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A CRITICAL REVIEW ON CELLULAR COMPONENT-BASED TECHNOLOGIES FOR RAPID DETECTION OF MICROBIAL QUALITY OF A PRODUCT: ATP BIOLUMINESCENCE AND FATTY ACID PROFILING

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Abstract

A Rapid Microbiological Method is an antidote to traditional compendial microbiological methods with its own assets and liabilities. As the intricacy of the drugs elevated in the pharmaceutical and biotechnology domains, these drugs requires critical, complicated handling and monitoring while ensuring the microbial quality. For example, microbial quality of Positron Emission Tomography drugs is defined by sterility test and Bacterial endotoxin test [1]. Sterility Testing can be performed by Adenosine Tri Phosphate Bioluminescence and Membrane Laser Scanning Fluorescence Cytometry as an alternative to the compendial Membrane Filtration method as they report results quickly by means of reducing the incubation period from 14 days to 5 days. On the other hand, Bacterial Endotoxin Test can be performed by Monocyte activation test and Recombinant factor C assay in preference to the classical Limulus Amebocyte Lysate Gel Clot Method as these techniques are highly sensitive, accurate and reproducible. The main restraint for afore mentioned methods in performing sterility test is that they are non-CFU based Rapid Microbiological Methods whereas membrane filtration is a CFU based classical method thereby ensuing a problem in establishing the acceptance criteria and equivalence during validation process [2]. Hence this review focuses on the comparison and contrast of different aspects related to rapid microbiological techniques particularly 'ATP Bioluminescence' for Bioburden testing and 'Fatty acid profiling' for identification of microorganisms. This may extend an ultimate solution for the existing limitations towards Rapid Microbiological Methods so as to acquire regulatory compliance.

Keywords: Rapid Microbiological Methods, Cellular Component-based technologies, ATP Bioluminescence, Fatty acid profiling, Validation.

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INTRODUCTION

The complexity and diversity of diseases in human beings demanded the need to discover the drugs starting from over the counter drugs, antibiotics to the orphan drugs [3]. This created a rapid fire between several pharmaceutical and biotechnological companies worldwide towards the research of quality assurance and quick product release of the respective drugs into the market. This led to the advent of Rapid Microbiological methods which availed its own importance by virtue of its notable attributes such as sensitivity and accuracy [4]. RMM's are highly validated and are not time and labor intensive [5]. Over the centuries in the field of microbiology, traditional methods adopted by Louis Pasteur and Robert Koch had been followed till date and were being updated at lumbering pace [6].

