# EFTBA Veterinary Newsletter 31

***Rhodococcus equi*** (Wikipedia)

**The nature of *Rhodococcus equi***

***August 2019***

. *Rhodococcus equi infection is recognized worldwide as a major cause of disease in foals.*

. *R. equi is a very potent germ which can both elude immunological mechanisms of its host and develop antibiotic resistance.*

. *The prophylaxis against R. equi is demanding and it is imperative to know its nature as well as possible.*

***"Many thanks to Mrs. Eva-Maria Bucher-Haefner, Moyglare Stud Farm, for her valued sponsorship of this newsletter."***

## Welcome to EFTBA's veterinary newsletter

Dear Breeder,

Over the last number of years Hanspeter Meier has educated us with no less than 31 veterinary newsletters. In this edition he discusses the very dangerous *Rhodococcus equi*, a disease familiar to all breeders. A very serious cause of pneumonia in young foals. The key to survival is to catch it early and treat it accordingly. The organism is commonly found in dry and dusty soil and symptoms include an increase in respiratory rate, lethargy, fever, cough and nasal discharge. I complement Hanspeter on a very informative article and encourage all breeders to read it.

Disease prevention is always front and centre and the work conducted by the EFTBA veterinary committee under the chairmanship of Des Leadon should not be underestimated. Adherence to our codes of practice is key to the prevention of disease and members are encouraged to promote high standards of biosecurity in their respective countries.

You are no doubt aware that a new Animal Health Law will come into effect in 2020 and European industry stakeholders are working hard to ensure that breeding animals are included in this law and restriction on movement of thoroughbreds are kept to a minimum.

Finally I would like to thank Moyglare Stud Farm for their continued support with our newsletters, wish you all every success in the forthcoming sales season and hope to see you all at our next meeting, which will take place in Newmarket on Tuesday 26th November 2019

Best regards

Joe Hernon

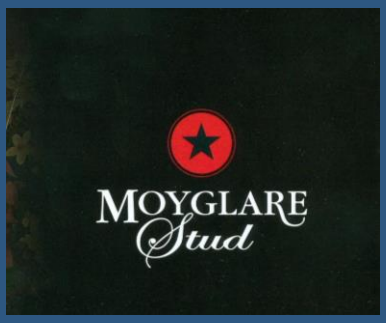
Chairman, EFTBA

## Editorial

Soundness certainly is the most important requirement for the well-being and the performance of our horses. But though we recognize this fact and try to prevent diseases as well as possible, some germs still are a great risk for our horses. Among them, *Rhodococcus equi* is a particularly troublesome bacterium, above all for our foals. The reasons for our great worries lie mainly in the very special nature of this germ. With this and following newsletters, we want to familiarize ourselves with the most distinctive peculiarities of *R. equi*.

Dr Hanspeter Meier

EFTBA veterinary advisor & Newsletter editor



## Introduction

The germ *Rhodococcus equi (R.equi)* is considered to be the most serious cause of pneumonia in foals 3 weeks to 6 months of age. In cases that we catch early, the foal can make a full recovery with proper and comprehensive treatment. However, under other circumstances, the mortality rate is high. We breeder therefore really want to protect our breeding stock as well as possible – but by trying to do so, we must realize that the responsible germ has very peculiar attributes. For instance, it certainly must find our stupefaction that the genus *Rhodococcus* comprises more than 40 species of organisms all over the world, but the only animal pathogen among the *Rhodococccci* is *R. equi* (Vázquez-Boland et al. 2019). Certainly, *R. equi* also has been isolated from a wide variety of other animals as e.g. cats, dogs, goats, cattle, pigs, wild boars, camelids, crocodiles and humans in immune-suppressive conditions. But *R. equi* is nevertheless one of the most important primary infectious agents of the horse (Hines 2014; Vázquez-Boland et al. 2019), whose nature we want to get to know as well as possible. With these intentions, we first must go into some details in the scientific literature and will come across quite a few pretty special expressions; those will be explained in the glossary in the annex at the end of the newsletter.

