TO SPECIATE OR NOT TO SPECIATE? THAT IS THE QUESTION...

By Bob Pietrowski

Increasingly, we are being encouraged by consultants and regulators to identify environmental isolates beyond the genus level and down to the species level. This involves additional time, resource and expense – and to what end? What additional value does speciation provide to us, to the patient or indeed to the regulators?

WHY DO WE IDENTIFY ENVIRONMENTAL ISOLATES?

Many years ago, a young Quality Control Manager (a Microbiologist) entered the office of the Production Manager with some exciting news. "Guess what!" he said, "We've isolated a very interesting organism from your clean room. It's a species of the genus *Jensenia*. This is a fascinating organism – it's a little like a *Mycobacterium* and a little like *Corynebacterium*. "That's really interesting", said the Production Manager (also a Microbiologist) in a sarcastic voice. "Now, answer me three simple questions; what is the risk associated with this organism, where is it coming from and what do I need to do to get rid of it? If you can't answer these questions, go away and don't come back until you can!"

Suitably humiliated, the young QC Manager went away with his tail between his legs. That young QC Manager was me and it is a lesson I have never forgotten!

We identify environmental isolates for very important, practical reasons:

- To assess the risk associated with the organism
- To establish the most probable origin of the contamination – where it is coming from
- To allow us to devise eradication strategies for the organism – to get rid of it

And additionally…

To allow us to recognise re-isolation of the same organism, either because our eradication strategy has failed or because we have re-introduced the organism into the manufacturing environment.

Is it really necessary to identify isolates down to the species level in order to satisfy these objectives? I believe that in the vast majority of situations, it is not!

THE IMPORTANCE OF COLONIAL MORPHOLOGY AND THE GRAM STAIN

Often, just looking at colonies on an agar plate is sufficient to carry out a sufficient level of identification. Rough colonies of *Bacillus sp*. are readily recognisable, as are the shiny, white or yellow colonies of *Staphylococcus* or *Micrococcus*. If the colonies are black or green and furry, we know that the organism is a mould; probably either *Aspergillus* or *Penicillium*.

For bacteria, our initial identification can be confirmed by a quick Gram stain. Thus, we can quickly say that the isolate is a Gram positive coccus (probably *Staphylococcus* or *Micrococcus*), a Gram positive rod (*Bacillus*) or a Gram negative short rod (if isolated from water, almost certainly a pseudomonad). This quick and rudimentary identification is sufficient to allow us to:

- Assess risk
- Determine the most probable origin
- Devise eradication strategies
- Recognise re-isolation

Table 1 summarises this information for a range of common organisms.

So why do we need to go further and identify isolates down to species?

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PEOPLE LIKE NAMES!

There is a common belief that, if we can put a second name to an organism, suddenly we know much more about it.

If we say we have isolated a *Bacillus* from our environment, our information appears incomplete. However, if we say we have isolated *Bacillus megat erium* from our environment, then we feel we have done a more complete job. But have we? What do we really know about *Bacillus megaterium* that allows us to take more effective action than we would take if we simply knew the organism was a *Bacillus*? The answer is almost certainly nothing! Giving the organisms a second name may give us a warm glow, but it doesn't normally help us to do our jobs better. And what is more, it has taken us several days and expensive equipment to get to this point!

Furthermore, how confident are we in our identification of the organism as *Bacillus megaterium?*

WHAT'S IN A NAME?

Identification is inextricably linked to classification (or taxonomy) and here we are faced with a major problem – the mindset of taxonomists. Bacterial taxonomists belong to one of two diametrically opposed groups; the Splitters and the Clumpers. Splitters classify organisms on the basis of how different they are from other organisms, whereas Clumpers tend to classify organisms on the basis of how similar they are to other organisms.

At the moment, the Splitters seem to have the upper hand, so that similar organisms are given very different names which tends to mask family similarities. Thus, what used to be *Bacillus stearothermophilus* is now called *Geobacillus stearothermophilus*. An inexperienced Microbiologist may, wrongly, assume the newly named *Geobacillus* does not share key characteristics with Bacillus species. Similarly, organisms that were formerly classified as *Pseudomonas* are now called *Burkholderia* and other weird and wonderful names, but they still share, as far as we are concerned, key characteristics with Pseudomonas species and our reaction to them should be essentially the same.

