Research Article

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Isoprenoid Quinone Composition of the Genus Microbacterium and Related Strains

Mohammed Nizar Battikhi*

De Montfort University, School of life Science, Leicester University Department of Microbiology, England

*Corresponding author: Moh'd Nizar Battikhi, 1017-1645 De Maisonneuve O, Montreal H3H 2N3, QC, Canada.

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Abstract

The aim of this study is to compare pattern of Isoprenoid quinine of unspecified bacterial isolates from dairy product to reference strains type species of the genus *Microbacterium*.

Method: Twenty-eight strains of coryneform and related bacteria were studied for isoprenoid quinine content. Extraction of 100 mg of freeze-dried cells by chloroform: methanol (2:1v/v), extract was then run onto analytical TLC plates coated with 0.5 mm Merck Silica Gel HF245.

Results: Out of the twenty-eight reference and unspecified strains *M. lacticum* and *M. flavum* are quite distinct and that the predominant of MK-8 in BL77/18, BL77/19 and BL77/20 support that these strains are related to *M. flavum*.

Keywords: Micro bacterium; Isoprenoid quinone; TLC

Introduction

Coryneform bacteria classification in Bergy's Manual of Bacteriology [1] accommodate different genera as *Cellulomonas*, *Arthrobacter*, *Corynebacterium* and *Microbacterium* described as incertae sedis. Other motile plant pathogens were added to coryneform group [2,3]. Numerical phonetic studies improved classification of coryneform [4,5]. Other markers were used to improve taxonomic position of coryneform bacteria such as DNA base composition determination [4,6-10], wall analysis [11-15] and mycolic acid analysis [2,15,16]. The use of additional marker (isoprenoid quinone) was recommended by [16,17] to improve classification of coryneform and related taxa. The analysis of the isoprenoid quinone of coryneform bacteria has been a useful aid to establish the taxonomic relatedness of bacterial strains [2, 3,17-20]. These quinines can be divided into menaquinones and ubiquinones abbreviation of their data is given in (Figure1a). Substantial investigation amongst coryneform have been carried out on the distribution of quinines bearing isoprenoid side chain. Bacterial quinones of this type can be sub-divided into menaquinones (2 methyl-3-polyisoprenyl 1-1,4 naphthoquinones and ubiquinones (co-enzyme Q; 12,3-dimethoxy-5-methyl-1-6-polyisoprenyl-1-1,4 benzoquinone (Figure1b). The menaquinones

(related to vitamin K) show structural variation at C-2 and ,more especially, at C-3. Variation at C-3 include the length and degree of un-saturation of the polyprenyl side-chain [22] and the presence of hydroxyl [21] and epoxide groups [22]. Similarly, ubiquinones show variation principally in the structure of polyprenyl sidechain [23]. Therefore, the structure of these compound might be of great value in classification of coryneform and related bacteria [23]. Other studies demonstrated that organisms can be clustered based on the isoprenoid side chain of the menaquinones [2,3,20,24,25]. From their analysis Minnikin et al [22] concluded that MK₈(H₂) was the major menaquinones of the animal associate *corynebacterium*. *Microbacterium flavum*, with consistently shows affinities with the animal associated corynebacteria in numerical phonetic studies, also contains MK₈(H₂). They also reported that Micro bacterium ammoniaphilum contained MK₉(H₂). Isoprenoid quinines are membrane-bound compounds (lipid molecules) found in nearly all living organisms. It marked structural variation depending upon the microbial taxon [27-30]. Therefore, organisms can be clustered based on isoprenoid side chain of their menaquinones. and hence differentiation between various taxa could be improved [3,17,18,27].

Figure1a: Menaquinone.

Figure1b: Ubiquinone.

Method

Twenty-eight strains of coryneform and related bacteria were collected for the study. These were obtained from public and private culture collections (Table 1). Each strain was given number for ease handling. Several strains representing different taxa defined

in study [4,5] where numerical phonetic surveys of coryneform and related bacteria were included as references. The unidentified strains were received from the National Institute of Research in Dairying, Sheffield, Reading and food Research institute, Norwich. All strains were originally obtained in freeze dried cultures.

Table 1: Menaquinone composition of strains.

Menaquinone Isoprenoid		MK6	MK7	MK8	MK9	MK10	MK11	MK12	MK13
<i>Bacteroid egesthii</i>	B6g				+	+++	++		
<i>Brevibacterium imperial</i>	C814					+	++	+++	+
<i>Brevibacterium splanchnices*</i>	B5				+	+++	+		
<i>Brochothrix thermosphactum</i>	C20	+	+++						
<i>Corynebacterium laevaniformans</i>	C625					+	+++	++	+
<i>Corynebacterium mediolanum</i>	C69					+	++	+++	+
<i>Corynebacterium okanaganae</i>	CSP					+	++	+++	+
<i>Corynebacterium oorii</i>	C617			+	+++				
<i>Corynebacterium oortii</i>	C618			+	++				
<i>Microbacterium lacticum</i>	C88			+	++	++	+++	++	++
<i>Microbacterium lacticum</i>	C89				+	+	+++	++	++
<i>Microbacterium lacticum</i>	C787		+	+	+	++	+++	++	++
<i>Microbacterium lacticum</i>	C788		+	+	+	++	+++	++	++
<i>Microbacterium lacticum</i>	C789		+	+	+	++	+++	++	++
<i>Microbacterium flavum</i>	C90		+	+++		+			
<i>Microbacterium lequefaciens</i>	C770			+	+	+	+++	++	+
<i>Microbacterium lequefaciens</i>	C771			+	+	+	+++	++	+