## The genus *Rhodococcus* and the species *Rhodococcus equi*

According to Melissa Hines (2014), *R. equi* infection was first described in horses in 1923 by the Swedish author Magnusson and today is recognized worldwide as a major cause of disease in foals. The most common clinical manifestation is pneumonia, although a number of other clinical problems also may occur. The disease has the potential to cause significant losses, especially on farms where it is endemic. Infrequently, *R. equi* also may cause infection in adult horses, generally thought to be associated with immunosuppression. The same phenomenon may also occur in immunocompromised human patients, particularly those suffering from infections with the human immunodeficiency virus (HIV). However, clinical disease is uncommon in these species, and the infections are often localized only.

Before going into details of the biology of *R. equi*, I would like to suggest a look at the 'pedigree' of this germ first, just the way we are used in studying sales catalogues.

However, by trying to realize this intention, I didn't have great success with my first step – the consultation of my veterinary dictionaries of 1974 and 1976 ('*The Horses Health from A to Z*' and '*Blacks Veterinary Dictionary*'). *R. equi* was not mentioned in either of them, for the simple reason that Magnusson initially called the responsible germ *Corynebacterium equi*. It was only in 1977 that Goodfellow and Alderson proposed to name it *Rhodococcus equi* (Vazquez-Boland and Meijer 2019).

But todays' scientific classification of these organisms (their 'family-tree') explains the situation:

***Rhodococcus equi***

Scientific classification (Wikipedia)

Kingdom: *Bacteria*
Phylum: *Actinobacteria*
Class: *Actinobacteria*
Order: *Actinomycetales*
Suborder: *Corynebacterineae*
Family: *Nocardiaceae*
Genus: *Rhodococcus*
Species: ***Rhodococcus equi***

The phylum *Actinobacteria* comprises more than 40 species of organisms, ubiquitously distributed in the environment, particularly in soil. For the selection of *Rhodococcus* species, please refer to the annex (state 2018).

According to Wikipedia, *Rhodococcus* is a genus of aerobic, nonsporulating, non-motile Gram-positive bacteria, a few species are pathogenic, but most are benign, and have been found to thrive in a broad range of environments, including soil, water, and eukaryotic cells (cells with a nucleus enclosed within membranes).

Wikipedia also explains that some strains of *Rhodococcus* are important owing to their ability to catabolize a wide range of compounds and produce bioactive steroids, acrylamide, and acrylic acid and their involvement in fossil fuel biodesulfurization.

They also can be used to convert cheap starting material into more valuable compounds (bioconversion) and – probably most interestingly – they also are able to metabolize harmful environmental pollutants, including toluene, naphthalene, herbicides, and polychlorinated biphenyls (PCBs). The PCBs are carcinogens in humans and animals and are known to be responsible for the environmental

contamination of rivers, buildings, parks, other sites and food supplies (Wikipedia).

Environmental pollution certainly is one of the great problems in our days and the discovery of the possibility of some species of *Rhodococcus* to biodegrade organic pollutants (so-called 'bioremediation') did find great attention. In the last years, *Rhodococcus* therefore has been investigated intensively; detailed information on these subjects can be found in the 368 pages of the book "*Biology of Rhodococcus*" (Alvarez et al. 2019).

This progress has been possible thanks to many properties of *Rhodococci*, as for instance:

- they are commonly found in the nature and can thrive under a variety of conditions
- they also are able to survive both in environments containing low oxygen concentrations and in oxygenated environments as well
- they can generate their own nutrients in environments with low nutrients
- they have a wide variety of catabolic pathways and many unique enzyme functions
- they are capable of accumulating heavy metal ions, such as radioactive caesium (allowing for easier removal from the environment)

etc. (Wikipedia).

Now, these are just very few (and very technical) hints to show us, that *Rhodococcus* is an extraordinary genus and that we have to deal with a most diverse and capable species (s. annex).

The main reason for the qualities of *Rhodococci* – or in our case – pathogenicity is that some of them have unusual **large genomes**, e.g. the 9.7 megabase pair genome of *R. jostii*.

