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Enzybiotics: Emerging Alternative to Biocontrol

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ABSTRACT

The discovery and development of antibiotics was one of the greatest successes of Medicine in the 20th century and allowed the control of many diseases caused by microorganisms. Nevertheless, it is necessary to search constantly for new therapeutic tools in the continuing fight against disease-causing microorganisms and this probably leads us to today's concept of enzybiotics. Although microorganism-degrading enzymes have been known since the beginning of the last century, their use was soon forgotten because of the widespread use of antibiotics. The term enzybiotic is a hybrid word from ''enzyme'' and ''antibiotic'' and refers to phages: that is, viruses that attack and lyse bacteria and that can potentially help us to fight bacterial diseases. If the concept of enzybiotic is extended to antifungal enzymes, an enormous potential in the struggle against microorganism-due diseases may become available in the foreseeable future.

Keywords: Enzybiotic; antibiotic; phage; drug therapy; drug resistance; Enzyme; protein; protein binding; biotechnology

Thus, there is an urgent need to find an alternative way to address the problem in an efficient course of action.

Enzybiotics

Overuse and abuse of antibiotics are largely responsible for the increasing prevalence of multi-drug resistant bacteria. Hence, the reseachers have come up with new class of antibiotics-enzybiotics, with novel mechanism of action against drug-resistant pathogens.

INTRODUCTION

Enzybiotics are an experimental antibiotic approach employing enzymes to combat pathogenic bacterial infections. The name is a combination of the words "enzyme" and "antibiotics" first coined in March 2001 by Nelson *et al.,* Many of the enzymes used as enzybiotics are lysins,

enzymes derived from bacterial viruses (or bacteriophages) used to release progeny bacteriophage from infected bacteria, though other natural or synthetic enzymes may be used. Over the last decade, a dramatic increase in the prevalence of antibiotic resistance has been noted in several medically significant bacterial species, especially *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Klebsiella pneumoniae*, as well as *Staphylococcus aureus*, coagulase-negative Staphylococci, Enterococci, and *Streptococcus pneumoniae* (Hawkey 2008). This unfavorable situation is further aggravated by a shortage of new classes of antibiotics with novel modes of action that are essential to contain the spread of antibiotic-resistant pathogens (Livermore 2004). In fact, some infectious disease experts have expressed concerns that we are returning to the pre-antibiotic era (Larson 2007). Therefore, there is an urgent need to develop novel antibacterial agents to eliminate multidrug-resistant bacteria (Breithaupt 1999). A very interesting class of novel (at least in terms of their formal clinical use) antibacterial is enzybiotics.

The term “enzybiotic” was used for the first time in a paper by Nelson *et al.*, (2001) to designate bacteriophage enzymes endowed with bacterial cell wall-degrading capacity that could be used as antibacterial agents. While some authors suggest that this name should refer to all enzymes exhibiting antibacterial and even antifungal activity (Veiga-Crespo *et al.*, 2007), for the purpose of this work, we will discuss only bacterial cell wall-degrading enzymes (regardless of their source). Other names that are used with respect to enzybiotics are lytic enzymes and peptidoglycan hydrolases. The latter refers to the major mode of action of enzybiotics, that is, the enzymatic cleavage of peptidoglycan covalent bonds, which results in the hypotonic lysis of a bacterial cell. Peptidoglycan hydrolases constitute an abundant class of enzymes and may be obtained from different sources, for instance, bacteriophages (lysins) and bacteria themselves (bacteriocins and autolysins). Yet another example of well-known enzybiotics are lysozymes, including hen egg white lysozyme and human lysozyme . Encoded by the bacteriophage genome they are synthesized at the end of the phage lytic cycle, headed for lysing host cells releasing newly produced virions. In addition to this “lysis from within”, endolysins from phages of gram-positive host are also able to swiftly lyse bacteria from exogenous application. They have wide application in pathogenic detection and development of diagnostics, as a means of bio-defence, eliminating food pathogens and in control of phytopathogens. This review discusses the widespread potential of various

bacteriophage lysins/enzybiotics in the perspective of future antibacterial drug development.

The significant characertics of enzybiotics are:

- ☐ Their novel approach for antibacterial action.
- ☐ Their ability to kill antibiotic-resistant bacteria, and
- ☐ Their low probability of developing bacterial resistance.

