



Review

Royal Jelly: An ancient remedy with remarkable antibacterial properties



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ABSTRACT

Royal Jelly (RJ), a honeybee hypopharyngeal gland secretion of young nurse and an exclusive nourishment for bee queen, has been used since ancient times for care and human health and it is still very important in traditional and folkloristic medicine, especially in Asia within the apitherapy.

Recently, RJ and its protein and lipid components have been subjected to several investigations on their antimicrobial activity due to extensive traditional uses and for a future application in medicine.

Antimicrobial activities of crude Royal Jelly, Royalisin, 10-hydroxy-2-decenoic acid, Jelleines, Major Royal Jelly Proteins against different bacteria have been reported. All these beehive products showed antimicrobial activities that lead their potential employment in several fields as natural additives. RJ and its derived compounds show a highest activity especially against Gram positive bacteria.

The purpose of this Review is to summarize the results of antimicrobial studies of Royal Jelly following the timescale of the researches. From the first scientific applications to the isolation of the single components in order to better understand its application in the past years and propose an employment in future studies as a natural antimicrobial agent.

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1. Introduction

Royal Jelly (RJ) is a glandular secretion white-yellowish (Fig. 1), gelatinous-viscous sour taste, with a slight characteristic smell of phenol (which gives it its characteristic flavour) produced from the hypopharyngeal and mandibular salivary glands of young nurse (bees aged between 5 and 14 days) (Chauvin, 1968; Fujita et al., 2013). RJ is the exclusive nourishment for all bee larvae, from hatching to the third day of life; those larvae which are selected

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to develop into queens are fed with RJ until the fifth day of larval life (the time at which the cell is operculated), and then RJ remains a dedicated feed for the queen bee alimentation for the duration of her life. Furthermore, RJ also has a significant impact on the life span: a worker bee lives around 45 days, while a queen bee could live up to five years during which is able to spawn in a day the equivalent of her weight in eggs (approximately 2000–3000 eggs per day for several years).

Storage conditions of RJ for its human employment is a critical point for maintain unchanged its properties; RJ is light and heat susceptible and undergoes oxidation to a direct contact with air (Bogdanov et al., 2004; Buttstedt et al., 2013; Kheyri et al., 2012; Sabatini et al., 2009; Scarselli et al., 2005; Zhang et al., 2012).

1.1. Historic background

The first historical notes about human employment of RJ date back to ancient Greece; Greeks thought that the “ambrosia”, the nectar which gave immortality to the gods of Olympus, was composed in part by RJ. At that time it was already consumed without knowing its specific effects, and historians reported that the honeycombs were shredded with inside honey, larvae, propolis, pollen and RJ and eaten fresh (Cassaignau, 1991; Mráz, 1995). Aristotle was the first to have discovered the function of RJ in the bees society and, by studying its effects in queen bee, he attributed to the consumption of RJ an increase of physical strength and, above all he supposed its role in an improvement of intellectual capacity; the breakfast of his school was exclusively made with honey and RJ (Domerego, 2001; Molan, 1999). In ancient Egypt, RJ was used like a cosmetic, which reached its zenith of notoriety with Cleopatra, as one of her personal beauty secrets. Furthermore, in that period RJ became a symbol of strength and majesty of the Pharaohs, which usually ate RJ (Emonet, 2001; Levet, 2008). In Asia, specifically in China, RJ is used in traditional medicine since ancient time. This product of beekeeping, which was produced exclusively in the sovereign gardens, was correlated with the longevity and the sexual force, even in old

age, of ancient dynasties of China (Cherbuliez and Domerego, 2003; Contessi, 2010). Jan Swammerdam (1637–1680), a Dutch naturalist, microscopist and entomologist, was the first to described the compound of nourishment in the royal cell and discovered that the “beehive chief” is a queen and not a king as supposed until the seventeenth century (Contessi, 2010; Viel and Doré, 2003). The French scientist René Antoine de Réaumur (1683–1757) coined the term “Royal Jelly” to name the feed of queen bee and he related the assumption of RJ with the exceptional growth of the queen (Cherbuliez and Domerego, 2003; Molan, 1999). In 1852 Reverend Langstroth, known as the father of American beekeeping, was the first to analysed chemically RJ, however he used methods did not guarantee a scientifically significant information (Domerego, 2001; Levet, 2008). Langstroth also proposed during the fifties the use of RJ as a commercial product, especially in areas where the production of honey was not profitable (Contessi, 2010; Viel and Doré, 2003). The use of RJ as a functional product and health enhancer was investigated since the early 60s, with the development of the “Apitherapy”. From then on, particularities and properties of RJ were discovered and RJ reached a widely used in therapy for both men and bee itself (Contessi, 2010; Molan, 1999).

2. Composition

RJ is an acid colloid (3.6–4.2 pH) composed mainly by water, sugar, proteins, lipids, vitamins and some mineral salts (Melliou and Chinou, 2005; Ramadan and Al-Ghamdi, 2012; Vecchi et al., 1993).

The major component is water, ranged from 60% to 70% (Caboni et al., 2004; Melliou and Chinou, 2005), followed by carbohydrates from 11% to 23% (Sabatini et al., 2009; Sesta, 2006), proteins from 9% to 18%, (Melliou and Chinou, 2005; Ramadan and Al-Ghamdi, 2012; Simuth, 2001), lipids from 4% to 8%, (Malka et al., 2009; Nagai and Inoue, 2005; Sabatini et al., 2009) and there are present in low amount vitamins and mineral salts with other unknown substances present in traces and all together could range from 0.8–3%

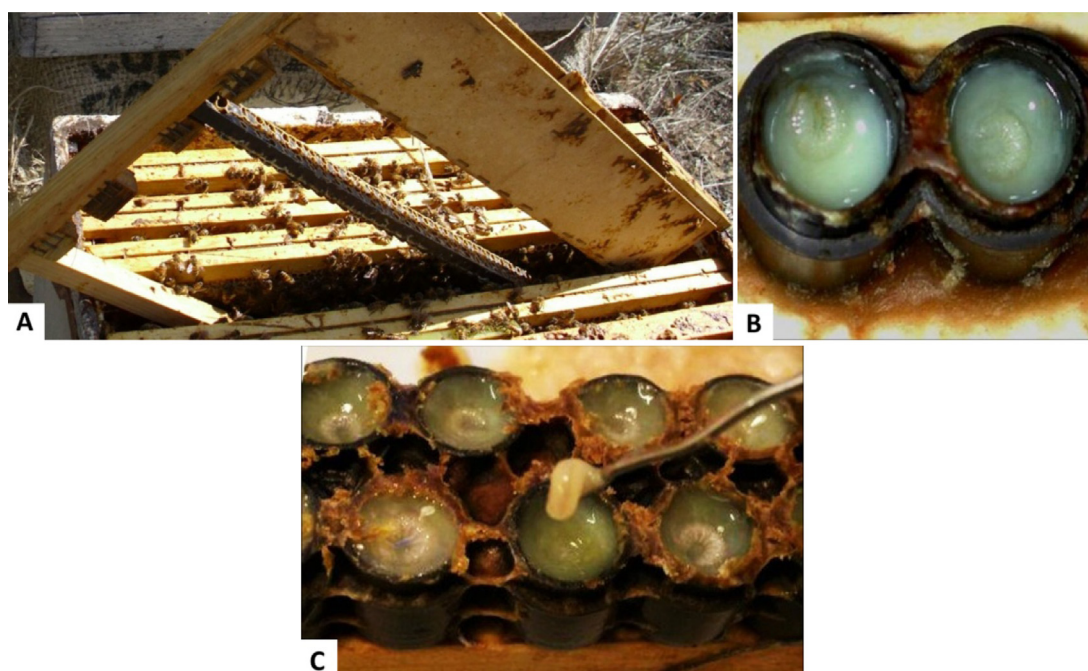


Fig. 1. Hive and royal cells with Royal Jelly and queen bee larvae.