Obviously, most of the *Rhodococci* are most useful germs in the nature and the industry, and so far **only two pathogenic species are known**, ***R. equi*** and ***R. fascians***. The latter causes the *leafy gall syndrome ("cauliflower disease")* in plants, above all in tobacco plants (Stes et al. 2013) – and *R. equi* is one of the most important foal pathogens (Wikipedia).

These facts very much remind the author of the times he spent in Lexington, 40 years ago, where the bloodstock usually was stabled in tobacco-barns. May there have been some confraternity among these two *rhodococci*? Who knows? – But all the facts as above surely invite or even oblige us to occupy ourselves with the molecular biology of these pathogens.

## The biology of *R. equi*

Due to the great importance of *Rhodococcus* for biotechnological processes (remediation, transformation and catalysis), these microorganisms are currently the subject of research in many countries all over the world. The number of publications and patents on *Rhodococci* has intensified significantly within the last years. The last chapter in the book "*Biology of Rhodococcus*" also deals with ***R. equi*** and we most probably can expect significant progress in regard to the research of ***the only animal pathogen among the rhodococci*** in the near future (Vázquez-Boland et al. 2019).

So far, we already know, that ***R. equi* is an *"intracellular parasite that replicates within a modified phagocytic vacuole in macrophages"*** (white blood cells) – a superb mechanism for its survival after infecting an organism (Fig. 1, 2 & 3)(Vázquez-Boland et al. 2019).

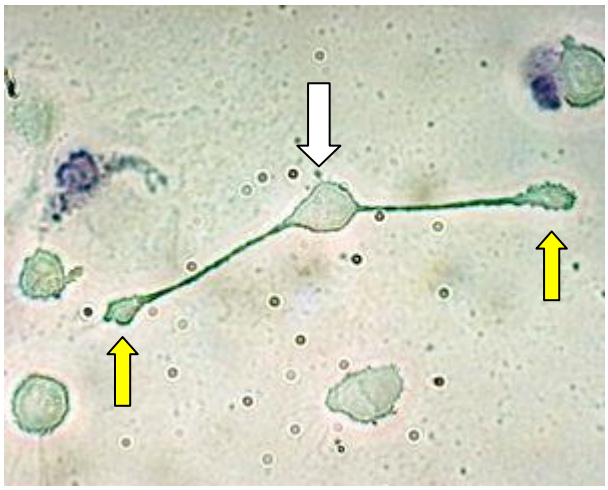


Fig. 1 A macrophage (white arrow) with two "arms" (pseudopodia)(yellow arrows)to engulf particles - e.g. *R. equi* (Wikipedia)

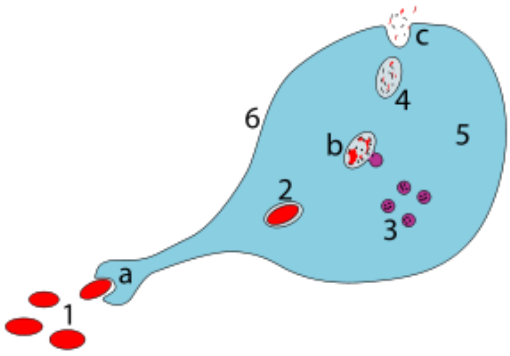



Fig. 2 **Usual** steps of a macrophage ingesting a pathogen (= phagocytosis): ./.

1) the macrophage (a) ingests the pathogen (1) (phagocytosis) and …
2) … a phagosome (2) is formed
3) lysosomes (3) break down the phagosome (b) by enzymes
4) waste material is expelled (c) (or assimilated),
(5 = Cytoplasm of macrophage)
(6 = Cell membrane of macrophage)

However, *R. equi* is an unusual germ and after being caught – **it isn't broken down by lysosomes** (Fig. 3). It protects itself by preventing the fusion with the lysosomes by acidification of the phagosome - like its close relative *Mycobacterium tuberculosis* (!).