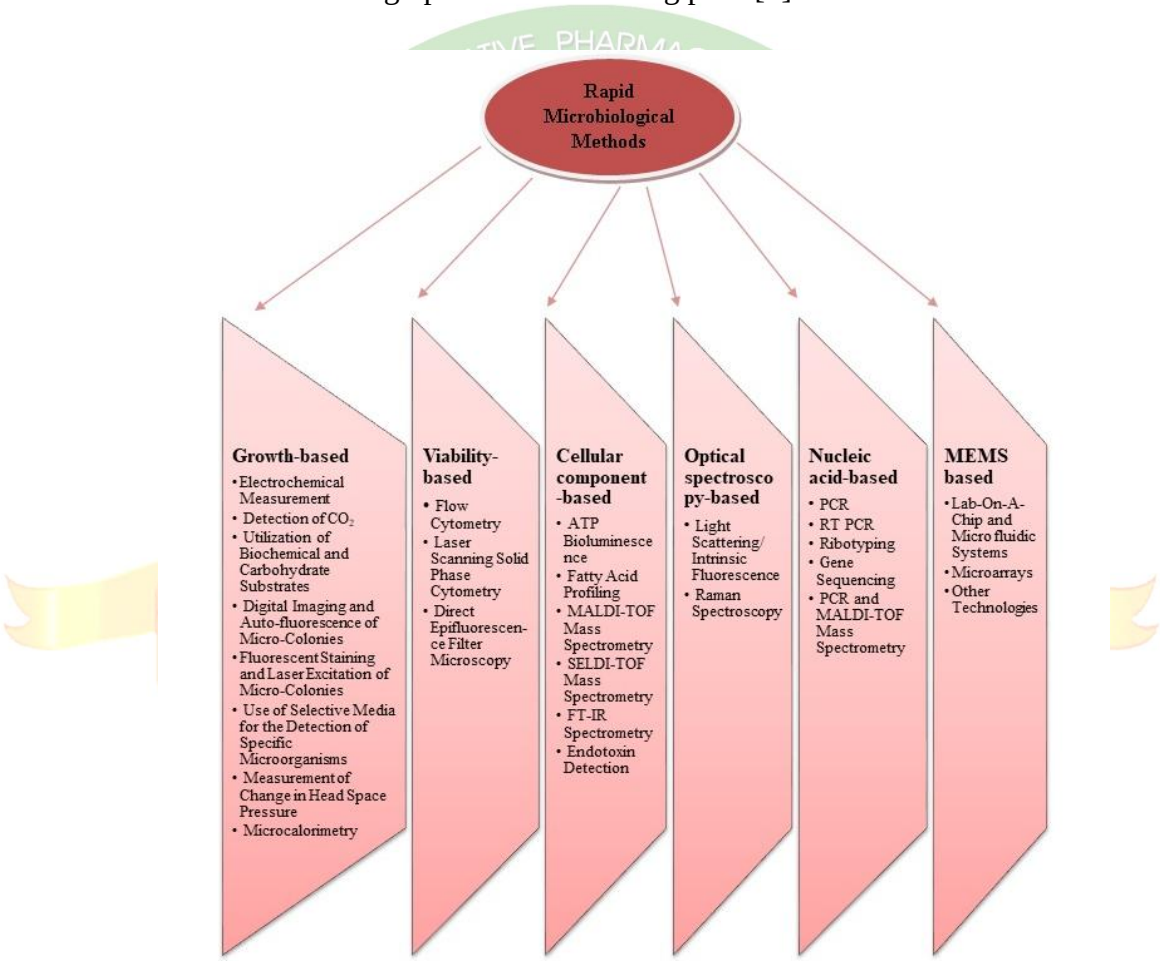

Fig. 1: Empirical classification of Rapid Microbiological Methods [7] (exclusive)

Basically any sample quality, by employing microbiological methods can be defined applying three types of tests namely qualitative, quantitative and identification tests [7]. Comprehensively, these tests are detection of presence or absence of microorganisms in the sample, Enumeration of microorganisms in the sample, Identification of specified pathogens in the sample [8]. Different RMM techniques have been developed to perform earlier termed

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microbiological methods and have to be selected depending upon vital quality feature of the product, validation prerequisites and GMP guidelines [9]. Practically, RMM's are classified as growth-based, viability-based, cellular component-based, Optical Spectroscopy, nucleic acid-based and Micro-Electro-Mechanical Systems (MEMS) based technologies [7] which is as shown in Figure 1. Traditional compendial methods have not been upgraded because of several reasons such as lack of awareness and vacillation on latest RMM's, validation difficulties in setting up the acceptance criteria, lack of equivalence between traditional and RMM methods and the affordability by many companies economically [10]. Hence, this review comprises of extensive elucidation on certain Cellular Component-based RMM techniques namely ATP Bioluminescence and Fatty acid profiling. This elaborate illustration on these two Cellular Component-based techniques has been incorporated to create and intensify the awareness, positive dominance and necessity of RMM in the field of advanced analytical Microbiology so as to increase the confidence level to adapt the RMM and also may lead to several solutions for the hurdles exists in the present day research of RMM's.

CELLULAR COMPONENT-BASED RAPID MICROBIOLOGICAL METHODS

ATP BIOLUMINESCENCE

Bioluminescence is a natural trait exhibited by distinct organisms irrespective of their cellular complexity, for their defense and survival [11]. This phenomenon in firefly is due to the crucial participation of ATP molecule in the reaction of converting the energy into light implying the term 'ATP Bioluminescence' which is explicitly displayed in Figure 2. Consequently, the intensity of light is directly proportional to the ATP utilized in the reaction [12]. Further, ATP detection sensitivity has been improved rapidly using Adenylate Kinase [13]. These two facts form the basis for rapid ATP detection devices in the field of microbiology to determine the microbial contamination and concentration. In this context, ATP bioluminescence has been applied prominently in sterility testing of pharmaceutical products rather than conventional methods [14]. The implementation of above technique is highlighted upon the recovery of even slow growing *Propionibacterium acnes* within 4-5 days instead of usual 14 days [15]. Furthermore, Bacterial bioluminescence is another exemplar with several applications in which $FMNH_2$ and NADH are the prime contributors [16,17] which is displayed precisely in Figure 3. Several aspects of Bacterial Bioluminescence are compared and contrast with ATP bioluminescence and listed in Table No: 1.

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Table 1: ATP bioluminescence Vs Bacterial bioluminescence

