessing the safety of probiotic products.

Following the correct identification of probiotic strains, those recognized as pathogenic to aquaculture organisms or listed as risk microorganisms should be discarded. For example, despite its promising results in live food production, a strain of *Cytophaga* had to be discarded because of possible risks to fish larvae and hazards associated with pathogens identified in category risk two by organizations like the American Biological Safety Association.

Also included on such category two lists are other bacteria genera proposed or sold as probiotics, including *Aeromonas*, *Enterobacter*, *Enterococcus*, *Streptococcus*, and *Vibrio*. Among *Bacillus*, *B. anthracis*, *B. cereus*, and *B. thuringiensis* are listed.


Enterococcus species are important bacteria for the ripening and aroma development of certain traditional cheeses and sausages, and are also used as probiotics. However, the role of enterococci in diseases like bacteraemia and endocarditis has raised questions on their safety. For example, an *Enterococcus* was found to cause severe mortalities among farmed turbot in northwest Spain.

Safety requires confirmation

Numerous probiotic strains are considered safe due to their common occurrence either in foods or as normal commensals in the human gut. This is the case of many lactic bacteria. On the other hand, strains isolated from aquatic environments and without close relation to well-studied species do not have this long history.

The potential of *Roseobacter* strains as probiotics has been reported, but the limited research carried out on this genus might hamper the identification of pathogens. For example, a member of the *Roseobacter Clade* bacteria has been implicated in Juvenile Oyster Disease outbreaks in Maine, USA.

Confirming the safety of such isolates to humans and the environment would require much time and effort. Having confirmed the safety of individual probiotic strains, these should be deposited in a recognized culture collection.

L E C T I O N S O F M I C R O O R G A N I S M S	DEPOSIT OF A BACTERIAL CULTURE STATEMENT ON RECEIPT AND ACCEPTANCE		 BCCM/LMG BACTERIA COLLECTION LABORATOIRUM VOOR MICROBIOLOGIE UNIVERSITEIT GENT KULLEIGANGENSTRAAT 45 B-9000 GENT - BELGIUM TEL: +32 (0)9 264 54 08 FAX: +32 (0)9 264 55 08 BCCM.LMG@UGENT.BE
	The International Directory Authority BCCM™/LMG accepts in safe deposit following culture:		
	<i>Bacillus licheniformis</i> KERN BL		
	received from:	Dr. O. Decamp INVE Technologies NV Hoogveld 93 9200 Dendermonde	
	received on:	June 17, 2005	
	Deposit number allocated:	LMG S-23092	
Viability: confirmed			
Purity: confirmed			

Individual probiotic strains with confirmed safety and purity should be registered in a recognized culture collection.

Antibiotic resistance

Most bacteria are resistant to some antibiotics. Bacteria multiply rapidly, going through many cell divisions a day. Some cells can acquire random genetic mutations that provide resistance to an antibiotic, giving them a competitive advantage when faced with that antibiotic.

Screening for antibiotic resistance aims to prevent strains that carry transmissible antibiotic resistance genes from disseminating the resistances to human or animal pathogens. For instance, in 2003, Gevers et al. reported that tetracycline-resistant *Lactobacillus* isolates were able to transfer in vitro this resistance to *Enterococcus faecalis* and *Lactococcus lactis* subsp. *Lactis*.

Bacillus testing

Bacillus are ubiquitous bacteria found not only in soil, fresh water, and sea water, but also in crustacean, fish, and even human gastrointestinal tracts. As mentioned above, some species of *Bacillus* are listed as risk microorganisms and should obviously be avoided. *B. cereus* strains can produce enterotoxins and an emetic toxin that causes food poisoning.

Certifying the absence of this species is desirable, but not sufficient. *Bacillus*-related food poisoning, although mainly associated with *B. cereus*, has also been linked to a lesser degree to *B. subtilis* or *B. licheniformis* because they have acquired the genes via horizontal gene transfer from *B. cereus*. Thanks to the intense research carried out on the *Bacillus* genus, commercial immunoassay kits or polymerase chain reaction assays for toxin genes are available to verify that *Bacillus* strains used as probiotics do not produce toxins.

Probiotic product safety

A commercial probiotic product requires a performing strain or combination of strains, but also an appropriate production method that decreases the risk of contamination during production, storage and, if required, preparation prior to application.

Microorganisms such as lactic bacteria and *Bacillus* can easily be produced at high concentration at a large scale. With their ability to form spores, *Bacillus* have the added advantage of a long storage duration under a range of conditions at low cost, whereas those that do not form spores would require

low temperature for liquid products or costly freeze-drying for dry products.

Producing bacteria at high concentrations in the factory removes the on-site brewing step that can lead to contamination and possible risk to humans or target organisms, or reduced product performance, a consequence of possible change in the composition of the product after brewing.

Editor's Note: Cited references are available from the authors.

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