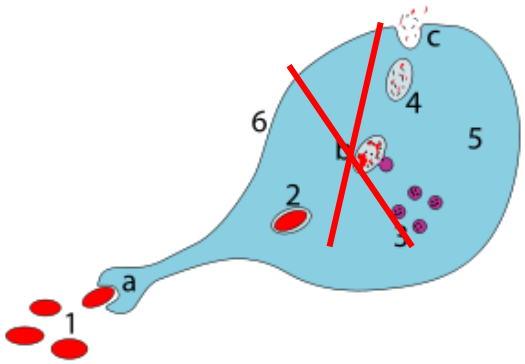



Fig. 3 *R. equi*–bacteria also are ingested by macrophages (1a), but their plasmid-genes encode proteins which prevent (by acidification of the phagosome 2), the fusion with the lysosomes.

Moreover, this extraordinary step allows ***R. equi* not only to survive - but even to multiply within macrophages**, where **it is also shielded from the immune system** – by the very cell that was supposed to kill it!

*R. equi* therefore is able to survive as an 'intracellular parasite' and doesn't that remind us of another troublemaker in our acquaintance - the Equine Herpes Virus (EHV)? EHV also is able to survive in cells of the organism of our horses, though by another mechanism (s. Newsletter 7, May 2012). In this ways, both germs avoid the elimination by the immune system, what also explains that we haven't got a vaccine against *R. equi* yet (what will be looked at in a further newsletter).

In this process, after about 48 hours, the macrophage is killed by necrosis, attracting additional phagocytic cells to the site of infection, eventually resulting in massive tissue damage – as we know it too well from post mortem examinations, sorry-to-say (Fig. 4).

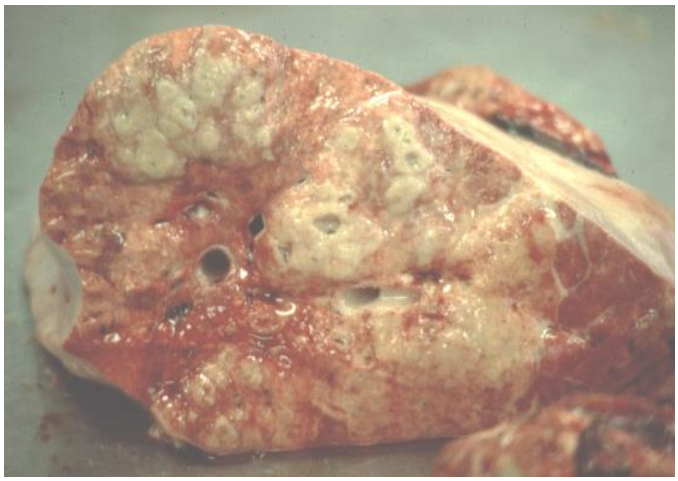


Fig. 4 Multiple pulmonary abscesses with caseous exudates characteristic of *R. equi* pneumonia (three-year old Arabian filly)

## Molecular epidemiology and determinants of virulence

What is the reason that *R. equi* is able to escape the immune system? I guess that any TB-breeder with his great interest in genetics has a suspicion – and you are right. The *Rhodococci* have a great genetic and catabolic diversity which isn't only due to the large chromosome but also to so-called 'plasmids'.

A **plasmid** is a small DNA molecule (79 - 100 kb) within a cell that is physically separated from the chromosomal DNA, but nevertheless carries genes as well and it also can replicate independently. The genes on these plasmids often benefit the survival of the microorganisms, e.g. genes which even provide antibiotic resistance. In *R. equi*, this fact already has been documented four years ago (Anastasi et al. 2015, Wikipedia).

The plasmid-genes are also essential for the ability of *R. equi* to survive in macrophages and the intracellular growth, the so-called 'conjugative virulence'. These virulence-genes encode antigens, with the name 'Vap proteins'; **vap** stands for **v**irulence **a**ssociated **p**rotein (Tripathi et al. 2012; Vázquez-Boland et al. 2019).

Equine strains are almost invariably associated with **VapA** virulence plasmids, and at least 12 vapA plasmids have been identified yet. However, specific research showed that VapA alone is not sufficient for virulence and that other plasmid genes are required (Giguère et al. 1999). – More research still has to be done here.