A. Hive for breeding queen bees and Royal Jelly production, constituted by only royal cells. **B.** Queen bee larvae during development in royal cells filled with Royal Jelly. **C.** Queen bee larvae removed by royal cells for the Royal Jelly collection.

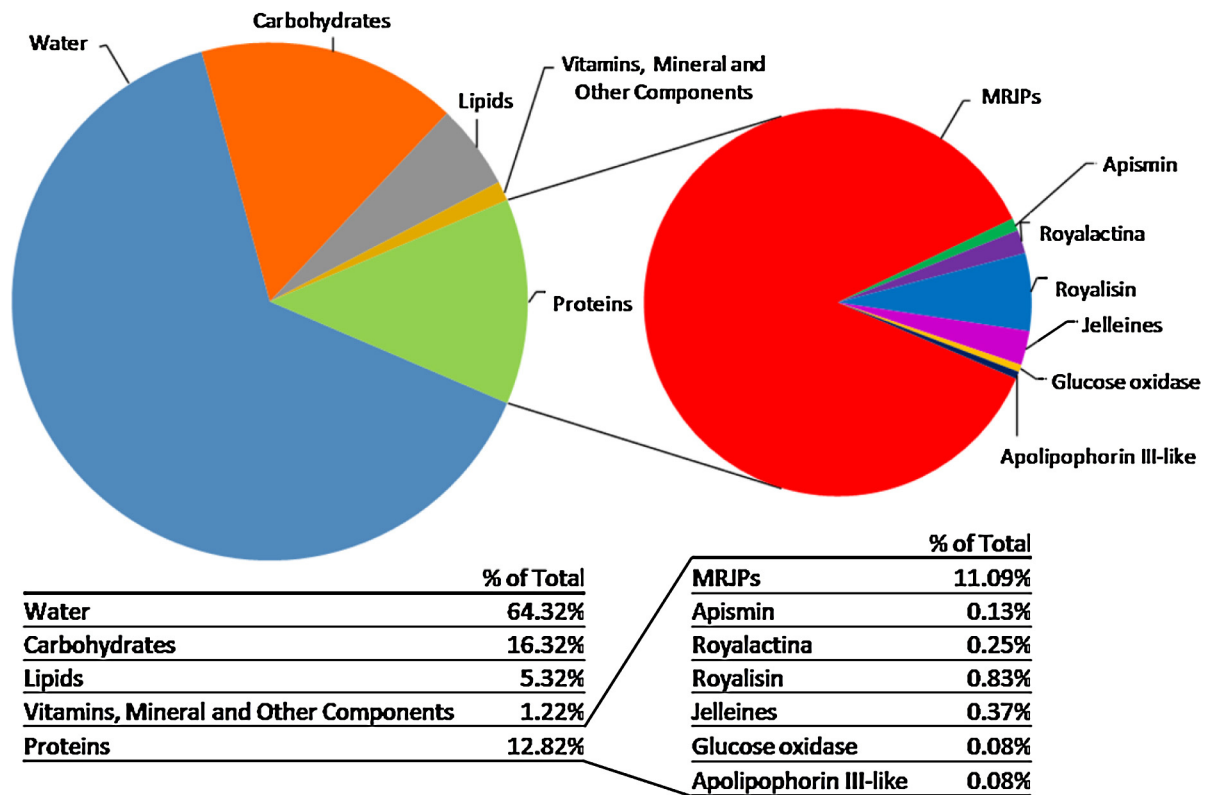


Fig. 2. Mean composition of Royal Jelly.

(Fig. 2) (Caboni et al., 2004; Lercker et al., 1992; Scarselli et al., 2005; Simúth et al., 2004; Zhang et al., 2012).

RJ composition could vary with seasonal and regional conditions of feeding (Antinelli et al., 2003; Attalla et al., 2007; Biondi et al., 2003; Chen and Chen, 1995; Sabatini et al., 2009), with metabolites and changes in the physiology of nurse bees as well as with the larval age (Abd-alla et al., 1995; Brouwers et al., 1987; Lercker et al., 1985, 1992), with bees genetic and race (Liu et al., 2008; Malka et al., 2009; Sano et al., 2004; Zheng et al., 2011), and above all could be modified from the storage conditions postharvest (Caboni et al., 2004; Li et al., 2008; Liu et al., 2008; Ragab and Ibrahim, 1999; Zheng et al., 2011). Several researches correlated these variations of the composition of RJ to its antimicrobial activity (Abd-alla et al., 1995; Li et al., 2008; Liu et al., 2008; Ragab and Ibrahim, 1999; Zheng et al., 2011).

Pollen grains are always presents in RJ as contaminant and could are very useful as indicators of geographical origin and may enriched RJ with some proteins from plants origin (Biondi et al., 2003; Chen and Chen, 1995; Scarselli et al., 2005; Simúth et al., 2004).

2.1. Carbohydrates

Carbohydrates represent about 30% of dry matter (Sabatini et al., 2009; Sesta, 2006) and they may be important indicators of the authenticity of RJ, through the analysis of minor sugars contained (Daniele and Casabianca, 2012; Lercker et al., 1981; Serra Bonvehí, 1992). The most abundant sugars, as in honey, are fructose, glucose and sucrose (Ramadan and Al-Ghamdi, 2012; Wytrychowski et al., 2012), but small traces of oligosaccharides such as maltose, trehalose, melibiose, ribose and other sugars can also be found (Finke, 2005; Kheyri et al., 2012; Lercker et al., 1981, 1985, 1986; Simuth, 2001).

2.2. Proteins

Proteins can reach about the 50% of dry matter of RJ (Bilikova et al., 2002; Buttstedt et al., 2014; Furusawa et al., 2008; Scarselli et al., 2005). During the last 20 years RJ was deeply investigated and several proteins have been identified.

Important protein components of RJ are those belonging to a family named Major Royal Jelly Proteins (MRJPs), or named apalbumins, that represent the 83–90% of protein component (Scarselli et al., 2005; Simuth, 2001). In this protein family have been identified eight proteins (MRJP 1–8) with molecular masses ranged from 49 to 87 KDa (Albert and Klaudiny, 2004; Albert et al., 1999a,b; Hanes and Simuth, 1992; Malecova et al., 2003; Moriyama et al., 2015).

