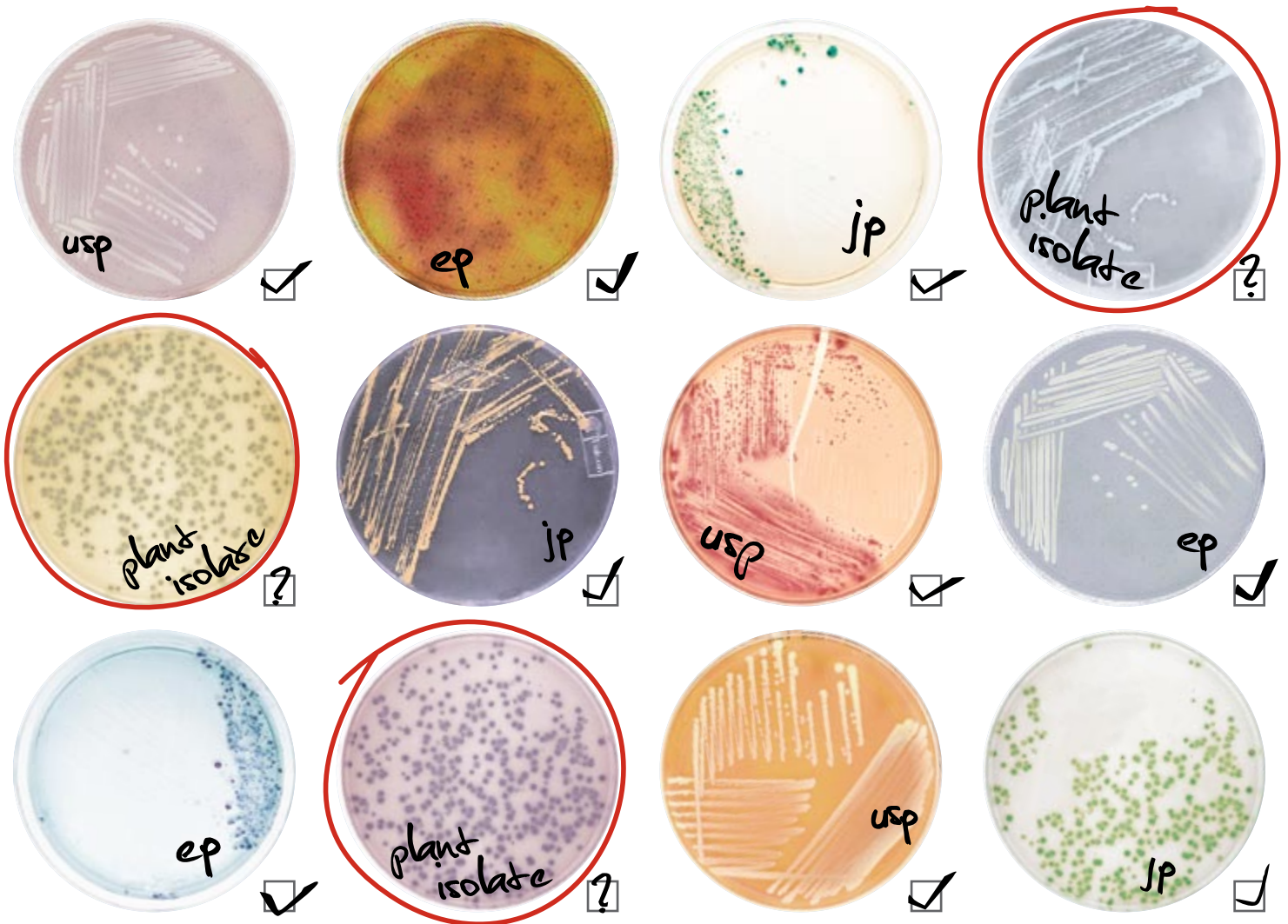


The Value of Plant Isolates in Pharma Quality

David Myatt, PhD and Charlotte Morgan, PhD, BTF, a bioMérieux company



What value are plant isolates in microbiological quality in the pharma industry?

Increasingly, pharmaceutical companies are including their own isolates in the battery of microorganisms that they use for media growth promotion testing and validation studies. These “plant isolates” are wild-type strains isolated during environmental monitoring, sterility and bioburden testing, and routine testing for contamination or spoilage. In so doing, these companies seek best microbiology practice, but it remains somewhat controversial. Some commentators argue that compendial methods do not mandate such an approach, others challenge its scientific merit, and some query the practicality. Notwithstanding a level of public debate, many companies are implementing standard operating procedures and grappling with the practicalities of strain selection, culture maintenance that sustains the cultural characteristics of “wild” plant isolates, a degree of regulatory uncertainty and, certainly, a paucity of guidance on how to achieve the desired outcome, whether that is simply compliance or genuine commitment to more challenging tests in pharmaceutical quality management.