Recent evidence suggests that the vaps also are involved in infectious tropism towards specific animal host species (horses, pigs, and cattle). This suggests the interesting notion that the species-specific virulence plasmid types of *R. equi* are subject to a strong selection determined by the host animal species. Given the strongly immunegenic character of the plasmid-encoded Vap proteins, it is possible that this selection is driven by host species-specific aspects of the immune response (Kohler et al. 2003; Vázquez-Boland et al. 2019).

However, in this field there are also still some open questions, as up to 23% of human isolates do not carry virulence plasmids (Ocampo-Sosa et al. 2007), but the disease in humans is clinically very similar to that seen in foals (purulent cavitary pneumonia). This observation indicates that although plasmid determinants are necessary for the colonization of the equine host, chromosomal factors also are involved in *R. equi* pathogenesis (Vázquez-Boland et al. 2019).

A preliminary analysis of the *R. equi* genome has been made in 2003 and the complete DNA sequence has been determined just recently; a detailed analysis is expected to be published soon (Vázquez-Boland et al. 2019). We all hope sincerely that these genome-based approaches will allow progress in understanding better the nature of *R. equi*.

## Conclusions

The contents of this NL certainly are an awful lot of theory – but they nevertheless are only a very short summary of the available literature on *R. equi*. But even the summary makes clear that its biology is a very complex subject with still quite a few questions to be answered. *R. equi* has an extraordinary efficient genome which allows many biological tasks (e.g. catalysis, remediation, transformation). This germ obviously is very versatile and one is tempted to suppose that *R. equi* may be the Thoroughbred among the germs.

To be able to cope with *R. equi* we really have to go into details and it might be advisable to follow the opinion of King Henry V.:

***In cases of defence, 'tis best to weigh the enemy more mighty than he seems.***

(Shakespeare W., 1599, King Henry V.)

Details to the etiology, epidemiology, pathogenesis, symptoms, diagnosis, treatment and prevention of *Rhodococcosis* will follow in further issues.

## References


Alvarez H.M. (2019) Biology of *Rhodococcus*, 2nd edition, Springer-Verlag, Berlin Heidelberg

Anastasi E., Giguère S., Berghaus L.J., Hondalus M.K., Willingham-Lane J.M., MacArthur I., Cohen N.D., Roberts M.C. and Vasquez-Boland J.A., (2015): Novel transferable erm(46) determinant responsible for emerging macrolide resistance in *Rhodococcus equi*, Journal of Antimicrobial Chemotherapy.

Giguère S., Hondalus M.K., Yager J.A., Darrah P., Mosser D.M., Prescott J.F. (1999): Role of the 85-kilobase plasmid and plasmid-encoded virulence-associated protein A in intracellular survival and virulence of *Rhodococcus equi*. Infect Immun 67:3548–3557

Hines M.T. (2014): *Rhodococcus equi*, in Sellon D.C. and Long M.T. Equine Infectious Diseases (Second Edition), Saunders Elsevier, St.Louis, Missouri, 281-295

Kohler A.K., Stone D.M., Hines M.T., Byrne B.A., Alperin D.C., Norton L.K., Hines S.A. (2003): *Rhodococcus equi* secreted antigens are immunogenic and stimulate a type 1 recall response in the lungs of horses immune to *R. equi* infection. Infect Immun 71:6329–6337

Magnusson H. (1923): Spezifische infektiöse Pneumonie beim Fohlen. Ein neuer Eitererreger beim Pferde. Arch. Wiss. Prakt. Tierheilkd. 50, 22-38

Ocampo-Sosa A.A., Lewis D.A., Navas J., Quigley F., Callejo R., Scortti M., Leadon D.P., Fogarty U., Vazquez-Boland J.A. (2007): Molecular epidemiology of *Rhodococcus equi* based on traA, vapA, and vapB virulence plasmid markers. J Infect Dis 196:763–769

Stes E., Francis I., Pertry I., Dolzblasz A., Depuydt S., Vereecke D. (2013): The leafy gall syndrome induced by *Rhodococcus fascians*. FEMS Microbiol. Lett. May 342(2), 187-194