The MRJPs plays an essential nutritional role in the diet of the queen bee (Tamura et al., 2009); MRJP 1, MRJP 4 and MRJP 5 represent the main intake of essential amino acids, as well MRJP 2, and MRJP 5 are the most important nitrogen reserve for its growth (Albert et al., 1999b; Schmitzova et al., 1998); moreover, MRJP 3 is a polymorphic protein, and this might also explain the role of MRJP 3 as nitrogen supply (Albert et al., 1999b).

The MRJPs could also play an important role in the production of other bee products, especially in formation of pollen-pellet and pollen-bread (Simuth, 2001); and it was demonstrated that they have a major role in the differentiation between queen bee and worker (Buttstedt et al., 2013).

MRJP 1 is also recovered in the honeybee neurons, this suggests another unknown function of the protein in addition to nurturing (Peixoto et al., 2009).

MRJP 1 and MRJP 2 have been characterized like major allergens in the RJ, and, in vivo, stimulate mouse macrophages TNF-alpha production (Rosmilah et al., 2008; Simúth et al., 2004).

Other proteins, present in lower amount than MRJPs, are Royalisin, Jelleines, Apisimin and Royalactina.

Several researches showed the antibacterial properties of Royalisins and proposed their uses as potential antimicrobial natural peptides (Bilikova et al., 2001; Fujiwara et al., 1990). Royalisins are amphipathic proteins (both hydrophobic and hydrophilic properties) composed of 51 residues, with net charge +2; the origin is unknown, but it is supposed that they could derive directly from honeybee. The peculiarity of its structure lies on its high content of cysteine (6 residues) and three intramolecular disulphide bridges which can give a compact structure exhibiting high stability at low pH and high temperature. Royalisins has extensive sequence homology with the sapecin (protein constituted from 40 amino acids, taken from embryonic *Sarcophaga peregrine* cells and phormicins from *Phormia terra novae* larvae (Fujiwara et al., 1990)).

As Royalisins, Jelleines, showed their antimicrobial effects in *in vitro* tests (Fontana et al., 2004; Romanelli et al., 2011). Jelleines formation could be the result of tryptic digestion of MRJP 1 by specific proteases. The Jelleines, despite having the structural base of the antimicrobial peptides and are characterized by hydrophobic residues, which influence the interactions with bacterial membranes, do not show similarity with other known antimicrobial peptides, including those produced by bees after a possible infection (apidaecine, abaecine, hymenoptaecine). Jelleine I (PFKISIH-NH₂) differ to Jelleine II (TPFKISIH-NH₂) only for a Thr (T) residue from C-terminal portion. This modification seems to vary the antibacterial activities of the two peptides, with a higher activity of Jelleine I than Jelleine II. Moreover, the removal of the residue Leu (L) at the N-terminus of Jelleine II with the formation of Jelleine IV (TPFKISIH-NH₂) determines the complete loss of antimicrobial activity. However, a significant decrease of activity was showed also when a residue Thr (T) C-terminal was replaced with Glu(E) as reported in the different sequence between Jelleine II and Jelleine III (EPFKISIH-NH₂). Because of the presence of an Arg (R) residue in position 373 and a Thr(T) residue in position 374 of the primary sequence of the MRJP-1 it can be supposed that the Jelleine II can be the product of digestion with trypsin of MRJP-1 (produced by the hypopharyngeal glands and secreted in the RJ). An action of exo-proteinase both on C-terminal to N-terminal tryptic fragment could lead, respectively, to the formation of Jelleines I and IV (Cabrera et al., 2014; Fontana et al., 2004).

Apisimin was also found, highly expressed, in the honeybee head and it was demonstrated its capacity to strongly bind MRJP 1 (Bilikova et al., 2002). Royalactina seems to induce the differentiation of the queen bee as well MRJP 1 (Kamakura, 2011).

Recently apolipophorin III-like protein was identified for the first time in RJ (Han et al., 2011). ApolipophorinIII-like protein is a lipid binding protein that may form protein-lipid complexes in order to carry lipids into aqueous environments (Fujita et al., 2012; Kim and Jin, 2015). ApolipophorinIII-like protein may contribute additional to the antibacterial properties of RJ and could also play a significant role in the development of immune responses of honeybee larvae (Fujita et al., 2012; Han et al., 2011).

Glucose oxidase enzyme (GOx) was also detected in RJ (Li et al., 2008; Sano et al., 2004). GOx that catalyses the oxidation of glucose to hydrogen peroxide was also detected in honey where showed a high antibacterial activity (Sagona et al., 2015).

Therefore, MRJPs, Royalisin, Jelleines, Apisimin, Royalactina, apolipophorinIII-like protein and glucose oxidase, present in RJ may contribute each other in virtue of their different chemical structure, to the development of queen bee and to the efficient immune systems of honeybees, and provided as well an effective protective action of RJ both in vivo and in vitro uses.

2.3. Lipids

The lipids are present from 3 to 19% of the RJ dry matter (Melliou and Chinou, 2005; Nabas et al., 2014; Ramadan and Al-Ghamdi, 2012).

Approximately 90% of lipids is constituted by fatty acids; the rest are neutral lipids, steroids, hydrocarbons and phenols (Nabas et al., 2014; Ramadan and Al-Ghamdi, 2012).

The fatty acids of RJ have 8–10 carbon atoms, usually either hydroxy fatty acids or dicarboxylic acids, unlike organic acids of most animal and plant materials (Kodai et al., 2007; Noda et al., 2005; Ramadan and Al-Ghamdi, 2012).

The analysis of the lipid components can be a criterion of the genuineness of the RJ, because an adulteration with honey or sugars, decrease the protein and lipid component, increase the concentration of minor sugars and makes RJ insoluble in alkaline medium (Boselli et al., 2003; Lercker et al., 1981; Li and Chen, 2003).

The main acid of fatty acid fraction is 10-hydroxy-2-decenoic (10-HDA) (Terada et al., 2011; Kitahara et al., 1995; Genc and Aslan, 1999), an unsaturated acid that seems to be involve in the antibacterial activity of RJ (Bloodworth et al., 1995; Nagai and Inoue, 2005).

10-HDA also showed to have an important biological role in the development of the colony strategies (Wu et al., 1991). Moreover, the 10-HDA content has been adopted as a marker for quality and freshness analysis of RJ (Antinelli et al., 2003; Ferioli et al., 2007).

Also the octanoic acid, present in lower amount than 10-HDA, seems to cover more than only a nutritional function; recently research showed that the octanoic acid is involved in the repellence action of queen cells against *Varroa destructor* (Nazzi et al., 2009).

2.4. Vitamins

RJ is very abundant in B group vitamins, mainly vitamin B5 followed by vitamins B1, B2, B6, B8, B9 and B12 (Li et al., 2012; Viuda-Martos et al., 2008). Vitamin PP and vitamin C are present only in small amounts (Liu et al., 2008; Melliou and Chinou, 2005; Nagai et al., 2001). Liposoluble vitamins such as vitamins A, D, E and K are absent (Li and Chen, 2003; Morita et al., 2012; Nagai and Inoue, 2005; Ramadan and Al-Ghamdi, 2012).

The vitamins content of RJ is subjected to seasonal changes as variation of the pollen of flowers collected by worker bees, as the mainly source of vitamins comes from the pollen (Biondi et al., 2003; Chen and Chen, 1995; Sabatini et al., 2009).