Wild-type Strains

By definition, strains found in nature. But in our context, we mean to discuss strains that are recently-isolated in a manufacturing context, either from a controlled manufacturing environment or, perhaps, a contaminant of raw materials or finished pharmaceutical product. These are strains that are not conditioned through serial subculture to growth on rich laboratory culture media and may exhibit unstable phenotypic characteristics associated with oligotrophy, desiccation or biofilm formation, namely traits that have enabled survival in harsh conditions and may not persist in strains that are serially passaged in rich culture media uncharacteristic of the environment from where they were isolated.

Trends in Use of Wild Isolates

Let's begin by agreeing that this really isn't anything new! Authorities on quality in pharmaceutical microbiology have been suggesting the merit of including wild-type isolates in media QC testing for many years (1, 2, 3) and auditors now issue FDA 483 observations in relation to this expectation.(4) Certainly, it's become a topical matter in recent years, with periodic debate in industry discussion forums and blogging sites.(5, 6, 7) While perspectives on the scientific merits vary, and whether it's a function of regulatory attention or best microbiological practice, use of plant isolates (or whatever you choose to call them) is now commonplace in pharmaceutical microbiology.

One author's own insight, gleaned from many conversations with practicing pharmaceutical microbiologists, clearly indicates that many big pharma companies and smaller ones alike are implementing (or already have) the use of a few of their own isolates to complement the compendial reference strains in growth promotion testing of environmental monitoring and sterility testing media, and sometimes in validation studies for new methods such as rapid microbiological methods (RMM) for sterility assurance testing. In most cases, these labs intend to make an annual assessment of the frequency of species amongst their environmental isolates and select either the two or three with highest frequency or the highest frequency isolate from each of the Gram positive, Gram negative and fungal isolate groups. Their intention is usually articulated in terms of

compliance (i.e., what auditors want) or best laboratory practice, even if they do not subscribe to the view that the use of these strains is a valuable exercise in verifying the performance of their culture media or test methods. It certainly seems that there is now a widely-perceived need for compliance here (in the absence of an FDA audit citation) given that the use of environmental isolates is strongly recommended in a number of compendial references and other authoritative documents.

It is also commonplace to see manufacturers of personal care products and nutraceuticals include extensive batteries of contaminant organisms (isolated from their raw materials or spoilage of their products) in studies to verify the efficacy of their preservative systems. Of course, conceptually, this is akin to the testing of non-sterile pharmaceuticals for objectionable organisms that often originate as contaminants in raw materials or from the manufacturing environment. Whatever the case, these practices are founded on the idea that these microorganisms are a better challenge to the microbiological method than the "standard" compendial strains.

Applications, Regulations and Recommendations

The compendial references for sterility tests, enumeration tests, specified microorganisms, and antimicrobial effectiveness tests (USP chapters <71>, <61>, <62> and <51> respectively) and the corresponding sections of the European Pharmacopoeia do not prescribe the use of environmental or other wild isolates. However, a number of compelling rec-

ommendations in this regard are made in guidelines issued by several authorities:

- Concerning the microbiological evaluation of controlled environments, USP <1116> says "for the Growth Promotion test, representative microflora isolated from the controlled environment... may also be used to test media."
- USP <1117> concerning Microbiological Best Laboratory Practice suggests "microorganisms used in growth-promotion testing...may include representative environmental isolates (but these latter are not to be construed as compendial requirements)."
- *FDA Guidance for Industry for Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice* (September 2004) says "The QC laboratory should determine if USP indicator organisms sufficiently represent production-related isolates. Environmental monitoring and sterility test isolates can be substituted (as appropriate) or added to the growth promotion challenge."
- *FDA Guidance for Industry concerning Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products* (draft guidance, February 2008) suggests, in relation to selecting a panel of appropriate challenge microorganisms for validating an RMM, the inclusion of "isolates detected in starting materials, isolates detected by in-process test-

Isolated Look at this Article

- Testing of plant isolates, or wild-type strains, is a regulatory expectation
- Arguments against such testing include practicalities related to repeatability, reproducibility in validations and cost
- The real value derives from significantly greater confidence in media, methods and systems that are validated and tested using strains that are more typical of target organisms than those referenced in compendial methods



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ing or during preliminary product testing, isolates detected by environmental monitoring of your manufacturing facility, [and] isolates from your production areas which represent low nutrient or high stress environments....”

- USP <1072> concerning Disinfectants and Antiseptics suggests “surface challenge tests using standard test microorganisms and microorganisms that are typical environmental isolates....”
- The Japanese Pharmacopoeia (XV, General Information section 11.4.1 concerned with Media Fill Tests) says in relation to selection of growth promotion testing organisms “one or two representative microorganisms which are frequently isolated in environmental monitoring should be used.”