S. No:	ATP Bioluminescence	Bacterial Bioluminescence
1	ATP bioluminescence is eukaryotic trait [18].	Bacterial Bioluminescence is prokaryotic triat [18].
2	The well understood eukaryotic bioluminescent systems are firefly systems particularly *Photinus pyralis*, sea pancy *Renilla reniformis*, marine copepod *Gaussia princeps* [19].	Prokaryotic systems such as *Photobacterium luminescens*, *Vibrio fischeri*, *V. harveyi*, *P. phosphoreum* and *P. leiognathi* are the efficient comprehend systems [19].
3	The cofactors involved in the firefly bioluminescence reaction are ATP and Mg^{2+} in the presence of molecular oxygen [16].	$FMNH_2$ and NADH are the cofactors that contribute in the bacterial bioluminescence in addition to the molecular oxygen [16].
4	Firefly luciferase is a Photinus-luciferin 4-monooxygenase of enzyme class EC 1.13.12.7 [20].	Bacterial luciferase belongs to the Flavin dependent monooxygenase family with enzyme class EC 1.14.13.8 [21].
5	FLuc is the firefly luciferase [22]	The operon that gives off enzyme, substrate and other necessary compounds for the respective bioluminescent reaction is *lux* operon [23].
6	Luciferase mediated light reaction releases the photon energy in the form of yellow-green light at a wavelength of 550-570 nm [19].	Blue-green light at a wavelength of 490 nm is emitted as the resultant of the luciferase catalyzed reaction [19].
7	Consistent emission of light even at lower cell concentrations made this ATP bioluminescence a powerful tool for different applications [24,25].	The light emission will be declined after reaching the stationary growth phase of organisms [26].
8	Firefly luciferase has better activity at temperatures above 22.5°C. Hence, it is suitable for the detection of organisms that require optimum growth temperatures [27].	As *Vibrio* luciferases are stable even at low temperatures thereby extending the application to the lower growth temperature favored organisms. On the other hand, *P.luminescens* luciferases have notable stability at higher growth temperatures [19].
9	This bioluminescence can be preferably applied to perform in vitro experiments. On the contrary, it can be extended to in vivo experiments owing to the fact that firefly can luminescent red light under acidic pH conditions, which has higher tissue penetration capacity [24,25].	This phenomenon is better competent towards in vivo experiments because it does not require external addition of substrate unlike firefly bioluminescence [24,25].
10	Transformation of large sized *luc* genes is difficult, which became a remarkable limitation to its successful application in in vivo studies [28].	Transformation of *lux* genes is quite easy and successful comparatively [29].

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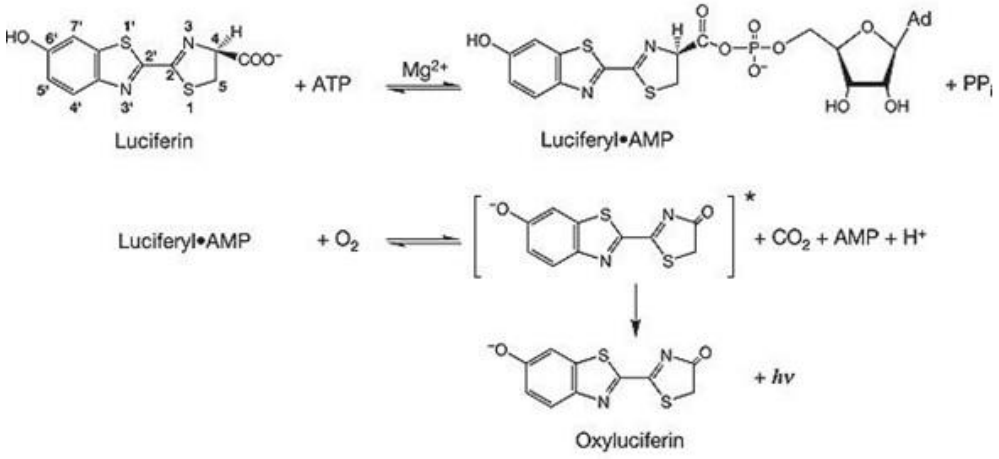

Fig. 2: Firefly luciferase mediated ATP Bioluminescence reaction [30]