2.5. Minerals and other minor elements

Minerals and other elements are about 4% to 8% of RJ dry matter (Sabatini et al., 2009). The main elements are K, P, S, Na, Ca, Al, Mg, Zn, Fe, Cu and Mn, but there are also traces of Ni, Cr, Sn, W, Sb, Bi and Ti (Benfenati et al., 1986; Li and Chen, 2003; Ramadan and Al-Ghamdi, 2012; Viuda-Martos et al., 2008). The presence of minerals is related (and therefore variability) by the source of the feed, the production period, the environment and biological factors of bees (Benfenati et al., 1986; Garcia-Amoedo and de Almeida-Muradian, 2007; Nation and Robinson, 1971; Sabatini et al., 2009).

Moreover, RJ contains several minor components classified under various chemical classes, such as heterocyclic substances, bipterine and neopterine (Bogdanov, 2012). In RJ were also found low amounts of free nucleotides (adenosine, guanosine, cytidine, and iridine), phosphates, ATP, ADP, AMP, acetylcholine and gluconic, benzoic, malic, citric, and lactic acids (Bogdanov, 2012; Matsuka, 1993; Sabatini et al., 2009). The functions of these components is still unclear, although their origin is assumed to arise from the nurse bee.

3. Mechanisms of antimicrobial peptides action

Antimicrobial peptides (AMPs) are fundamental defence biomolecules that could protect the host from bacteria, viruses or fungi (Beutler, 2004; Gallo and Nizet, 2003; Zasloff, 2002). They have been preserved evolutionarily in their innate immune response, which represents the first line of defence in most living organisms (Hancock and Lehrer, 1998; Brogden et al., 2003).

AMPs are polypeptides of variable length containing from 10 to 50 amino acids, so relatively short, and they have a positive charge that goes from 2 to 9 (most commonly 4 or 6), due to an excess of basic residues of lysine, arginine and histidine (Ebenhan et al., 2014; Splith and Neundorff, 2011). These properties allow the interaction between AMPs and microbial surfaces (negatively charged), and to the cell membrane penetration by bilayer phospholipids head groups (Brogden et al., 2007; Pandey et al., 2011).

The sequence of amino acids of the peptide has a significant role; in fact the presence of an amino acid or its substitution with another one, even with similar chemical properties, may change the effectiveness of peptide as its antimicrobial activity (Maróti et al., 2011; Pandey et al., 2011).

The interaction between the AMPs and the surface of the bacterial cell membrane seems to be strictly correlated to the electrostatic interactions of the AMPs sequence and the structure of the bacterial membrane surface. Furthermore, even the secondary structure of the peptide (α -helix or β -sheet) plays an important role primarily when the electrostatic interactions can not be established due to the distance between the charged groups (Scott et al., 1999; Yang et al., 2001; Zhao et al., 2001).

Several studies on the mechanism of action of AMPs have revealed different ways in which these substances exerted their effect (Bulet et al., 1999). Although the antibacterial properties of many peptides present in RJ were demonstrated however their mode of actions were not been yet clarified in details.

The classic way which AMPs exert their action is by the ability to interact with cells membrane determining a permeabilization (Boman, 1994; Brogden, 2005; Huang et al., 2000).

There are three different models to describe possible AMPs mechanisms of action against bacteria: barrel-stave model, carpet-like model, and toroidal pore model (Li et al., 2012).

The barrel-stave model contemplates the formation of pores in the hydrophobic core of the membrane created by a circular assembly of AMPs where their hydrophobic domains pointing toward the lipid chains of the membrane while the hydrophilic toward the interior of the pore (Li et al., 2012; Maróti et al., 2011; Shai, 2002).

In the carpet-like model the AMPs initially interact with the external surface of the membrane, subsequently the charged region of the peptide interacts with the anionic phospholipids forming a carpet, which extends on the surface of the target membrane. This mode of action cause a reducing of the lipid layer surface and a consequent membrane disruption with collapse of the lipid structure. The toroidal pore model also presents the formation of pores in the membrane like barrel-stave model, but in this case the phospholipids assumed a completely curvature as a double layer. In this process the lines of the double layer becomes a continuous structure, with the consequent formation on a pore. The toroidal pore model is an intermediate case between the two previously described models and in some cases it is difficult to establish a clear distinction. In the barrel-stave model and in the toroidal pore model the peptide causes a rearrangement of the polar heads of phospholipids by bundling the amphipathic helices and forming a transmembrane pore which the hydrophilic part of the peptide facing the lumen of the pore (Li et al., 2012; Matsuzaki et al., 1996). Currently a fourth model was described, the aggregate channel model. Several studies indicate that permeabilization of the cell membrane alone may not be enough to kill bacteria (as predict

in the other models). Like the carpet model there is no formation of pores in the cell membrane. After the formation of a binding between the peptide and the phospholipid head groups the peptide reaches the inner part of the cell without modified the membrane (a mechanism of transport through the lipid bilayer without the formation of a stable channel). Once inside, the peptide can interact with the targets (Pálffy et al., 2009; Xiao et al., 2015). Unfortunately, the type of aggregates that provide the insertion of the peptide inside the membrane is not well defined, so it is more difficult to predict molecular properties who can favour this mechanism (Herbig et al., 2005; Li et al., 2012).

Besides the ability to interact with bacterial membranes, the AMPs could have other intracellular target (Ahn et al., 2006); in fact they can bind DNA, RNA and proteins and inhibit synthesis of different essential cell constituents as cell wall, DNA, RNA and proteins (Lan et al., 2010; Li et al., 2012). Moreover, AMPs can interfere with bacterial cytokinesis by cell filamentation by an unique mechanisms to translocation inside the cell in order to alter the cytoplasmic membrane septum formation (Brown and Hancock, 2006; Lan et al., 2010; Li et al., 2012).

Many constituent of RJ are ascribable to the antimicrobial peptides category such as MRJPs, Royalisin, Jelleines, Apismin, Royalactin, and apolipoprotein III-like. The natural origin of these compounds could be a potential added value to different products as *in vitro* use. RJ, as well its by-products, could find a major role in the control of microorganisms growth both for their proven activities and for the low amounts needed.

4. Antibacterial activity

The presence of antimicrobial properties of RJ against Gram positive and Gram negative bacteria was scientifically showed for the first time by McCleskey and Melampy (1939).

Subsequent studies of Hinglais et al. (1955), Butenandt and Rembold (1957), Blum et al. (1959), Iizuka and Koyama (1964) and Muratova et al. (1967) reported the effects of RJ and 10-HDA against many bacteria, including *Escherichia coli* and *Micrococcus pyogenes*.

In 1990, Fujiwara et al. isolated and purified Royalisin from RJ. MIC (Minimum Inhibitory Concentration) evaluation of crude RJ showed that both Gram positive and Gram negative tested bacteria posses a low resistance of to this substance (Table 1).

Royalisin MIC evaluation reported a strong antibacterial activity against Gram positive bacteria, but not against Gram negative (Table 2). Bacterial strains belonging to *Bifidobacterium*, *Clostridium*, *Corynebacterium*, *Lactobacillus*, *Leuconostoc*, *Staphylococcus* and *Streptococcus* genera showed an inhibitory concentration of Royalisin comparable with the effective concentrations of several antibiotic classes.

