Antimicrobial activity of fatty acids against Bacillus larvae, the causative agent of American foulbrood disease

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Original article

Antimicrobial activity of fatty acids against *Bacillus larvae*, the causative agent of American foulbrood disease

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Summary — Various saturated and unsaturated free fatty acids were tested for their antibiotic activity against *Bacillus larvae*, the causative agent of American foulbrood disease of honey bee larvae. Saturated compounds containing > 14 carbons were inactive. Antimicrobial activity could be restored to the longer chain length compounds by the addition of one or more double bonds. The antibiotic activity of fatty acids may make them suitable for use in the prevention and control of foulbrood diseases of honey bees.

foulbrood / antimicrobial activity / fatty acids / *Bacillus larvae*

INTRODUCTION

The report by Shimanuki *et al* (1992) that an ethanolic extract of chalkbrood mummies inhibited the growth of *Melissococcus pluton* and *Bacillus larvae*, the causative agents of European and American foulbrood diseases of honey bee larvae, respectively, led us to seek the identity of the active compound(s). In the previous paper (Feldlaufer *et al*, 1993) we reported the isolation of an active antimicrobial agent identified as linoleic acid from mycelia and spores of the chalkbrood fungus, *Ascosphaera apis*. In an extension of this study, we have tested various saturated and unsaturated free fatty acids for antibiotic activity against *B larvae* in an effort to correlate antimicrobial activity with fatty acid structure.

MATERIALS AND METHODS

Fatty acids

Fatty acids were obtained from Nu-Chek Prep, Inc (Elysian, MN, USA). Stock solutions (10 mg/ml) and dilutions (1:10, 1:100, and 1:1 000) were prepared in benzene.

Bioassay

Twenty-five µl of the test solutions were applied to paper discs with an electronic pipet, supplying final amounts of 250, 25, 2.5 and 0.25 µg of fatty acid per disc. After drying, the discs were placed in the center of agar plates containing *B larvae* spores. Discs treated with benzene served as controls. Active compounds exhibited measurable zones of inhibition after 3 d. Full details of the bioassay and incubation conditions have been previously described (Feldlaufer *et al,* 1993).

Nomenclature

Common names for fatty acids are used where available (Weete, 1974, 1980). Abbreviated symbols are also used; the number preceding the colon indicates the number of carbon atoms in the fatty acid, while that following the colon represents the number of double bonds. For example, 18:0 would refer to stearic acid, while 18:2 would refer to linoleic acid. Configuration of double bonds is *cis*, unless otherwise stated.

RESULTS

The antimicrobial activity of various saturated and unsaturated fatty acids against *B larvae* is shown in table I. Among the saturated fatty acids, activity increased with chain length from 6 carbons to 12 carbons, and then decreased. Activity was greatest with lauric acid (12:0), and any saturated fatty acid > 14 carbons in length was inactive (15:0 through 18:0).

Activity could be enhanced or restored to the longer chain lengths by the addition of a double bond. For example, while myristic acid (14:0) was only slightly active (10 mm inhibition zone), myristoleic acid (14:1) exhibited antimicrobial activity equal to that of lauric acid (80 mm). Additionally, while palmitic acid (16:0) was inactive, the corresponding unsaturated fatty acid, palmitoleic (16:1) was quite active (72 mm). However, once fatty acid chain length consisted of or exceeded 18 carbons, more than one double bond was necessary to inhibit bacterial growth. Polyunsaturated fatty acids, consisting of 3, 4 or 6 double bonds, also showed activity even when the chain length consisted of 22 carbons, though this activity was somewhat reduced (see table I). Among the active unsaturated fatty acids, the *cis* configuration was always more active than the *trans* configuration.

Since unsaturated fatty acids are susceptible to oxidation and are therefore considerably less stable than their saturated counterparts, we tested only several of the active fatty acids for antimicrobial activity at lower concentrations (table II). While all of those tested inhibited *B larvae* at a dose of 25 µg per disc, only lauric (12:0), tridecanoic (13:0), palmitoleic (16:1) and linoleic (18:2) acid exhibited activity at 2.5 µg per disc. No fatty acid tested was active at the lowest dose (0.25 µg).