While none of these can be construed as a mandatory requirement, here are many calls to consider the relevance of plant isolates in growth promotion testing, validation studies and challenge testing. Presumably, this selection of references represents a much greater number of experienced individuals on expert panels who’ve co-authored these documents in conjunction with the regulatory agencies that have published them. So, it seems fair to say that there are widely-held views that plant isolates are relevant.

Costs and Value

Arguments against the inclusion of plant isolates in pharmaceutical microbiology are varied and include the practicalities of standardizing such isolates for repeatability and reproducibility in validations, and the challenge of preserving these strains in a culture collection.(7) We would add to that list the considerable challenges related to expertise and specialized resources needed to manage a culture collection of plant isolates so that they’re minimally compromised by subculture and preservation. This is an increasingly acute issue in pharma where everyone, including microbiological quality laboratories, is asked to do

much more with much less in tougher economic times.

Experience in culture collection management and culture preservation techniques is increasingly rare when many laboratories opt to purchase strains from recognized collections or commercial suppliers. Beyond that, time and competencies needed to prepare standardized suspensions by serial dilution are also increasingly scarce. Toted up against the costs of these activities are lab space, acquisition and installation costs, qualification and validation projects, and maintenance and user training demanded by various pieces of laboratory equipment like ultra low temperature freezers, freeze-dryers, spectrophotometers and data management systems needed for a competent culture collection. There are also costs of specialized laboratory reagents and consumable items and their procurement, qualification, documentation, storage and wastage. The costs mount up dramatically. In this context, commercially-available quantitative microbiological controls produced with compendial reference strains have grown in popularity, and leading brand products are Certified Reference Materials according to ISO Guide 34 accreditation. Such products offer labs the option of “outsourcing” laborious, time-consuming, expensive and error-prone activities associated with maintaining cultures and preparing inocula for routine growth promotion tests and validations studies.

But, having outsourced these activities, those wanting to incorporate plant isolates in their testing are now challenged to reinstate skills, time and other resources needed to maintain and prepare them. Additionally, it could be argued that the expertise and facilities needed to preserve plant isolates markedly exceeds those demanded for compendial strains. For instance, the optimal culturing conditions required for the compendial strains are well known and documented within the industry, advice is

on hand. Whereas, when preserving and culturing a plant isolate, it is unknown whether the environmental strain will be as robust or have the same culturing requirements as a known culture collection strain of the particular plant isolate species, so it can quite often be a case of “trial and error” and therefore time-consuming and expensive.

Practicalities in Implementation

The contention that exists about use of plant isolate derives from a general lack of knowledge of how these wild strains differ from culture collection strains that have been serially subcultured to such an extent that they are “adapted” to rich culture media. The nature of the differences is poorly understood, as are the mechanisms involved. Certainly, serial subculture drives a process of *in vitro* evolution where there is natural selection for clones that grow most luxuriantly on rich media, but the stability of what could be defined as “wild” attributes, and consequent phenotypic and physiological changes, and how quickly these emerge in serially-subcultured strains, is generally not understood.

In this context, the safest approach is to minimize the serial subculture of plant isolates. Compendial references suggest that culture collection strains should be five or fewer subcultures (passages) from the culture originally sourced from a reference culture collection. This “five passages” rule has been extrapolated to plant isolates, but again in the interests of conservatism, we suggest that minimizing serial subculture is the only way likely to minimize the risk of significant strain evolution that would compromise a strain’s merit as a stringent challenge to media fertility or RMM performance. So, it could be argued that the ideal candidates will be minimally subcultured plant isolates, with phenotypic characteristics stabilized through sophisticated preservation techniques, and standardized to deliver a reliable inoculum for consistent growth promotion testing and ►



for repeatable and reproducible validation studies. That is an ambitious objective! Few laboratories have the competencies and resources outlined above to accomplish this, and it is a fact that some very useful techniques are the subject of patents or proprietary know-how. There

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are three main techniques to reliably store microbial cultures, namely ultra low freezing at below -70°C , cryopreservation in liquid nitrogen, or freeze drying (lyophilization). Each technique has advantages and disadvantages. However, selection of a technique is more often based on the availability of equipment and expertise than on the suitability of the technique to the particular strain to be preserved. Some strains survive well in a frozen matrix, whereas others can only be frozen in liquid nitrogen for long term survival. Freeze drying is the technique of choice for long term microbial preservation (8), for cells that can tolerate freezing and drying, but this technique can be too harsh for more fragile microbes (e.g., mycoplasma). It must be kept in mind, no matter which technique is used, there is still a degree of selection happening during storage, and viability cannot be sustained indefinitely, and therefore the longer the storage, the greater the possibility of genetic or phenotypic drift. It is for this reason that extensive profiling of strains prior to storage should occur, so that any change in the strain can be detected by comparison with the original profile. The value of experience in the cataloguing and storage of strains in a culture collection can be easily under-estimated, hence the reason why most companies that hold commercially important microbial strains invest in back-up storage of their strains at off-site facilities with relevant expertise and capabilities.