For the purpose of this work, we shall discuss the major groups of enzybiotics, including lysins, bacteriocins, autolysins, and lysozymes, in the context of their potential medical applications.

SOURCES OF ENZYBIOTICS

Many PLY endolysins are identified and isolated from a variety of bacterial species, among them, few are discussed here.

Encoded by PLY genes 3 endolysin proteins from *Bacillus cereus* bacteriophage bastille, TP21 and TP12 have also been produced in *E.coli*. these were isolated as recombinant proteins and purified by two step chromatography. All the three enzymes rapidly and specifically lysed several Bacillus species with highest lytic activity against *B.cereus and B.toringenes* ply12 and PLY 21 were chemically N-acetylmuramoyl-L-alanine amidases. Each of lytic enzymes (PLYBA,41.1 kDa;PLY21,29.5 Kda, PLY1227.7 Kda) show significant heterogenity in their amino acid sequence and molecular weight with only little similarity. Phage lysin protein display that the catalytic/enzymatic ability is due to the N-termianl medium which resemble with the cell wall hydrolyses and autolysin(CW1SP) of *B.subtilis* while the C terminal of proteins are responsible for specific recognition and binding with the peptidoglycan of *Bacillus spp.* The close relationship of the phage lytic enzyme and cell wall autolysin reflects an indication towards horizontal gene transfer or sharing among various bacillus phages in their host (Loessner *et al*., 1997; Potter *et al*., 2007*).*

Bacillus anthracis prophage BA02 endolysin is another PLY endolysins encoded by the Bacillus anthracis genome.plyL ia an N-acetylmuramoyi-L-alanine amidase capable of cleaning the cell wall of several *Bacillus* species when applied exogenously. It is observed that the catalytic domain of plyL cleaves more efficiently than the full length protein. Cell wall binding domain showed strong binding *to B.cereus* comparative to other species like endolysins (PIY2L) of B. Cereus phage, TP21. Studies shows that the C terminal domain sometimes inhibits the activity

of catalytic domain through intramolecular interactions but targeting of the enzyme to the cell wall externally is not prerequisite of its lytic activity. This fact may be helpful while considering endolysins as therapeutic agents.

P1YC is a bacteriophage lysin containing two sub unit PLYCA and PLYCB , which altogether exert murein hydrolyse action against *Sterptococcous pneumoniae cell* wall. This prevents colonisation of group a *Streptococci* in the upper respiratory tract of mice and leads to bacterial exclusion by killing the microorganism.

Lysteria moncytogens bacteriophage also encode lytic endolysins enzymes which harbours specifically hydrolyzing cross linking enzymes bridges for Listeria peptidoglycan.

Two endolysisns, PLY118 ,A30.8 kDa L-alanoyl-D-glutamtae peptidase and PLY511,A36.5 kDa N-acetylmuramoyl-L-alanine amidase have been used with the aim of bio preservation properties based against *L. monocytogenes* in the food specifically in dairy starter cultures. Endolysins ply118 and ply511 are used for production of lytic enzymes by genetic fussion with *Lactococcus* lactic MG1363. Therefore ply511 was fused with the s1pa nucleotide sequence encoding the lactobacillus s layer protein signal peptide. Expression of s1pa-ply511 from psl-pl511 resulted in secretion of functional ply511 enzyme from L.lactice which shows unduly strong lytic activities due to frame shift mutation occurred in final secretory products. Surprisingly, the resulting mutant polypeptide strongly increased its lytic activity. Immunoblotting enzyme experiment indicated that the enzyme caused rapid lysis of *L.monocytogen* cells.

Mur-lh is a broad spectrum endolysins obtained from temprate bacteriophage (i)-0303 0f *Lactobacillus helveticas* carz 303 strain. The lysin encoding iys genes of this bacteriophage was clone using a library of of 4-0303 in *E.coli* DH5a. The lys gene sequence has 1122 by encoding a protein of 373 amino acid (murlh) with lytic activity and molecular mass of 40.2 Kda. More mur-lh endolysins was expressed in *E.coli* BL21, its N-terminal sequence showed catalytic activity and caused hydrolysis of *L. havetica* CNRZ303 cell walls. Endolysins mur-lh posseses N-acetyl muramidase activity which provides broad spectrum of lytic activity against different species such as *Bacillus subtilis, thermophillic lactobacicili* and *lactococci, Clavibacterium* linens and *Enterococcus faecium.*

SENSITIVE TARGETS OF THERAPY

Endolysins act specifically against its target bacteria either in narrow range or in broad spectrum. Literature reveals examples of various bacteriocidal phage enzymes. Recombinant phage endolysins inhibit various pathogens and have recently been asserted as alternative antimicrobials for treatment of bacterial infections due to gram-positive bacteria (Fischetti, 2003; Leossner, 2005). The effectiveness of phage lysins in clearing bacterial infections have been well documented in mouse models (Leoffler *et al*.,2001) and also in transgenic plants.