DISCUSSION

Our isolation of linoleic acid as an antimicrobial agent from the fungus *Ascosphaera apis* (Feldlaufer *et al,* 1993), led us to examine a variety of saturated and unsaturated fatty acids for antibiotic activity. Our results are in general agreement with others that have investigated fatty acids as antimicrobial agents (see Kabara, 1978; Kabara *et al,* 1972). We found that both lauric and tridecanoic acid were the most active saturated fatty acids, while palmitoleic and linoleic acid were most active of the unsaturates at the lower doses tested. Introduction of a double bond or multiple double bonds is necessary to maintain antibiotic activity once the chain length of the fatty acid exceeds 14 carbons. Double bonds in

Table I. Antimicrobial activity of various saturated and unsaturated fatty acids against *Bacillus larvae*.

Fatty acids [a]		*Position of the double bond* [b]	*Inhibition zone (mm)* [c]
6:0	(caproic)		I
8:0	(caprylic)		18
9:0	(pelargonic)		40
10:0	(capric)		54
11:0	(undecanoic)		60
12:0	(lauric)		80
13:0	(tridecanoic)		40
14:0	(myristic)		10
14:1	(myristoleic)	9	80
15:0	(pentadecanoic)		I
16:0	(palmitic)		I
16:1	(palmitoleic)	9	72
16:1	(palmitelaidic)	9 *t*	30
17:0	(heptadecanoic)		I
18:0	(stearic)		I
18:1	(petroselinic)	6	I
18:1	(petroselaidic)	6 *t*	I
18:1	(oleic)	9	I
18:1	(elaidic)	9 *t*	I
18:1	(vaccenic)	11	I
18:1	(transvaccenic)	11 *t*	I
18:1	(ricinoleic)	9 (12-Hydroxy)	60
18:1	(ricinelaidic)	9 *t* (12-Hydroxy)	45
18:2	(linoleic)	9,12	68
18:2	(linoelaidic)	9*t*,12 *t*	40
18:3	(linolenic)	9,12,15	52
18:3	(γ-linolenic)	6,9,12	55
20:1	(11-transeicosaenoic)		28
20:2	(11,14-eicosadienoic)		I
20:3	(homo γ-linolenic)	8,11,14	40
20:3	(11,14,17-eicosatrienoic)		40
20:4	(arachidonic)	5,8,11,14	50
22:1	(erucic)	13	I
22:1	(brassidic)	13 *t*	I
22:2	(13,16-docosadienoic)		28
22:3	(13,16,19-docosatrienoic)		40
22:4	(7,10,13,16-docosatetraenoic)		50
22:6	(4,7,10,13,16,19-docosahexenoic)		52
Benzene	(control)		I

[a] The first number is the number of carbons followed by the number of double bonds. Common or systematic names are given in parentheses. [b] Carbonyl carbon is number 1. All configurations are *cis* unless designated as *trans (t)*. [c] Fatty acids were bioassayed against *Bacillus larvae*. Treated paper discs (250 μg fatty acid in benzene) were placed in the center of agar plates containing *B larvae* spores. After 3 d the diameter (mm) of the circular zone of inhibition was measured (average of at least 4 replicates). I : inactive.

Table II. Antimicrobial activity of selected fatty acids against *Bacillus larvae*. *

Fatty acids	*Dosage (µg/disc)* 250	25	2.5	0.25
	Inhibition zone (mm)			
10:0 (capric)	54	I	I	I
11:0 (undecanoic)	60	18	I	I
12:0 (lauric)	80	34	14	I
13:0 (tridecanoic)	40	30	14	I
14:1 (myristoleic)	80	60	I	I
16:1 (palmitoleic)	72	50	18	I
18:2 (linoleic)	68	38	4	I

* Explanation as in table I (except for dosage).

the *cis* configuration bring the carboxyl end of the molecule closer to the methyl end by bending the molecule, so the overall length of the molecule is probably important in maintaining antibiotic action.