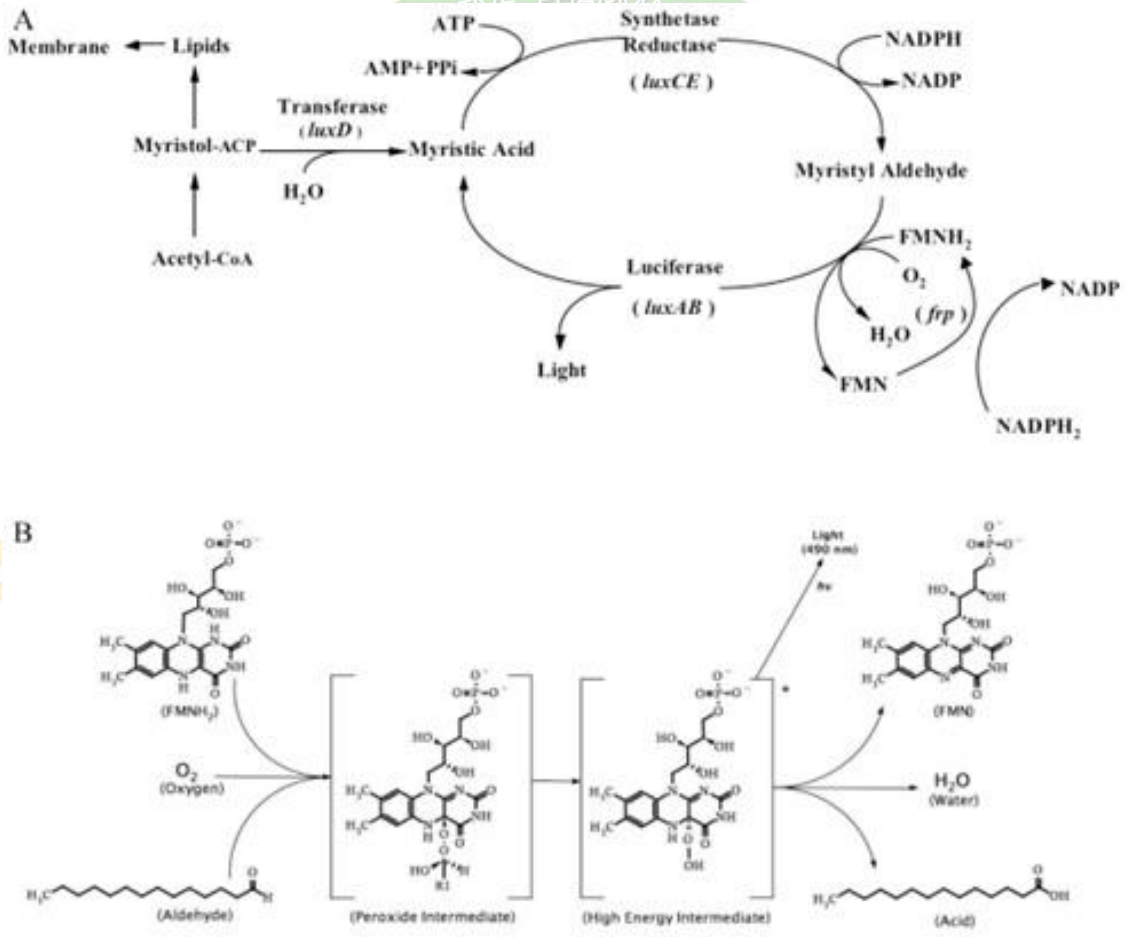

Fig. 3: Bacterial luciferase mediated Bioluminescence reaction [25]

The spotlight of current research of this technique has been pivoted on the identification of divergent bioluminescent mechanisms [31], development of different luciferin analogs and luciferase mutants [32] and validation perspectives [33]. Approximately, 30 diverse bioluminescent mechanisms have been reported till date [31] and 9 luciferin molecules have

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been understood till date [32] and are displayed in Figure 4. In recent years, bioluminescence similar to that of firefly in terms of ATP dependence have been identified in earthworm species namely *Fridericia heliota* along with the recognition of bioluminescence in higher fungi which needs only oxygen excluding any other cofactors with the involvement of a luciferin precursor, 'Hispidin' [31].

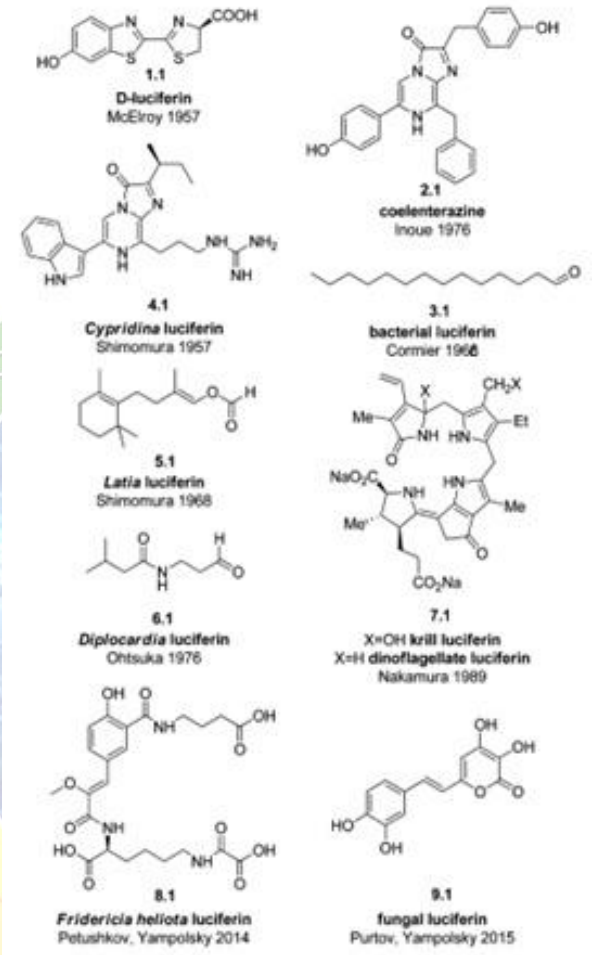

Fig. 4: Meticulously understood 9 luciferin molecules till date [32]