Staphylococcus aureus, Streptococcus uberus and Streptococcus agalactiae bacteriophage endolysins have been applied in mastitis in cow's treatment (Donovan *et al*., 2006) with profitable results.

Bacteriophage k1-5 encodes two different proteins originating from tails fibres capable of infecting k1 and k5 strains of Escherichia coli by replicating within it. Similarly, bacteriophage t4 tail lysozyme also acts as lysin enzymes. Bacteriophage phi3626 produces murein hydrolase enzyme lysis system against many strains of *Clostridium perfringes* (Zimmer *et al*.,2002*). Staphylococcus aureus and Streptococcus agalactiae*, causal agent of mastitis mainly in high lactating cattle are also pathogenic for humans .To check the bacterial infection , *S. Agalactiae* bacteriophage B30 induced two endolysins have been used. When these two novel antimicrobials 182-amino-acid length endolysins were allowed to fuse with the lystostaphin protein of *Staphylococcus simulans*, this fusion exhibited lytic activity for streptococcal as well as S. aureus pathogens. Immunohistochemical studies have shown that fussion proteins remain active in milk against bacteria with no harmful effect on the cells. It can successfully be used as an alternative to broad-range antibiotics against clinical infections since the fusion peptidoglycan hydrolase acts selectively as multi-pathogen targeting antimicrobial agent. One recombinant endolysins 11 is capable of hydrolysing not only heat killed *Staphylococci* but also *Staphylococci* biofilms. Another phage lysin Lysk is a recombinant endolysins protein exerting lytic activity aginst clinically relevant as well as methicllin-resistant *Staphylococcus aureus* (Navarre *et al*., 1999 O' Flaherty *et al*., 2005 Donovan *et al*., 2006). The endolysins Lysh5 from the *Staphylococcus aureus* bacteriophage 01-15 resembled other murein hydrolases encoded by *Staphylococcal* phages. It rapidly lyses Bovin and human *S. aureus* and human *staphylococcus epidemydis* strains in pasteurised milk (Loessner *et al.,* 1998,1999. O'flaherty *et al*., 2005).

MODE OF ACTION

Enzybiotics majorly belong to the class peptidoglycan hydrolases. When these enzymes are added exogenously to Gram positive bacteria they cause rapid disintegration of the cell wall as there is no outer membrane present to hinder their action. But, in Gram negative bacteria outer membrane obstructs their way to the cell wall, thus, limiting their activity. Enzybiotics have narrow host range; therefore, they selectively target their pathogenic hosts without affecting the surrounding micro flora.

MECHANISM OF ACTION

Bacteriophages, depending upon structure follow two methods to release their progeny virions from host bacterial cells:

Filamentous phages are released through bacterial cell walls without killing bacterial cell.

Non filamentous phages make use of specific lysine enzymes to either inhibit the synthesis of peptidoglycan(single stranded RNA or DNA phage encoded enzymes)in the cell wall of bacteria or hydrolyze the built peptidoglycan by means of a holin-endolysin system(stranded DNA phage encoded enzymes).

Endolysins or lysins need a second protein Holin to find their substrate molecule in the cell wall. Lysins remains in the cytosol till the late stage of the lytic cycle and hydrolyse the peptidoglycan of the bacterial cell wall when Holin form pores in the inner membrane of the infected host cell. This results in access of lysin to the peptidoglycan causing rapid cell lysis thus releasing mature phage progeny (Wang *et al*.,2000).