The difference in antibacterial effectiveness between RJ and Royalisin could be explained by the presence of other compounds, such as 10-HDA, which were completely lost during this peptide splitting.

Further studies on antibacterial activity of Royalisin carried out by Bilikova et al. (2001). These Authors investigated the specific action of Royalisin against Gram positive bacteria (Table 2). The aim of that study was to verify the action of the peptide against aetiological agent of American foulbrood, *Paenibacillus larvae* subsp. *larvae*. The results showed effective action of Royalisin against *Bacillus subtilis* and *Paenibacillus larvae* subsp. *larvae* while no inhibition was determined for *Micrococcus luteus* (*Sarcina lutea*).

Shen et al. (2010, 2012) isolated and purified recombinant Royalisins expressed by *Escherichia coli* after fusing in a vector the *Apis cerana* cDNAs encoding for different Royalisin forms. These recombinant Royalisins showed higher antibacterial activity against Gram positive bacteria than Gram negative bacteria (MIC

Table 1
Antibacterial activities of Royal Jelly.

Bacterial strains		MIC	MBC	Method	Reference
<i>Bacillus cereus</i>		12.5 mg/ml		AI	Ratanavalachai and Wongchai (2002)
<i>Bacillus subtilis</i>	RCMBA 6005	7.8–500.0 µg/ml		AWD	Moselhy et al. (2013)
<i>Bacteroides fragilis</i>		nd		BM	Fujiwara et al. (1990)
<i>Bacteroides vulgatus</i>		nd		BM	Fujiwara et al. (1990)
<i>Bifidobacterium adolescentis</i>	ATCC 15703	10.0 µg/ml		BM	Fujiwara et al. (1990)
<i>Bifidobacterium bifidum</i>	ATCC 15696	10.0 µg/ml		BM	Fujiwara et al. (1990)
<i>Bifidobacterium breve</i>	ATCC 15700	10.0 µg/ml		BM	Fujiwara et al. (1990)
<i>Bifidobacterium infantis</i>	ATCC 15697	10.0 µg/ml		BM	Fujiwara et al. (1990)
<i>Bifidobacterium longum</i>	ATCC 15707	10.0 µg/ml		BM	Fujiwara et al. (1990)
<i>Enterococcus faecium</i>		50.0–70.0 w/w ^c		AWD	Garcia et al. (2013)
<i>Enterococcus faecalis</i>		40.0–100.0 w/w ^c		AWD	Garcia et al. (2013)
		40.0–80.0 w/w ^c		AWD	Garcia et al. (2013)
		50.0–90.0 w/w ^c		AWD	Garcia et al. (2013)
	ATCC 29212	60.0–80.0 w/w ^c		AWD	Garcia et al. (2013)
		3.7–7.6 mg/ml	125.0–> 250.0 mg/ml	BD	Garcia et al. (2010)
	ATCC 29212	5.0–13.7 mg/ml	>250.0 mg/ml	BD	Garcia et al. (2010)
<i>Escherichia coli</i>		13.5 mg/ml		AI	Ratanavalachai and Wongchai (2002)
		60.0–100.0 w/w ^c		AWD	Garcia et al. (2013)
	RCMBA 5003	500.0 µg/ml		AWD	Moselhy et al. (2013)
		2.0 v/v ¹		BD	Boukrra et al. (2009)
		7.0–7.1 mg/ml	> 250.0 mg/ml	BD	Garcia et al. (2010)
	IID 861	10.0 µg/ml		BM	Fujiwara et al. (1990)
	ATCC 29532	12.0 mm ^b		DP	Eshraghi (2005)
<i>Klebsiella pneumoniae</i>		80.0–100.0 w/w ^c		AWD	Garcia et al. (2013)
		8.0–8.1 mg/ml	125.0–250.0 mg/ml	BD	Garcia et al. (2010)
	IFO-3321	nd		BM	Fujiwara et al. (1990)
<i>Lactobacillus acidophilus</i>	ATCC 314	10.0 µg/ml		BM	Fujiwara et al. (1990)
	ATCC 4356	10.0 µg/ml		BM	Fujiwara et al. (1990)
<i>Lactobacillus helveticus</i> subsp. <i>Jugurti</i>		10.0 µg/ml		BM	Fujiwara et al. (1990)
<i>Micrococcus luteus</i>	ATCC 9341	40.0–60.0 w/w ^c		AWD	Garcia et al. (2013)
	ATCC 9341	7.5–11.8 mg/ml	125.0 mg/ml	BD	Garcia et al. (2010)
<i>Micrococcus luteus</i> (<i>Sarcina lutea</i>)		0.3 mg/ml		AI	Ratanavalachai and Wongchai (2002)
<i>Proteus vulgaris</i>		15.5 mg/ml		AI	Ratanavalachai and Wongchai (2002)
<i>Pseudomonas aeruginosa</i>		15.5 mg/ml		AI	Ratanavalachai and Wongchai (2002)
	ATCC 27853	4.0 v/v ^a		AI	Boukrra (2008)
		70.0–100.0 w/w ^c		AWD	Garcia et al. (2013)
		60.0–100.0 w/w ^c		AWD	Garcia et al. (2013)
	RCMBA 1002	nd		AWD	Moselhy et al. (2013)
		3.3–14.4 mg/ml	63.0–250.0 mg/ml	BD	Garcia et al. (2010)
<i>Salmonella infantis</i>		10.0 µg/ml		BM	Fujiwara et al. (1990)
<i>Salmonella typhi</i>		14.5 mg/ml		AI	Ratanavalachai and Wongchai (2002)
<i>Salmonella typhimurium</i>		10.0 µg/ml		BM	Fujiwara et al. (1990)
<i>Shigella flexneri</i>		14.5 mg/ml		AI	Ratanavalachai and Wongchai (2002)
<i>Staphylococcus aureus</i>		12.5 mg/ml		AI	Ratanavalachai and Wongchai (2002)
	RCMBA 2004	15.6–500.0 µg/ml		AWD	Moselhy et al. (2013)
		1.7 v/v ^a		BD	Boukrra et al. (2009)
	ATCC 14776	15.0 mm ^b		DP	Eshraghi (2005)
<i>Staphylococcus aureus</i> MR 1		40.0–70.0 w/w ^c		AWD	Garcia et al. (2013)
		8.0–14.5 mg/ml	125.0 mg/ml	BD	Garcia et al. (2010)
<i>Staphylococcus aureus</i> MR 2		30.0–70.0 w/w ^c		AWD	Garcia et al. (2013)
		8.0–12.5 mg/ml	125.0–250.0 mg/ml	BD	Garcia et al. (2010)
<i>Staphylococcus aureus</i> MS 1	ATCC 25923	20.0–80.0 w/w ^c		AWD	Garcia et al. (2013)
	ATCC 25923	7.8–9.0 mg/ml	125.0–> 250.0 mg/ml	BD	Garcia et al. (2010)
<i>Staphylococcus aureus</i> MS 2		40.0–80.0 w/w ^c		AWD	Garcia et al. (2013)
		3.4–8.8 mg/ml	125.0–> 250.0 mg/ml	BD	Garcia et al. (2010)
<i>Staphylococcus epidermidis</i>		40.0–80.0 w/w ^c		AWD	Garcia et al. (2013)
		8.7–10.3 mg/ml	125.0 mg/ml	BD	Garcia et al. (2010)
<i>Streptococcus agalactiae</i>		50.0–100.0 w/w ^c		AWD	Garcia et al. (2013)
		70.0–90.0 w/w ^c		AWD	Garcia et al. (2013)
	ATCC 27956	50.0–90.0 w/w ^c		AWD	Garcia et al. (2013)
<i>Streptococcus dysgalactiae</i>		80.0–100.0 w/w ^c		AWD	Garcia et al. (2013)
		80.0–90.0 w/w ^c		AWD	Garcia et al. (2013)
	ATCC 27957	50.0–100.0 w/w ^c		AWD	Garcia et al. (2013)
<i>Streptococcus uberis</i>		60.0–70.0 w/w ^c		AWD	Garcia et al. (2013)
		5.8–14.5 mg/ml	250.0–> 250.0 mg/ml	BD	Garcia et al. (2010)
<i>Streptomyces griseus</i>	ATCC 11746	14.0 mm ^b		DP	Eshraghi (2005)