JP2/1/6							+	+++	++
JP2/6/5						+	+++	++	
JP2/1/1						+	++	+++	
JP2/1/21						+	++	+++	
JP2/1/11			++	+++					
JP2/1/20		+	++	+++					
JP2/1/15		+	++	+++					
JP2/1/3		+	++	+++					
JP2/1/18				+++	++				
JP2/1/19			+	+++	++				
JP2/1/20			+	+++	++				

General Growth Media

Strains were routinely subculture at three weeks intervals onto nutrient agar (Difco) plates and stored at 4°C. After 2-3 days incubation at 37 °C. For broth culture Nutrient broth Media (Oxoid) media was and culture were stored at lyophilized aliquots in glass vials for future used were they resuscitated by transfer small amount of nutrient onto nutrient agar plates and incubated for 7 days. Nutrient agar (Difco) and nutrient broth (Oxoid) were used for growing all isolates. All media were sterilized by autoclaving at 121 °C for 15min. Culture were incubated for 24-48 H at 37 °C. Cultures were harvested by centrifugation at 8,000 rpm for 20min, in an MSE high speed M-18 centrifuge, washed in distilled water and re-harvested Organism were lyophilized and stored anhydrously as a fine powder until required and freeze dried for future used. Isoprenoid quinone analysis done by extraction of 100mg portions freeze dried organisms by a method described by Collins et al. [17] that proved to be satisfactory for the analysis of lipids. At the preparation phase, components with chromatograph with vitamin K were the only isoprenoid quinines which were detected. Ultraviolet spectroscopy of the eluted quinines showed absorption maxima at 242, 248, 260, 270 and 326nm (Figure 2), feature which are in accordance with the published data for menaquinones [28] where 100mg of freeze dried cells were mixed with 20ml chloroform: methanol (2:1 v/v) in a 50ml conical flask and the suspension stirred for 16-18h in the dark (isoprenoid quinine are susceptible to strong light). Organisms were removed by filtration and the extracts were evaporated to dryness under reduced pressure and teem below 37 °C. Components were detected by observing chromatograms under short-wave (254nm) ultra-violet light. The dried extracts were prepared as described in above then were re-suspended in small volume of chloroform: ethanol (2:1 v/v) and spotted onto analytical TLC plates coated with 0.5mm Merck Silica Gel HF245 (prepared by coating plates with slurry contains 40g gel HF245 per 100ml distilled water and drying overnight at 65 °C).

Plates were developed in petroleum ether (BP 60-80): diethyl ether (85:15 v/v). In this solvent system, the menaquinones migrate further (Rf 0.7) than ubiquinones (Rf 0.4). Vitamin K (sigma) an Ubiquinone's -50 (BDH) were included as standard. The isoprenoid quinines were also isolated by eluting with chloroform from preparative TLC plates (1 mm, Merck silica Gel HF245) using the same solvent system. The quinines were detected by brief

irradiation with ultraviolet light (245nm) when they appeared as dark brown spots on a bright green fluorescent background. Eluted samples were evaporated to dryness by a method based on Collins et al [17] proved to be satisfactory for analysis of lipids at the preparation phase, components with chromatographed with vitamin K were the only isoprenoid quinines which were detected. Ultraviolet spectroscopy of the eluted quinones showed maxima at 242, 248, 269, 270 and 326nm (Figure 2) feature which are in accordance with the published data from menaquinones [28]. The isoprenoid quinine was separated by reverse phase partition chromatography using appropriate standards. Components were detected by observing chromatogram under ultraviolet light (254nm).

Figure 2: Absorption spectrum of isoprenoid quinine extracted from *M. lactecum*.

Result

In describing the menaquinones of strains, the following nomenclature was adopted. The symbol MK denoting menaquinone is followed by a number (e.g. 8, 9 or 10) denoting the number of isoprene units attached as a side chain (Table 2). A wide range of manaquinones with varying numbers of isoprene units were characterized within the group of the menaquinones although only one type predominated of *Corynebacterium*, oortii (strain C617 and C618) was MK9, a result in agreement with other study [20]]. Likewise, the major quinine of *Microbacterium flavum* (C90) was MK8, again in agreement with published data [17,29]. In this study the menaquinones of 28 reference strains of Micro bacterium,

Corynebacterium and unknown isolates were examined. The five strains of *M. lacticum* (C88,C,89,C787,C788.C789) tested were all contain eleven and twelve isoprene units as the predominant side chain of their menaquinone in contrast *M. flavum* (C90) was shown to contain MK-8 as the predominant menaquinones which is in agreement with other study by [2]. These observations agree with conclusion that *M. lacticum* and *M. flavum* are quite distinct, likewise the major menaquinone (MK-7) of *Brochothrix thermosphacta* (*Microbacterium thermosphactum*) support the other evidence that this species is quite distinct from both *M. lacticum* and *M. flavum*. The presence of MK-11 and MK12 in *Corynebacterium laevaniformans* (C626) agrees with other study [20]. Strains