In Holin-Endolysin system, phage requires both the Holin and Lysin for host cell lysis. Nevertheless, when Lysins are employed as recombinant enzymes and applied exogenously to gram-positive bacteria they are well capable of causing rapid lysis as no outer membrane is present to inhibit their access to the cell wall. In Gram-negative bacteria, the use of endolysins as antibacterial is limited as outer membrane hinders the access to exogenous lysins towards the cell wall peptidoglycan. Phage lysins selectively target specific pathogenic bacteria without affecting surrounding commensal micro flora due to narrow host range (Leossner *et al.*, 1995; Leossner, 2005).

Bacteriophage murein hydrolase enzyme display high specificity towards the cell wall of host bacteria due to presence of well-defined cell wall binding domain that affix the endolysins to its substrate. Bacteriophage induces host cell lysis with the help of two proteins, Endolysins and Holins.

Endolysins, a kind of muralytic enzyme accumulate in the cytosol during the vegetative cycle and degrade the bacterial cell wall with the help of holin proteins which are accrued inside the cytoplasmic membrane.

Holins as membrane proteins remain in the membrane until a specific programmed time when the membrane becomes abruptly permeable to the endolysins.

Destruction of the murein of the cell wall and cellular bursting are immediate consequences of lytic actions of endolysins. As holin genes direct the length of the infected cycle of lytic phages by means of holin proteins hence they are subject of deep evolutionary interest. Though actions of holins is regulated by a number of diverse proteins, they represent one of the most sundry functional groups, with more than 1000 known or putative holin sequences (Wang *et al*., 2000).

Lysis of the host cell wall with the Lyz endolysins of bacteriophage P1 is mediated by an N-terminal Trans membrane Domain (TMD), without involving a holin. The N-terminal domain of Lyz is capable of exporting the endolysins to the membranes but also facilitates its release into the periplasm (Donovan *et al*., 2006).

Endolysins have applications in specific enrichment of microbial cells by their magnetic separation and immobilisation. This novel application is based on affinity of cell wall binding domains (CBDs) of phage encoded peptidoglycan hydrolases for host bacterial cell wall. Such polypeptide endolysins exclusively recognise the specific ligands on the gram-positive cell wall such as *Bacillus cereus, L. Monocytogenes* and *Clostridium perfringens* with high affinity . the CBD-based magnetic separation (CBD-MS) procedure has shown significant results when paramagnetic beads coated with recombinant Listeria phage endolysins derived CBD molecules could capture and detect more than 90% of the viable *L monocytogenes*; from artificially as well as naturally contaminated food samples that too within 20 to 40 minutes. Presence of other microorganisms in the same solution did not interfere with the isolation procedure and needs less time, hence considered as superior to the already established traditional standard procedures.

Neisseria gonorrhoeae encodes At1A proteins with peptidoglycan transglycolase homologous property, processing peptidoglycan lytic activity similar to endolysins of bacteriophage (Kohler *et al* 2007).

MAJOR CLASSES OF ENZYBIOTICS

1. **Bacteriocins:** Bacteriocins are proteinaceous extracellular substanaces that are produced by both Gram positive and Gram negative species. They are either produced spontaneously or induced by certain chemicals such as mitomycin C. They inhibit the growth of similar or closely related bacterial strains. These are narrow spectrum class of antibiotics. Their lethal activity involves adsorption to the specific receptors on the exterior of specific bacteria, followed by metabolic, biological and morphological changes resulting in the killing of bacteria. Bacteriocins are produced by non-pathogenic bacteria that normally reside in human body. Antibiotic use results in the loss of these harmless and useful bacteria, paving way for the opportunistic pathogens to invade the human body.
2. **Lysins:** Lysins, also known as **endolysins** or **murein hydrolases** are peptidoglycan degrading enzymes released by bacteriophages that help in lysis of bacterial cell wall at the end of lytic cycle to release the progeny phage particles. They are increasingly being used as antibacterial agent owning to their high efficacy and specificity. They have characteristic lysis or cell-wall binding domain and degrade peptidoglycan with glycosidase, amidase, endopeptidase, or lytic tansglycosylase activities. They are species specific but some broad spectrum lysins have also been reported. They are highly effective against Gram positive bacteria as the outer membrane is absent as against Gram negative where outer membrane is present. The main mode of antibacterial action of lysins is the enzymatic cleavage of the covalent bonds in peptidoglycan. Depending on their enzymatic specificities, lysins fall into five major classes: N-acetylmuramoyl-L-alanine amidases, endopeptidases, N-acetylmuramidases (lysozymes), endo-β-N-acetylglucosaminidases, and lytic transglycosylases.
3. **Lysozymes:** Also known as muramidase or N-acetylmuramide glycanhydrolase, globular protein of 129 amino acid residues. They belong to the class glycoside hydrolase that catalyse the hydrolysis of 1,4-β-linkages. They cleave the 1,4-β-linkages between N-acetyl