nd: value not determined.

AI: Agar Infusion; AWD: Agar Well Diffusion; BM: Broth Medium; BD: Broth Dilution; DP: Drop Plate; DT:

^a Volume/volume of RJ on Muller Hinton agar medium.^b RJ concentration 330 mg/ml.^c Weight/weight of RJ on water.

Table 2
Antibacterial activities of Royalisin.

Bacterial strains		MIC	MBC	Method	Reference
<i>Bacillus subtilis</i>		5.4–108.0 µg/ml		DT	Bilikova et al. (2001)
	CMCC 63501	9.83 mm ^a		DT	Shen et al. (2010)
	CMCC 63501	10.53 mm ^b		DT	Shen et al. (2010)
	CMCC 63501	62.5 µg/ml ^c		MA	Shen et al. (2012)
<i>Bacteroides fragilis</i>		nd		BM	Fujiwara et al. (1990)
<i>Bacteroides vulgatus</i>		nd		BM	Fujiwara et al. (1990)
<i>Bifidobacterium adolescentis</i>	ATCC 15703	1.0 µM		BM	Fujiwara et al. (1990)
<i>Bifidobacterium bifidum</i>	ATCC 15696	1.0 µM		BM	Fujiwara et al. (1990)
<i>Bifidobacterium breve</i>	ATCC 15700	1.0 µM		BM	Fujiwara et al. (1990)
<i>Bifidobacterium infantis</i>	ATCC 15697	1.0 µM		BM	Fujiwara et al. (1990)
<i>Bifidobacterium longum</i>	ATCC 15707	1.0 µM		BM	Fujiwara et al. (1990)
<i>Clostridium perfringens</i>	ATCC 13124	1.0 µM		BM	Fujiwara et al. (1990)
<i>Clostridium tetani</i>	ATCC 19406	250.0 µg/ml ^c		MA	Shen et al. (2012)
<i>Corynebacterium pyogenes</i>		1.0 µM		BM	Fujiwara et al. (1990)
<i>Escherichia coli</i>		nd		DT	Bilikova et al. (2001)
		nd	nd	MA	Bilikova et al. (2015)
		nd ^d	nd ^d	MA	Bilikova et al. (2015)
		nd ^e	nd ^e	MA	Bilikova et al. (2015)
		nd ^f	nd ^f	MA	Bilikova et al. (2015)
	IID 861	nd		BM	Fujiwara et al. (1990)
	CGMCC1.1139	>2000.0 µg/ml ^c		MA	Shen et al. (2012)
<i>Klebsiella pneumoniae</i>	IFO-3321	nd		BM	Fujiwara et al. (1990)
<i>Lactobacillus acidophilus</i>	ATCC 314	1.0 µM		BM	Fujiwara et al. (1990)
	ATCC 4356	1.0 µM		BM	Fujiwara et al. (1990)
<i>Lactobacillus bulgaricus</i>	ATCC 11841	1.0 µM		BM	Fujiwara et al. (1990)
<i>Lactobacillus helveticus</i> subsp. <i>jugurti</i>		1.0 µM		BM	Fujiwara et al. (1990)
<i>Lactobacillus lactis</i>	ATCC 8000	1.0 µM		BM	Fujiwara et al. (1990)
<i>Lactobacillus leichmannii</i>	ATCC 7830	1.0 µM		BM	Fujiwara et al. (1990)
<i>Leuconostoc cremoris</i>	ATCC 19254	1.0 µM		BM	Fujiwara et al. (1990)
<i>Micrococcus luteus</i> (<i>Sarcina lutea</i>)		nd		DT	Bilikova et al. (2001)
	CMCC 28001	15.07 mm ^a		DT	Shen et al. (2010)
	CMCC 28001	16.70 mm ^b		DT	Shen et al. (2010)
	CMCC 28001	125.0 µg/ml ^c		MA	Shen et al. (2012)
<i>Paenibacillus larvae</i> subsp. <i>larvae</i>		6.0 µg/ml	15.0 µg/ml	MA	Bilikova et al. (2015)
		10.0 µg/ml ^d	nd ^d	MA	Bilikova et al. (2015)
		10.0 µg/ml ^e	20.0 µg/ml ^e	MA	Bilikova et al. (2015)
		50.0 µg/ml ^f	nd ^f	MA	Bilikova et al. (2015)
	ATCC 5084	5.4–108.0 µg/ml		DT	Bilikova et al. (2001)
	ATCC 5085	5.4–108.0 µg/ml		DT	Bilikova et al. (2001)
	ATCC 5086	5.4–108.0 µg/ml		DT	Bilikova et al. (2001)
<i>Proteus vulgaris</i>	CGMCC1.1527	>2000.0 µg/ml ^c		MA	Shen et al. (2012)
<i>Pseudomonas aeruginosa</i>		10.0 µg/ml	15.0 µg/ml	MA	Bilikova et al. (2015)
		nd ^d	nd ^d	MA	Bilikova et al. (2015)
		11.0 µg/ml ^e	17.0 µg/ml ^e	MA	Bilikova et al. (2015)
		nd ^f	nd ^f	MA	Bilikova et al. (2015)
<i>Salmonella choleraesuis</i>		9.0 µg/ml	11.0 µg/ml	MA	Bilikova et al. (2015)
		20.0 µg/ml ^d	nd ^d	MA	Bilikova et al. (2015)
		20.0 µg/ml ^e	18.0 µg/ml ^e	MA	Bilikova et al. (2015)
		nd ^f	nd ^f	MA	Bilikova et al. (2015)
<i>Salmonella infantis</i>		nd		BM	Fujiwara et al. (1990)
<i>Salmonella typhimurium</i>		nd		BM	Fujiwara et al. (1990)
	CGMCC1.1190	>2000.0 µg/ml ^c		MA	Shen et al. (2012)
<i>Serratia marcescens</i>		nd		DT	Bilikova et al. (2001)
<i>Staphylococcus aureus</i>		7.5 µg/ml	13.0 µg/ml	MA	Bilikova et al. (2015)
		20.0 µg/ml ^d	nd ^d	MA	Bilikova et al. (2015)
		9.5 µg/ml ^e	13.5 µg/ml ^e	MA	Bilikova et al. (2015)
		20.0 µg/ml ^f	nd ^f	MA	Bilikova et al. (2015)
	SC-D	1.0 µM		BM	Fujiwara et al. (1990)
	CMCC 26003	11.53 mm ^a		DT	Shen et al. (2010)
	CMCC 26003	10.50 mm ^b		DT	Shen et al. (2010)
	CMCC 26003	250.0 µg/ml ^c		MA	Shen et al. (2012)
<i>Staphylococcus intermedius</i> B		4.0 µg/ml	6.5 µg/ml	MA	Bilikova et al. (2015)
		nd ^d	nd ^d	MA	Bilikova et al. (2015)
		4.6 µg/ml ^e	7.0 µg/ml ^e	MA	Bilikova et al. (2015)
		nd ^f	nd ^f	MA	Bilikova et al. (2015)
<i>Staphylococcus xylosus</i>		10.5 µg/ml	12.0 µg/ml	MA	Bilikova et al. (2015)
		nd ^d	nd ^d	MA	Bilikova et al. (2015)
		18.0 µg/ml ^e	19.0 µg/ml ^e	MA	Bilikova et al. (2015)