Synthetic luciferin substrate molecules such as aminoluciferins [34], dehydroluciferin, decarboxy- and dehydroxyluciferins, 5,5-dimethylluciferin, synthetic luciferin compounds with replaced benzothiazole fragments, hydrazide D-luciferin [35] and brominated luciferins [36] have been innovated. These synthetic luciferin analogues have depicted eclectic peculiarities such as emission of intense light with increased wave length, pH stability, thermostability and improved specific activity when compared to D-luciferin, depending upon the mutant and engineered luciferases [32] and such pairs can be obtained by the procedure according to Figure 5. For instance, brominated luciferins exhibited intense red light emission than D-luciferin with high tissue penetrating capacity by means of recombinant firefly

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luciferase [36]. Irrespective of highlights and challenges of ATP bioluminescence, it has been raised as current rapid testing tool for various applications [14]. In pharmaceutical industry, ATP bioluminescence has been prominently used as a rapid method in sterility testing as it reduces the incubation period to 5 days from 14 days allowing the detection of even 1 CFU, whereas bacterial bioluminescence is better suitable in microbial limit testing of water, providing continuous real time monitoring of microbial action and alert levels [37]. This is a bit laborious by means of ATP bioluminescence as it requires external addition of substrate [24,25].

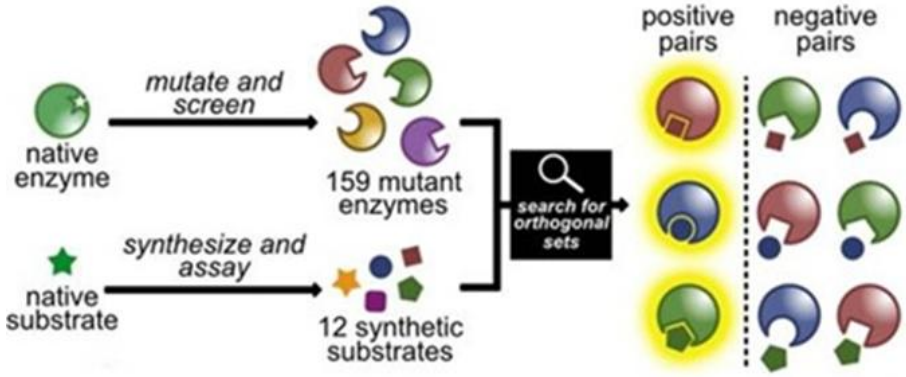

Fig. 5: Schematic for obtaining orthogonal luciferin analogues-mutated enzyme pairs [38]

Validation perspectives

The major constraint during validation of ATP bioluminescence is the interfering components affecting ATP-light reaction [39]. Robustness of ATP detection systems has been affected by the stressed environment under which the validation has to be performed. Rapid Sterility test medium which is an agar instead of broth [40] and sample-bearing swabs have been employed to overcome the above problem [33]. Concurrently, the above agar medium has positive impact on the specificity of the rapid method by recovering a wide variety of stressed microorganisms than the usual liquid media [40] including non-cultivable eukaryotic integrants [39]. There has been no noticeable statistical significance for Limit of detection between conventional and rapid sterility tests [40]. Limit of detection is very low for pure cells. Synchronously, gram positive cells have showed an appreciable limit of detection than the gram negative bacterial cells. The sensitivity towards gram negative cells can be enhanced partially by sonication [33]. Low level ATP concentration is the concern in deviating accuracy and precision of RLU values during sterility tests [33,41].

FATTY ACID PROFILING

Fatty acid profiling has been emerged as a rapid method for the spotting of numerous microorganisms in which fatty acids serves as means of identification [42], as lipids are

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prominent cellular components [43]. The initial step in profiling the fatty acids is the extraction of lipids from cells followed by deriving of fatty acids from lipids and analyzing the samples employing appropriate analytical techniques by means of modifying them to form fatty acid methyl esters (FAME's) [44] which is clearly depicted in the Figure 6.

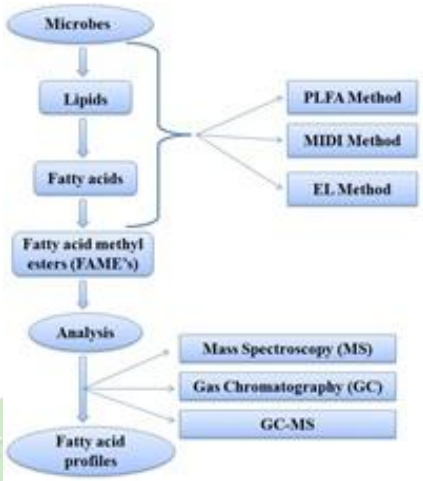

Fig. 6: General schematic of fatty acid profiling [44] (exclusive)