Accordingly, specialist service providers with the appropriate focus, experience

and facilities now offer to acquire from labs their minimally subcultured strains and return standardized inocula to cover a year (or more) of testing with minimized risk of compromising the strains' "wild" traits. Leaders in this field use the most sophisticated techniques available

for strain preservation, standardization and delivery and seek to provide premium service to match the regulatory and operational context that is peculiar to microbiological quality in the pharma industry. When assessing the capabilities of such service providers, we recommend a thorough review of their track-record with a wide range of compendial strains, the potential for their technologies to minimize the *in vitro* evolution or "adaptation" of plant isolates, and their ability to provide plant isolates in formats that are consistent with and as convenient as the compendial strains they supply.

Conclusion and Future Trends

We've outlined here our perception that there is now a very strong trend to increased use of plant isolates to challenge pharmaceutical microbiology media, methods and systems, both in routine QC testing and validation. We've observed the regulatory pressures for greater compliance in this area, but acknowledge the practical and economic challenges that accompany a commitment to the routine use of plant isolates. Nevertheless, given the ethical, legal and economic imperatives that compel rigorous quality management in the pharmaceutical industry, we find few convincing arguments against the use of plant isolates to more effectively challenge the media and methods used in pharmaceutical quality. It is not costless to do so and must therefore deliver real value. We suggest that real value derives from significantly greater confidence in media, methods and systems that are validated and tested using strains that are more

typical of target organisms than those referenced in compendial methods, that is, strains acquired from culture collections where they've been serially passaged under atypical conditions for many years. Certainly, the very isolation of wild-type strains and their minimal subculture for preservation are selective pressures that threaten the traits we'd hope to retain in the strains we use to challenge our microbiology tests, but we don't see practical alternatives beyond use of the most sophisticated techniques to preserve strains as close to their primary isolation as possible. Accordingly, we argue that there is real value, albeit difficult to quantify, in maximizing confidence that media and methods we use in pharmaceutical microbiology are effective and reliable to the greatest extent that we're practically able to demonstrate, and therefore contribute more assurance of the quality of our pharmaceuticals, medical devices and personal care products.

It seems inevitable that there will be sustained or increased attention paid to critical environment monitoring, detection of objectionable organisms and other microbiological practices intended to minimize contamination and adverse outcomes from the use of therapeutic and nutritional products. There is little indication from regulators or any other authorities in the pharma industry that vigilance will decline or expectations will be relaxed. Increasingly litigious developed markets, growing healthcare standards and expectations in emerging markets all have the effect of encouraging greater regulation, despite economic pressures. We would also expect to see greater use of methods not based on microbial growth, where these RMMs need to be shown, through extensive validation, to be equivalent to traditional compendial methods, at least according to current requirements.(9) For instance, is a slow growing plant isolate (that takes >5 days to grow in traditional culture) detectable by a RMM? This is why, if such plant isolates are found, they may be relevant for method validations. Indeed, such questions only serve to fuel discussions about the merits of using plant isolates to ►

complement culture collection strains in important pharmaceutical microbiology testing. But, it seems the tide of opinion now shows that many have accepted the value of additional strains selected for their relevance in individual sites and products. The challenge remains to execute this practice well, so its value is realized, either through investment in the competencies and skills required to reap the value of plant isolate strains preserved as close to their original state as possible, or by partnering with service providers whose focus, expertise and experience in the specialized area of microbiological strain preservation can provide stable and quantitative plant isolate strains that are minimally passaged since isolation.

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About the Authors

David Myatt has more than 20 years experience in diagnostic and industrial microbiology, having held senior roles in quality management, marketing and commercial leadership in global microbiology companies. His pharma microbiology experience began with

a QA management role implementing a quality system, cGMP and compliance with international regulatory standards. His subsequent commercial roles in the pharma and bioprocessing markets included focus on microbiological culture media, pharmaceutical quality control, critical environmental monitoring, and biopharma production. He holds a PhD in Microbiology and an MBA in International Business and Marketing. He leads strategic marketing at BTF, a bioMérieux company in Australia. To contact email david.myatt@btf.biomerieux.com



Charlotte Morgan has more than 10 years experience in research related to precise detection and dispensing of microbial cells and maintaining viability through techniques such as freeze drying. Her role as a principle researcher in the development of the commercial microbial reference material "BioBall" has developed into managing a team of dedicated scientists to expand the BioBall range and the techniques to improve long term precise preservation of microbial cells. She holds a Masters in Water Microbiology and a PhD in Microbiology. She is currently a R&D Manager at BTF, a bioMérieux company in Australia. To contact, email charlotte.morgan@btf.biomerieux.com.



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