Table 2 (Continued)

Bacterial strains	MIC	MBC	Method	Reference
<i>Streptococcus alactolyticus</i>	nd ^f	nd ^f	MA	Bilikova et al. (2015)
	9.0 µg/ml	11.0 µg/ml	MA	Bilikova et al. (2015)
	nd ^d	nd ^d	MA	Bilikova et al. (2015)
	12.0 µg/ml ^e	18.0 µg/ml ^e	MA	Bilikova et al. (2015)
	nd ^f	nd ^f	MA	Bilikova et al. (2015)
<i>Streptococcus thermophilus</i>	1.0 µM		BM	Fujiwara et al. (1990)
<i>Vibrio parahaemolyticus</i>	4.0 µg/ml	6.5 µg/ml	MA	Bilikova et al. (2015)
	8.0 µg/ml ^d	nd ^d	MA	Bilikova et al. (2015)
	4.6 µg/ml ^e	7.0 µg/ml ^e	MA	Bilikova et al. (2015)
	8.0 µg/ml ^f	nd ^f	MA	Bilikova et al. (2015)

nd: value not determined.

BM: Broth Medium; DT: Diffusion Test; MA: Microplate Assay.

^a 2 mg/ml of fusion protein from pre-pro-Acc-royalisin.

^b 2 mg/ml of fusion protein from mature Acc-royalisin.

^c Recombinant Acc-royalisin.

^d Royalisin treated with DTT.

^e Royalisin-D.

^f Royalisin-D treated with DTT.

over 2000.0 µl/ml, Table 2); unfortunately no solid consideration could be formulated about the differences between the native and recombinant Royalisins for the different method of determination of the inhibitory activity.

In 2002, in Thailand, Ratanavalachai and Wongchai tested the antibacterial activities of crude RJ, and both the lipid and defatted extracts. Authors also evaluated different storage combination time/temperature in order to enhance RJ conservation. RJ freshly picked was stored at room temperature (25–27 °C), refrigerated temperature (2–4 °C) and deep frozen (–18 °C) for 12 h, 24 h and 3 days and subsequently was tested against several bacteria.

Results showed that conservation of RJ at frozen temperature did not affect antibacterial and bacteriostatic activities (Table 1). Instead, Authors observed a decrease in RJ antibacterial activity during storage time at all tested temperatures.

Fontana et al. (2004) identified in RJ employing mass spectrometry four antimicrobial peptides that were called Jelleines.

The most relevant peptides with antimicrobial activity were Jelleines I and II, followed by Jelleine III, which has not demonstrated activity against all the microorganisms, and finally the Jelleine IV that has not given any evidence of activity (Table 3).

Few researches were carried out on antibacterial activities of Jelleines and their modified forms. Romanelli et al. (2011) showed that Jelleine I, II and III inhibit bacterial growth while the modifications of the structure in C and N terminals of these peptides caused a decreasing of activity (Table 3). Results of Capparelli et al. (2012) showed a wide range of activity against *Staphylococcus epidermidis* from 30 to 300 µg/ml for C-terminal modified peptides.

Brudzynski and Sjaarda (2015), in a recent study about the evaluation of honey glycoproteins against *Escherichia coli* and *Bacillus subtilis*, attributed the antibacterial activities to the presence of MRJP-1 and likely the presence of Jelleines.

In 2005 Eshraghi investigated the different antibacterial properties of the crude RJ, the ether-non-soluble fraction and the ether-soluble fraction against different bacteria. The results showed a clear inhibitory effect of crude RJ; *Staphylococcus aureus* strain was the most sensible followed by *Streptomyces griseus* and *Escherichia coli* (Table 1).

The ether-soluble fraction of RJ showed a greater antibacterial action than the crude RJ, while the ether-non-soluble fraction, containing the Royalisin, was found to be less effective, even of crude RJ.

According to the experiment results, the antibacterial RJ action could be attributed to the ether-soluble fraction, i.e. the part containing lipids and fatty acids including 10-HDA, and not to the

ether-non-soluble fraction, that includes Royalisin (inhibition zone of 10-HDA are reported in Table 4) (Eshraghi, 2005).

Recently, Boukraa (2008) and Boukraa et al. (2009) evaluated antibacterial effect of RJ against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. Results showed that all tested bacterial strains were susceptible to RJ (Table 1). Antibacterial activity against *Pseudomonas aeruginosa* is probably related to the action of RJ demonstrated by Lerrer et al., 2007 that seems abrogate lectin-dependent infection-preceding by *Pseudomonas aeruginosa* adhesion.

RJ is also effective against some bacteria implicated in infection of skin wounds, as shown by the study conducted in Argentina on two different RJ samples in 2010 by Garcia et al. The MIC values of the two samples of RJ were reported in Table 1 as a range. Interesting results were reported as concern the inhibition and the bactericidal effects: the MIC values were around twenty times lower than MBC values. The observed differences in the values of MIC and MBC may be related to the RJ components associated to the geographical area or genetic variability between bee colonies.

In 2013 Garcia et al. evaluated the antibacterial activity of four Argentinean RJs, 10-HDA, ether-soluble fraction and fat-free RJ (Table 1 and Table 4). The results showed that the different samples of RJ tested had a significant antibacterial activity on almost all bacterial strains examined, with remarkable MIC values; both RJ and 10-HDA showed lower activity against Gram negative bacteria, as *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* than against Gram positive bacteria.

In 2013, Moselhy et al. studied the antibacterial activity of Egyptian RJs (two samples collected in two different period, ie camphor and citrus seasons) and a Chinese RJ.

Authors reported that Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) were more sensitive to all three samples of RJ compared to Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) (Table 1).