Three methods have been employed to derive fatty acid methyl esters from the microbial samples as mentioned in Figure 6. The brief description of the above three methods are as follows:

PLFA Method

Phospholipids gained interest as they can act as a measure of living cells. Thus the phospholipids have been targeted for estimating the viable biomass. Also, taxonomical classification and estimating physiological stress of microbes have been possible using Phospholipid Fatty Acid (PLFA) method [45]. PLFA analysis comprises of four basic steps namely: 1) Lipid extraction in which single phase solvent extraction has been employed.2) Fractionation of total lipid by means of silicic acid chromatography, 3) Deriving Fatty Acid Methyl Esters (FAME's) upon methanolic transesterification and 4) Separation and quantification of FAME's [46]. The critical limitation of PLFA analysis is towards the mixed cultures. This method cannot identify specific microbes till its species level in mixed cultures and thereby can be applicable only to estimate the overall biomass communities present in the sample [47]. The traditional extraction method has become efficient by adopting two phase extraction which is also known as Bligh and Dyer method involved with mild treatment with chloroform, methanol and water [48]. Further, lipid extraction has been still improved by means of citrate buffer instead of phosphate buffer, acetate buffer, tris and water [49]. On the other hand, a new fractionation column has been developed competitive to the silicic acid

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column which is well known as solid phase extraction column [50]. Later, PLFA method has been out-turned to be efficient in terms of time, capital and reproducibility upon scaling down the volumes of both the sample and other auxiliary compounds involved [51]. PLFA analysis has been a notable technique in identifying both fungal and bacterial communities using the same sample [52]. PLFA technique has become more reliable even to distinguish substrate level utilization differences in the microbial communities particularly in the soil samples by labelling the substrates using stable isotopes such as ^{13}C [46,53].

MIDI Method

Basically, MIDI method involves five basic steps, that are 1) Harvesting of microbial cells, 2) Saponification by applying sodium hydroxide, methanol and distilled water in appropriate proportions 3) Methylation by means of hydrochloric acid and methyl alcohol, 4) Extraction using hexane and methyl tert-butyl ether and 5) Washing with sodium hydroxide and further analysis [54]. Extraction using hexane has been helpful in efficient extraction of highly volatile fatty acids [55]. MIDI method when compared to PLFA method has this unique step of saponification, is for better extraction of fatty acids to avoid any interfering compounds during further analysis [56]. MIDI method can identify microorganisms till the species level in an effective manner than the PLFA method even within small amounts of sample and can perform whole lipid analysis. Also, MIDI method can identify hydroxy fatty acids which are the signature fatty acids for certain microbes such as acetic acid bacteria, *Pseudomonas* sp, *Enterobacteriaceae* and Dimethylacetals which have been the evidence for stringent anaerobic conditions respectively unlike PLFA method [57].

EL Method

Ester Linked (EL) Method gained its own importance with the merit of extracting fatty acids without the need for prior separation of lipids from samples. This attribute made this EL method as less time consuming and simpler method than PLFA method. Also, this direct extraction of fatty acids facilitated the analysis of other fatty acids from glycolipids and neutral lipids along with PLFA's [58]. EL method has four steps. They are: 1) Extraction and methylation of fatty acids with 0.2 M KOH, a mild alkaline reagent in methanol. 2) Neutralization of pH employing 1.0 M acetic acid followed by separation of FAME's by means of centrifugation after the addition of hexane. 3) Evaporation for better recovery of FAME's using nitrogen gas [44]. The nitrogen gas exposure of FAME's also reduces the auto oxidation of polyunsaturated fatty acids [59] 4) Dissolution of FAME's in the mixture of hexane and methyl-tert-butyl ether of ratio 1:1 and further analysis of FAME's. Washing step is absent in EL method unlike MIDI method. The MIDI method can extract non-targeted fatty

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acids during the identification of microbial fatty acids. This limitation has been resolved by EL method. EL method has been reported as efficient in dealing with long-branched chain fatty acids where as MIDI method has been noted as sensible in the case of hydroxy fatty acids [44]. Even though EL method has shown rational results, PLFA method has been highlighted as better method for fungal biomass estimation due to several interference factors to EL method, depending upon fungal survival atmospheres [58]. Substantially, EL method is highly sensitive than MIDI method [60].

Important targeted organisms during each and every validation and quality control associated tests of products in pharmaceutical industry are: 1) *Aspergillus niger*, 2) *Bacillus subtilis*, 3) Bile tolerant gram negative bacteria (For example, *Aeromonas hydrophila and Lactobacillus brevis*), 4) *Candida albicans*, 5) *Clostridium sporogenes*, 6) *Escherichia coli*, 7) *Pseudomonas aeruginosa*, 8) *Salmonella enterica* and 9) *Staphylococcus aureus* [10]. The major fatty acid composition of the above mentioned organisms acquired by fatty acid profiling techniques is as follows in the Table No: 2.