Tripathi V.N., Harding W.C., Willingham-Lane J.M., and Hondalus M.K. (2012): Conjugal Transfer of a Virulence Plasmid in the Opportunistic Intracellular Actinomycete *Rhodococcus equi*. Journal of Bacteriology, Dec. 194(24), 6790-6801

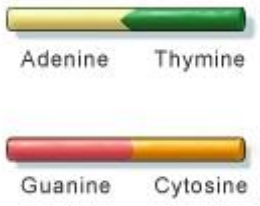
Vázquez-Boland J.A., Letek M., Valero-Rello A., González P., Scortti M. and Fogarty U. (2019): *Rhodococus equi* and Its Pathogenic Mechanisms, in Alvarez H.M. (2019) Biology of *Rhodococcus*, Second Edition, Springer-Verlag Berlin Heidelberg

Vázquez-Boland J.A. and Meijer W.G. (2019): The pathogenic actinobacterium *Rhodococcus equi*: what's in a name? Molecular Microbiology, Jul., Vol. 112(1), 1-15

# Annex

**Species of *Rhodococcus*** (> 40) (Wikipedia)

*R. aerolatus* (Hwang et al. 2015)
*R. aetherivorans* (Goodfellow et al. 2004)
*R. agglutinans* (Guo et al. 2015)
*R. aurantiacus* (Tsukamura and Yano 1985 ; ex Tsukamura and Mizuno 1971)
*R. artemisiae* (Zhao et al. 2012)
*R. baikonurensis* (Li, et al. 2004)
*R. biphenylivorans* (Su et al. 2015)
*R. boritolerans*
*R. canchipurensis* (Nimaichand et al. 2013)
*R. cerastii* (Kämpfer et al. 2013)
*R. cercidiphylli* (Li et al. 2012)
*R. coprophilus* (Rowbotham and Cross 1979)
*R. corynebacterioides* (Serrano, et al. 1972; Yassin and Schaal 2005
syn: Nocardia corynebacterioides (Serrano et al. 1972)
*R. defluvii* (Kämpfer et al. 2014)
*R. electrodiphilus* (Ramaprasad et al. 2018)
*R. enclensis* (Dastager et al. 2014)
***R. equi*** (Magnusson 1923; Goodfellow and Alderson 1977)
*R. erythropolis* (Gray and Thornton 1928; Goodfellow and Alderson 1979)
*R. fascians* (Tilford 1936; Goodfellow 1984)
syn: *Rhodococcus luteus* (ex Söhngen 1913; Nesterenko et al. 1982)
*R. globerulus* (Goodfellow et al. 1985)
*R. gordoniae* (Jones et al. 2004)
*R. hoagii* (Kämpfer et al. 2014)
*R. imtechensis* (Ghosh et al. 2006)
*R. jialingiae* (Wang et al. 2010)
*R. jostii* (Takeuchi et al. 2002, identified as producing a lignin digesting enzyme, it was the first isolated from a bacterium rather than a fungus)
*R. koreensis* (Yoon et al. 2000)
*R. kroppenstedtii* (Mayilraj et al. 2006)
*R. kunmingensis* (Wang et al. 2008)
*R. kyotonensis* (Li et al. 2007)
*R. maanshanensis* (Zhang et al. 2002)
*R. marinonascens* (Helmke and Weyland 1984)
*R. nanhaiensis*
*R. olei* (Chaudhary and Kim 2018)
*R. opacus* (Klatte et al. 1995)
*R. percolatus* (Briglia et al. 1996)
*R. phenolicus* (Rehfuss and Urban 2006)
*R. polyvorum* (Li et al. 2012)
*R. pyridinivorans* (Yoon et al. 2000)
*R. qingshengii* (Xu et al. 2007)
*R. rhodochrous* (Zopf 1891; Tsukamura 1974)
*R. rhodnii* (Goodfellow and Alderson 1979)
(syn.: *Nocardia rhodnii*)