In addition, the samples of Egyptian RJ were found to have a more effective antibacterial action than Chinese RJ. The bactericidal or bacteriostatic action of RJ is closely linked to the geographical origin, the related botanical species and the genetic variability between colonies (Boukraa and Sulaiman, 2009; Garcia et al., 2010; Garcia et al., 2013; Garcia-Amoedo and de Almeida-Muradian, 2007).

Another confirmation of the effectiveness of RJ against *Staphylococcus aureus* strains was recently reported by an in vivo study carried out on rats by Gunaldi et al. (2014).

Table 3
The inhibitory activities of Jelleines against bacteria.

Bacterial strains		MIC ^a				Reference
		Jelleine I	Jelleine II	Jelleine III	Jelleine IV	
<i>Bacillus cereus</i>		nd	nd	nd	nd	Fontana et al. (2004)
<i>Bacillus pumilis</i>		nd	nd	nd	nd	Fontana et al. (2004)
<i>Bacillus subtilis</i>	CCT 2471	10.0	30.0	nd	nd	Fontana et al. (2004)
<i>Bacillus thuringiensis</i>		nd	nd	nd	nd	Fontana et al. (2004)
<i>Enterobacter cloacae</i>	ATCC 23355	10.0	15.0	nd	nd	Fontana et al. (2004)
<i>Escherichia coli</i>	CCT 1371	2.5	15.0	15.0	nd	Fontana et al. (2004)
<i>Klebsiella pneumoniae</i>	ATCC 13883	10.0	15.0	nd	nd	Fontana et al. (2004)
<i>Listeria monocytogenes</i>		≥200.0	200.0			Romanelli et al. (2011)
<i>Proteus mirabilis</i>		nd	nd	nd	nd	Fontana et al. (2004)
<i>Pseudomonas aeruginosa</i>	ATCC 27853	10.0	15.0	30.0	nd	Fontana et al. (2004)
<i>Salmonella enterica</i> Paratyphi		≥200.0	200.0			Romanelli et al. (2011)
<i>Staphylococcus aureus</i>	ATCC 6535	10.0	15.0	30.0	nd	Fontana et al. (2004)
		≥200.0	200.0			Romanelli et al. (2011)
<i>Staphylococcus saprophyticus</i>		15.0	10.0	30.0	nd	Fontana et al. (2004)

nd: value not determined.

^a MIC values (μg/ml) obtained with Microplate Assay method.

Table 4
The inhibitory activities of 10-hydroxy-2-decenoic acid against bacteria.

Bacterial strains		MIC	Method	Reference
<i>Streptomyces griseus</i>	ATCC 11746	29.0 mm ^a	DP	Eshraghi (2005)
<i>Staphylococcus aureus</i>	ATCC 14776	40.0 mm ^a	DP	Eshraghi (2005)
<i>Staphylococcus aureus</i> MS 1	ATCC 25923	1.9 mg/ml	AWD	Garcia et al. (2013)
<i>Staphylococcus aureus</i> MS 2		1.9 mg/ml	AWD	Garcia et al. (2013)
<i>Staphylococcus aureus</i> MR 1		1.9 mg/ml	AWD	Garcia et al. (2013)
<i>Staphylococcus aureus</i> MR 2		2.3 mg/ml	AWD	Garcia et al. (2013)
<i>Escherichia coli</i>	ATCC 29532	22.0 mm ^a	DP	Eshraghi (2005)
		nd	AWD	Garcia et al. (2013)
<i>Enterococcus faecalis</i>	ATCC 29212	2.3 mg/ml	AWD	Garcia et al. (2013)
		1.9 mg/ml	AWD	Garcia et al. (2013)
		1.9 mg/ml	AWD	Garcia et al. (2013)
		2.3 mg/ml	AWD	Garcia et al. (2013)
<i>Enterococcus faecium</i>		2.3 mg/ml	AWD	Garcia et al. (2013)
<i>Streptococcus uberis</i>		2.3 mg/ml	AWD	Garcia et al. (2013)
<i>Streptococcus agalactiae</i>	ATCC 27956	nd	AWD	Garcia et al. (2013)
		2.3 mg/ml	AWD	Garcia et al. (2013)
		0.9 mg/ml	AWD	Garcia et al. (2013)
<i>Klebsiella pneumoniae</i>		nd	AWD	Garcia et al. (2013)
<i>Pseudomonas aeruginosa</i>		nd	AWD	Garcia et al. (2013)

nd: value not determined.

DP: Drop Plate; AWD: Agar Well Diffusion.

^a Ether-soluble fraction of RJ concentration 30 mg/ml.

Rats with spinal implant inoculated with the bacteria and treated with RJ showed a decrease in severity of the infection if compared with the rats without RJ addition.

Bilikova et al. (2015) analysed Royalisin and Royalisin-D, a recombinant shortened form constructed in order to correlate the structure to the antimicrobial activity. Royalisin-D was structured as a reduced form of Royalisin that lacks of 11 amino acids at the C-terminal (Tseng et al., 2011).

In addition to investigate the importance of the disulfide bonds in Royalisin, the two peptide were treated with dithiothreitol (DTT) as a reducing agent of the disulfide bonds. The action of each peptide, crude and treated with DTT, against each microorganism was evaluated with MIC and MBC (Table 2).

All bacteria were susceptible to the peptides, with the exception of *Escherichia coli*. Moreover, the activity of Royalisin and Royalisin-D were very similar to each other, while a significant and important difference was noted for the two peptides treated with DTT. In fact Royalisin and Royalisin-D treated with DTT showed a decreased inhibitory and bactericidal effects. These results highlight the importance of the disulfide bonds of Royalisin.

5. Conclusions

From available literature Royal Jelly and its derivate components, such as Royalisin, Jelleines and 10-hydroxy-2-decenoic acid (10-HDA), showed a high activity against Gram positive bacteria while their effectiveness decrease against Gram negative. Moreover, several studies carried out on Royal Jelly showed that this product is also effective against many multidrug resistant bacteria, such as MRSA (methicillin-resistant *Staphylococcus aureus*). This is particularly important since one of the major public health problems is currently represented right from the onset of an increasing number of antibiotic resistant bacteria. The indiscriminate use of antibiotics has led to the selection of resistant clones many for which it is often not provided an adequate therapy. Multidrug resistant bacteria management request an increasing attention to the antibacterial molecules/products used. For this reason researches in recent years has been directed toward the discovery of new antimicrobial substances, particularly natural substances such as plant extracts, essential oils and antimicrobial peptides isolated from many different animals. The interest in beehive products is also further enhanced by the fact that these products have always

represented an important resource such as functional foods, which have not only the nutritional function, but also nutraceutical or rather to improve and promote human health due to the presence of molecules that prevent or fight various disease states.

On the basis of the results obtained by several studies about the antibacterial properties of Royal Jelly, it seems clear that this beehive product could be a potential subject of further investigation by the scientific world.

The new findings regarding its active components, their inner mechanisms of action and the possibility of isolation and purification of the pure substances, represent a starting point for the formulation of new products for therapeutic and pharmacological uses as an alternative to conventional antibiotics. Natural peptides, as Major Royal Jelly Proteins, could be taken into account as potential alternatives. The use of RJ could lead to the realization of nutraceutical products with a remarkable added value.

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