Table 2: Major fatty acid composition of specified organisms obtained by Fatty acid profiling techniques

Reference Number	Organism		Composition of major fatty acids	
			In smaller amounts (<2%)	**In large amounts (>2%)**
[61]	*Aspergillus niger*		Straight chain fatty acids: C16:1	Straight chain fatty acids: C16:0, C18:0, C18:1, C18:2, C18:3
[62]	*Bacillus subtilis*		Straight chain fatty acids: C14:0, C15:0^{Me}	Straight chain fatty acids: C16:0, C18:1, C18:0, C19:0^{Me}
[63,64]	Bile tolerant gram negative bacteria	*Aeromonas hydrophila*	Straight chain fatty acids: C15:0, C18:2 Hydroxy fatty acids: OH C15:0 Cyclic fatty acids: cyc C17:0 Branched chain fatty acids: i-C15:0, i-C17:0, a-C17:0	Straight chain fatty acids: C12:0, C14:0, C16:1, C16:0, C18:1, C18:0 Hydroxy fatty acids: OH C13:0 Branched chain fatty acids: i-C17:1
		Lactobacillus brevis	Straight chain fatty acids: C12:0, C15:0, C17:0, C20:1 Cyclic fatty acids: cyc-C19:0	Straight chain fatty acids: C14:0, C16:1, C16:0, C18:1, C18:0
[55, 65]	*Candida albicans*		Straight chain fatty acids: C12:0, C14:0, C15:0, C17:0, C18:3, C24:0 Hydroxy fatty acids: 2-OH C16:0 Branched chain fatty acids: a-C17:0	Straight chain fatty acids: C16:1, C16:0, C18:2, C18:1, C18:0
[66, 67]	*Clostridium sporogenes*		Straight chain fatty acids: C10:0, C12:0, C13:0, C17:0 Hydroxy fatty acids: 3-OH C16:0, 3-OH C14:0	Straight chain fatty acids: C14:0, C14:1, C15:0, C16:0, C16:1, C18:0, C18:1 Hydroxy fatty acids: 2-OH

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		Dimethylacetals: C14:1 cis 7 DMA, C16:0 DMA, C15:0 DMA	C11:1 Branched chain fatty acids: C15:0, C16:0 Dimethylacetals: C14:0 DMA, C16:1 cis 9 DMA, C18:1 cis 9 DMA
[62]	*Escherichia coli*	Straight chain fatty acids: C10:0 Cyclic fatty acids: cyc-C19:0	Straight chain fatty acids: C12:0, C14:0, C16:0, C16:1, C18:0, C18:1 Hydroxy fatty acids: OH C14:0 Cyclic fatty acids: cyc-C17:0
[68]	*Pseudomonas aeruginosa*	Straight chain fatty acids: C14:0, C15:0, C17:0, C18:0, C19:0	Straight chain fatty acids: C12:0, C16:0, C16:1, C18:1 Hydroxy fatty acids: 3-OH C10:0, 2-OH C12:0, 3-OH C12:0 Cyclic fatty acids: cyc-C17:0, cyc-C19:0
[69]	*Salmonella enterica*	Straight chain fatty acids: C15:0, C17:0, C18:0, C19:0 Hydroxy fatty acids: 2-OH C14:0 Cyclic fatty acids: cyc-C17:0, cyc-C19:0	Straight chain fatty acids: C12:0, C14:0, C16:0, C16:1, C18:1 Hydroxy fatty acids: 3-OH C14:0
[70]	*Staphylococcus aureus*	Straight chain fatty acids: C14:0, C19:0 Branched chain fatty acids: i-C14:0, i-C16:0, i-C18:0, i-C19:0	Straight chain fatty acids: C16:0, C18:0, C20:0 Branched chain fatty acids: i-C15:0, a-C15:0, i-C17:0, a-C17:0, a-C19:0

- Short hand representation of fatty acid is 'CN:M', in which C-carbon, N-Number of carbon atoms and M-Number of double bonds present; short hand representation of hydroxyl group is n-OH, in which n-Position of the hydroxyl group present; Short hand representation of DMA is 'CN:M cis m DMA' where DMA-dimethylacetals, m-Position of the double bond; Abbreviations: Me-methyl; cyc-Cyclic; i-iso; a-anteiso.
- The above fatty acid compositions of organisms may change depending upon various environmental factors.

Detection techniques of FAME's

"Gas chromatography (GC) is a predominant method for fatty acid analysis''. Reasons:

GC has been developed and adopted as predominant technique among most of the analytical techniques for the analysis of fatty acids because it has not only been simple in terms of cost, equipment handling and sample preparation but also rapid in detecting FAME's relatively. As pure targeted lipid extraction is difficult, this may result in interferences during analysis. In this context, GC is a better option than any other techniques such as NMR spectroscopy [71].