| Glossary | |
|---|---|
| **base pair (bp)** | A base pair (bp) is a unit consisting of two nucleobases bound to each other by hydrogen bonds. They form the building blocks of the DNA double helix and contribute to the folded structure of both DNA and RNA.<br><br>Example of a base-paired DNA sequence:<br>ATCGATTGAGCTCTAGCG<br>TAGCTAACTCGAGATCGC<br>A = Adenine T = Thymine G = Guanine C = Cytosine<br>  |
| **biocatalysis** | biocatalysis refers to the use of living biological systems or their parts to speed up (catalyze)chemical reactions. In biocatalytic processes, natural catalysts, perform chemical transformations on organic compounds. The employment of enzymes and whole cells have been important for many industries for centuries. The most obvious uses have been in the food and drink businesses where the production of **wine, beer, cheese** etc. is dependent on the effects of the micro-organisms. |
| **bioconversion** | also known as *biotransformation*, is the conversion of organic materials, such as plant or animal waste, into usable products or energy sources by biological processes or agents, such as certain microorganisms. One example is the industrial production of cortisone, which one step is the bioconversion of progesterone (to 11-alpha-Hydroxyprogesterone)by *Rhizopus nigricans*. Another example of bioconversion is the conversion of organic materials, such as plant or animal waste, into usable products or energy sources by biological processes or agents, such as certain micro-organisms or enzymes. |
| **biodegrade** | to decay naturally and in way that is not harmful |
| **bioremediation** | bioremediation is a process used to treat contaminated media, includeing water, soil and subsurface material, by altering environmental conditions to stimulate growth of microorganisms and degrade the target pollutants. In many cases, bioremediation is less expensive and more sustainable than other alternatives. Biological treatment is a similar approach used to treat wastes including wastewater, industrial waste and solid waste. |
| **biotransformation** | also known as bioconversion is the chemical modification (or modifications) made by an organism on a chemical compound. Recently its application is seen as an efficient, cost effective, and easily applicable approach for the valorization of agricultural wastes with potentials of enhancing existing bioactive components and synthesis of new compounds. |
| **kb (= kbp) kilo base pairs** | kilo base pair (kbp) is a unit of length of nucleic acids, equal to one thousand base pairs = 1'000 bp |
| **macrophage** | macrophages (Greek: 'big eaters') are a type of white blood cell, of the immune system, that engulfs and digests microbes, cellular debris, foreign substances, cancer cells, and anything else that does not have |

| | |
|---|---|
| | the type of proteins specific to healthy body cells on its surface in a process called phagocytosis. |
| **Mb (= Mbp) mega base pairs** | mega base pair (Mbp) is a unit of length of nucleic acids, equal to one million base pairs or to one thousand kilobase pairs = 1'000'000 bp. |
| **phagocytosis** | phagocytosis (Greek: 'to eat', and 'cell') is the process by which a cell uses its plasma membrane to engulf a large particle (≥ 0.5 µm), giving rise to an internal compartment called the phagosome. The engulfing of a pathogen by a phagocyte In a multicellular organism's immune system, phagocytosis is a major mechanism used to remove pathogens and cell debris. The ingested material is then digested in the phagosome. Bacteria, dead tissue cells, and small mineral particles are all examples of objects that may be phagocytized. |
| **plasmid** | A plasmid is a small DNA molecule within a cell that is physically separated from the chromosmal DNA which can replicate independently They are most commonly found as small circular, double-stranded DNA molecules in bacteria. |
| ***R. equi*** | *Rhodococcus equi* |
| **saprophyte** | organisms or micro-organisms that live on dead or decomposing matter or waste |

**If there is any area you would like covered in these very informative newsletters you should contact Kerry on kryan@itba.ie and she will forward your request on.**

**Joe Hernon, Chairman EFTBA**

***European Federation of Thoroughbred Breeders' Associations EFTBA***
***c/o Irish Thoroughbred Breeders' Association***
***ITBA HQ, Greenhills, Kill, Co. Kildare, IRELAND***
***Tel: +353 45 877 543***
***Fax: + 353 45 877 429***
***E-Mail: info@eftba.eu Web Site www.eftba.eu***