JP2/1/6 and strain JP2/1/5 were both contain MK-11 and MK12, which is similar to *M. lacticum* that showed resemblances. *M. liquefaciens* (C770, C771), *Corynebacterium mediolanum* (C69) and *Brevibacterium imperialis* (C25) all shown to contain MK-11 and MK-12 a feature consistent with earlier study [19]. The unnamed strains used in this study showed that the predominant of MK-8 in BL77/18. BL77/19 and BL77/20 support that these strains are related to *M. flavum*. The similar menaquinones profiles of strains JP2/1/1 and JP2/1/21 which contain MK-11 and MK-12 supports their taxonomic relatedness but suggest that they may not closely related to *M. flavum*. The predominant of MK-8 in strains.

Table 2: Isoprenoid quinone of coryneform and related bacteria (Abstracted from Minnikin et al. [3]).

Major isoprenoid quinone	Organisms
MK-9(H2)	<i>Arthobacter albidus</i>
	<i>Arthobacter globiformis</i>
	<i>Arthobacter ureafaciens</i>
	<i>Brevibacterium ammoniagenes</i>
	<i>Brevibacterium flavum</i>
	<i>Brevibacterium lactofermentum</i>
	<i>Brevibacterium roseum</i>
	<i>Corynebacterium bovis</i>
	<i>Corynebacterium glutamicum</i>
	<i>Corynebacterium melassecola</i>
	<i>Corynebacterium xerosis</i>
	<i>Microbacterium ammoniaphilum</i>
	<i>Rhodococcus bronchiale</i>
	<i>Rhodococcus corallines</i>
MK-8(H2)	<i>Arthobacter roseoparaffinis</i>
	<i>Brevibacterium linens</i>
	<i>Corynebacterium diphtheria</i>
	<i>Corynebacterium equi</i>
	<i>Corynebacterium fasciens</i>
	<i>Corynebacterium murium</i>
	<i>Corynebacterium ulcerans</i>
	<i>Microbacterium flavum</i>
	<i>Rhodococcus erythropolis</i>
MK-8(H4)	<i>Arthobacter simplex</i>
	<i>Brevibacterium lipolyticum</i>
MK9-(H4)	<i>Cellomonas fimi</i>
	<i>Cellomonas flavigena</i>
	<i>Oerskovia turbata</i>
MK-11	<i>Brevibacterium testaceum</i>
Q-10	<i>Corynebacterium autotrophicum</i>
	<i>Microbacterium flavum</i>

Discussion

The analysis of isoprenoid quinones of coryneform bacteria has been a useful aid to establishing the taxonomic relatedness of strains [2,18-21,26]. These quinones can be divided into menaquinones and ubiquinones. By far the majority of coryneform contains menaquinones although different groups produce menaquinones with different numbers of isoprene units and may also differ in their

degree of saturation. These differences appear to be taxonomically significant [26]. Thus, organism regarded as closely related on other evidence (numerical taxonomic data, cell-wall composition,) often have similar menaquinones while unrelated organisms frequently contain different components.

In this study the menaquinones of 28 strains were examined. The five strains of *M. lacticum* (C88, C89, C787, C788, C789) used

all contained eleven and twelve isoprene units as the predominant side chain of their menaquinones. In contrast *M. flavum* (C90) was shown to contain MK-8 as the predominant menaquinone which agrees with other study [26]. These observations agree with the conclusion that *M. lacticum* and *M. flavum* are quite distinct. Likewise, the major menaquinone (ML-7) of *Brochothrix thermosphata* (*Microbacterium thermosphactum*) supports the other evidence that this species is quite distinct from both *M. lacticum* and *M. flavum*. The presence of MK-11 and MK-12 in *Corynebacterium* lve K-11 and MK-12 which showed their relatedness to each other and similar to *M. lacticum*. The significant of manaquinone in taxonomy was reported by Collins et al [18].

They showed that, amongst other criteria, the curtobacteria can be divided into two groups based on their menaquinones. The significant of menaquinone marker in taxonomy was clearly appeared by showing relatedness of unknown isolates as BL77/18, BL77/19, BL77/20 to each other and to *M. flavum* since they showed MK-8. Likewise, the similar menaquinones profiles of strain JP2/1/1 and JP2/1/21 which contain MK-12 supports their similar taxonomic position but they are closely related to *M. flavum*. Likewise, our result in this study showed the presence of MK-9 in strains JP2/1/11, JP2/1/20, JP2/1/15 and JP2/1/3 which suggest resemblance of these strains.

Conclusion

This study showed the significant of isoprenoid quinine as marker of clustering different strains according to their menaquinone content which may lay base line for future taxonomy. However, other markers may be need for further support.

Acknowledgement

None.

Conflict of Interest

No conflict of interest.

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