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"Fatty acids have to be converted to FAME's". Reasons:

The fatty acids have been converted to FAME's to make them volatile thus enabling them to undergo gas chromatographic analysis conveniently, along with the flexible free fatty acid detection [72]. Few polyunsaturated fatty acids (PUFA's) are highly polar compounds which can form hydrogen bonds with the stationary phase during analysis, resulting adsorption of these compounds onto the column. Hence this problem can be resolved by converting them to FAME's thereby neutralizing their carboxyl groups. This will also facilitate better differentiation among PUFA's by means of their degree, position and configuration of unsaturation [73].

Different detection techniques for fatty acid profiling:

GC is a versatile technique for fatty acid profiling. The efficiency of gas chromatographic separation of fatty acids mainly depends on polarity of stationary phase, selection of column and detector. With this perspective, capillary column has been claimed as better option than the packed column [73]. GC has been coupled with Flame ionization detector (FID), Mass spectrometry (MS) [71,74] and Vacuum ultra violet (VUV) detector [75,76] to obtain better fatty acid profiles. GC-FID has been recorded as efficient in detecting saturated fatty acids where as GC-MS has been versatile towards unsaturated fatty acids comparatively [71]. MS has been noted as successful technique to obtain spectrums of spores with ionic peaks of both dipicolinic acid and other fatty acids [77]. One dimensional GC-MS has limited efficiency while dealing with diverse PUFA's as they can co-elute during the detection. This limitation has been rectified by two-dimensional gas chromatography (GC-GC) with better information on unsaturation of fatty acids [74]. Even though conventional detectors such as FID and MS have shown predominant advantages in GC analysis of fatty acids, they bear few limitations namely difficulty in differentiating certain isomeric, isobaric and small chemically labile compounds along with the requirement of columns with complex compositions. These limitations have been resolved by VUV detector. VUV detector have acquired importance with notable features such as deconvolution of co-eluted signals [76], time effective and better differentiation of *cis*/*trans* isomers as photons in spectral range of this detector can trace almost every small energy excitations in different compounds [75,76]. Near infrared reflectance spectroscopy (NIRS) have been addressed as a potential alternative to GC for fatty acid analysis of microalgae and various foods as NIRS is reported as non-destructive, less time consuming and less complicated technique comparatively [78,79]. But this technique has not been utilized yet in fatty acid profiling of microorganisms.

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Validation perspectives

Any validation process holds certain parameters such as Accuracy, Limit of detection, Limit of quantification, Linearity, Precision, Robustness and Specificity [80]. GC has been validated and declared as a rapid method for fatty acid profiling by the development of various afore mentioned generations of detectors. Recently GC-FID involving simple sample preparation has been validated and declared as successful application for rapid profiling of oleic acid and related fatty acids upon evaluating certain validation parameters significantly [81]. The cross-validation studies of PLFA profiles by computational neural networks and linear discriminant analysis revealed that the PLFA profiles can be analyzed with better sensitivity by computational neural networks than traditional statistical methods [82]. During validation of MIDI method, *Bacillus subtilis* ATCC 6633 and *Stenotrophomonas maltophilia* ATCC 13637 have been selected as prominent challenging organisms. *Bacillus subtilis* is the most sensitive organism which can easily reveal problems in sample preparation procedure of MIDI method. As *Stenotrophomonas maltophilia* has complex fatty acid composition among aerobic bacteria, it can also trace any issues while dealing with complex fatty acids, during each and every step of sample preparation for GC analysis [83]. MIDI method has been validated and been proclaimed as sensitive in profiling fungal fatty acids particularly in differentiating different species and genera of *Rhizoctonia*-like fungi [84].

CONCLUSION

ATP Bioluminescence and Fatty acid Profiling are the rapid Cellular Component-based methods. ATP bioluminescence is a well-known and effectively validated technique for measuring the cellular viability among other cellular component-based technologies. Fatty acid profiling is for fingerprinting fatty acid profiles for the identification of various microorganisms. Fatty acid profiling for identification of microorganisms in pharmaceutical field is under the process of validation and have not been well-documented yet. Technically, any RMM technique being highly advantageous in fastidious definition of microbial quality of a product resulting in early product release, has its own disadvantages. Difficulties in installation, validation and maintenance of RMM systems can be balanced by the fact that RMM is not labor-intensive. As RMM will reduce the man power requirement, concerned professionals have to update themselves with relative technical knowledge or can be trained and employed in R&D Department to improve and maintain consistency in the scope of development and validation of rapid microbiological methods. Thereby in the long run, limitations of RMM can be conceded. Hence, this review can pave the way for successful application of ATP Bioluminescence and Fatty acid Profiling in each and every industry

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which conducts microbial examinations and also to extend the research on RMM such that it can replace a traditional method in total.

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