The demonstration that certain saturated and unsaturated fatty acids inhibit the bacterium responsible for American foulbrood disease of honey bees should stimulate research directed at a practical application. As control agents, fatty acids would be safe and environmentally-sound. In addition, their relatively low cost would make them prime candidates for use in prevention programs.

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Résumé — Activité antimicrobienne d'acides gras contre *Bacillus larvae*, agent de la loque américaine. L'activité antibiotique de l'acide linoléique contre la bactérie responsable de la loque américaine chez les abeilles nous a conduit à examiner l'activité antimicrobienne d'autres acides gras libres saturés et insaturés. Le test biologique a consisté à exposer des spores de *Bacillus larvae* à des disques de papier traités avec 250 µg des composés étudiés et à mesurer la zone d'inhibition de croissance au bout de 3 j (tableau I). Il en ressort que l'activité antimicrobienne des acides gras saturés augmente avec la longueur de la chaîne jusqu'à 12 carbones (acide laurique). Les acides gras saturés dont la chaîne contient plus de 14 carbones sont inactifs. L'activité peut être accrue, ou restaurée chez les composés qui ont les plus grandes longueurs de chaîne, en ajoutant une ou plusieurs doubles liaisons. Certains des composés les plus actifs ont été testés à des doses inférieures (tableau II). Tous les acides gras testés ont été actifs à 25 µg par disque, tandis que seuls les acides laurique, tridécanoïque, palmitoléique et linoléique sont restés actifs à la dose de 2,5 µg par disque. La preuve que les acides gras saturés et insaturés inhibent la croissance de la bactérie de la loque américaine devrait encourager les recherches en vue d'une application pratique. Les acides gras sont sans danger, respectueux de l'environnement et bon marché.

loque américaine / *Bacillus larvae* / acide gras / antimicrobien

Zusammenfassung — Die antimikrobielle Aktivität von Fettsäuren gegen *Bacillus larvae*, dem Erreger der Amerikanischen Faulbrut. Die antimikrobielle Aktivität der Linolsäure gegen die für Faulbrutkrankheiten der Honigbiene verantwortlichen Bakterien führte uns zur Untersuchung weiterer gesättigter und ungesättig-

ter Fettsäuren hinsichtlich ihrer mikrobiellen Aktivität. Es wurden Biotests durchgeführt, bei denen Sporen von *Bacillus larvae* mit Papierscheiben in Berührung gebracht wurden, die mit 250 µg der Prüfsubstanz getränkt waren; nach 3 Tagen wurde die Hemmzone gemessen (Tabelle I). Dabei ergab sich, daß die antimikrobielle Aktivität der gesättigten Fettsäuren mit der Kettenlänge bis zu 12 Kohlenstoff-Atomen (Laurinsäure) zunimmt. Gesättigte Fettsäuren von einer Kettenlänge von mehr als 14 C-Atomen waren inaktiv. Die Aktivität konnte durch Einfügung einer oder mehrerer Doppelbindungen gesteigert, bzw bei den längeren Ketten wiederhergestellt werden. Einige der stärker aktiven Verbindungen wurden einem Biotest in niedrigeren Dosen unterworfen (Tabelle II). Alle getesteten Substanzen waren bei 25 µg pro Scheibe aktiv, während Laurin-Iridekan-, Palmitolein- und Linol-Säure schon bei 2,5 µg pro Scheibe aktiv waren. Der Nachweis der Hemmwirkung gesättigter und ungesättigter Fettsäuren auf das Wachstum des Bakteriums der Amerikanischen Faulbrut sollte Forschungen in Richtung der praktischen Anwendung ermutigen. Fettsäuren sind sicher, umweltverträglich und sie sind billig.

***Bacillus larvae* / Faulbrut / Antimikrobielle Aktivität / Fettsäuren**

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