This problem is exacerbated by our attempts to identify organisms using proprietary systems such as API, Vitek, ribotyping etc. These increasingly sophisticated techniques, allied to a classification philosophy which tends to accentuate differences rather than similarities, makes it more likely that identification procedures will produce less clear identification and more likely that the same organism, when put through the same system on two different occasions, will be identified differently on each occasion.

Table 1 Characteristics of Some Common Environmental Isolates

ORGANISM	BACILLUS	STAPHYLOCOCCUS	MOULDS	PSEUDOMONAS
COLONIAL MORPHOLOGY	Smooth or rough, white or cream colonies	White, cream or yellow domed colonies	White, black or green hairy large spreading colonies	Cream or white colonies, sometimes surrounded by greenish pigment in agar
GRAM STAIN	Gram Positive Rod	Gram Positive Coccus	N/A	Gram Negative Rod
RISK	Spores highly resistant to killing by heat and disinfectants	General contamination risk. Some species toxigenic	Spores, although not heat resistant, permit rapid spread of contamination	Endotoxin risk, some species resistant to disinfectants, some toxigenic
ORIGIN	Soil, dust, cardboard, wood etc.	Human skin	Soil, dust, cardboard, wood etc.	Water and moist environments
ERADICATION STRATEGY	Check effectiveness of disinfection of materials and trollies entering area via transfer hatches etc	Check effectiveness of gowning procedures personal hygiene, glove disinfection procedures	Check effectiveness of disinfection of materials and trollies entering area via transfer hatches etc	Remove standing water, check cleaning materials, sanitise water systems

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Thus, in Week 1 we may isolate a Gram positive rod from our environment which, upon speciation, is found to be *Bacillus pietrowskiensis*. In Week 2, we isolate a Gram positive rod which we identify as *Bacillus beggii*. Are we really sure that there are in fact two different organisms and not the same organisms misidentified?

And anyway, does it really matter? We have isolated a Gram positive rod from our environment on two consecutive weeks, which tells us that we have a problem controlling *Bacillus* species!

Misidentification or organisms is not uncommon and we would be well advised to treat all identifications with a healthy degree of caution.

Recently, the Microbiology department of a European pharmaceutical company sent a Gram positive rod which had been isolated in their facility to a laboratory of international repute for identification by ribotyping. Several weeks later, the laboratory sent back a report stating that the organism was a species of *Bacillus* which had previously only ever been isolated from fermented Korean seafood! As the product had no links to fermentation,

Korea or seafood it is highly likely that the identification was, to say the least, questionable and unhelpful. It also took a lot of time to obtain, as well as a lot of money!

SO IS THERE A PLACE FOR SPECIATION?

There are two major benefits from speciation

- It satisfies regulatory expectations
- It can assist investigation into the origins of organisms and identification or re-contamination incidents.

The regulatory expectation for speciation is clear and cannot be simply ignored. Having said that, I believe that the frequency of speciation should be restricted to incidents when it provides real value. For example, if a Gram positive rod is isolated form a bulk product or a Sterility Test unit, speciation of the organism and isolates from the manufacturing and testing environments can assist in determining where the contaminant was introduced.

Similarly, speciation of isolates from water samples can assist in determining whether our eradication strategies have been effective.

My advice, though, is not to put too much importance on what is sometimes an unreliable practice.

IN SUMMARY

- Don't overestimate the value of speciation – in many cases it does not provide practical value
- Don't underestimate the risk of incorrect speciation, and of inappropriate conclusions drawn therefrom.
- Speciation can slow down your reaction to problems. Don't wait for completion of speciation to instigate corrective actions; you already have sufficient information from Gram stain etc to determine risk, most probable origin and eradication strategies
- Be prepared to defend your practices to regulators, by the use of good science.

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