muramic acid and N-acetyl-D-glucosamine in peptidoglycan structure. Lysozymes have been found in secretion like tears, mucus, saliva and human milk. Egg white (albumin) is an abundant source of lysozyme. It significantly contributes to innate immunity of humans . Lysozymes are most powerful natural antibacterial and antiviral substance. It also exhibits anti-inflammatory, anti-cancer and immunomodulatory activities. Generally, lysozyme is capable of killing only Gram-positive bacteria, while Gram-negative bacteria are resistant owing to the presence of the outer membrane. However, several exceptions to this rule have been reported, including both lysozyme resistant Gram-positive bacteria (e.g., some strains of *S. aureus* and *E. faecalis*) and lysozyme-sensitive Gram-negative bacteria (e.g., *Capnocytophaga gingivalis*). It is also worth mentioning that several modifications of the lysozyme molecule have been developed to enable the enzyme to kill Gram-negative bacteria. These are essentially based on coupling lysozyme to molecules facilitating the penetration of the outer membrane (e.g., fatty acids and hydrophobic peptides) (Ibrahim *et al.,* 2002; Masschalck and Michiels 2003).

4. **Autolysins:** This enzyme hydrolysis the biological component or tissue in which it is produced. These are found in all peptidoglycan containing bacteria. The enzyme functions similar to lysozyme. It cleaves the 1,4-β-linkage between N-acetyl glucosamine and N-acetyl muramic acid. They break down the peptidoglycan matrix and assist the bacterial cell in growth and cell division. They present a promising target for the development of new type of antibiotics. Atl is the major lysin of *Staphylococcus epidermidis* and *S.aureus* playing an important role in the separation of cell and in virulence, their virulence are also attenuated. For the development of new types of antibiotics, autolysins represent a promising target (Zoll *et al.*,2010). For the pathogenesis of infections that are invasive in nature, presence of pneumolysin appears to be more critical. In case of all isolates of *Streptococcus pneumonia* presence of lytA gene signifies that autolysin is an obligate necessity for this organism irrespective of the isolation site (Nelson *et al.*,2001).
5. **Defensins** and **Cathelicidins:** These are antimicrobial peptides found in the lysosomes of macrophages and polymorphonuclear leukocytes and keratinocytes. They are a part of mammalian innate immunity system that help fight bacterial infections. Cathelicidins obliterate the lipoprotein membranes of microbes enveloped in phagosomes after fusion

with lysosomes in macrophages. The defensins as well as cathelidicins can be exploited as enzybiotics among other families of antimicrobial peptide genes. In the innate immunity, such enzymes that are endogenous in nature play crucial role and forms the first line of defence for protecting the internal as well as external surface of the host (Wang et al., 2011).

6. **Virion-associated peptidoglycan hydrolases (VAPGH):** These are phage encoded lytic enzymes that disintegrate the peptidoglycan of the bacterial cell wall during infection. Their mode of action involves the generation of small hole through which the phage tail enters, crosses the cell envelop and releases the phage genetic material at the onset of the infection cycle. VAPGHs are highly specific, thermostable and have high modular organization. They serve as a potential candidate for the use as enzybiotics.

Examples

Various enzybiotics with their host range, source and types are summarized below.

Enzybiotic name	**Enzybiotic class**	**Source**	**Enzymatic specificity**	**Antibacterial range**
Ply C	Lysin	Phage C1	Amidase	*S. pyrogens* group C and E
P al	Lysin	Phage DP	Amidase	*S. pneumonia*
Lambda SA 2-E	Endolysin	Staphylococcal phage	Endopeptidase	*S.aureus*
P ly G	Lysin	Phage Gamma	Amidase	*B.anthracis*
Lyt A	Autolysin	S. pneumoniae	Amidase	*S.pneumoniae*
Lysostaphin	Bacteriocin	S. simulans	Endopeptidase	*S.aureus*, *Staphylococci*
Hen egg white lysozyme	Lysozyme	Hen's white	Muraminase	Gram positive bacteria

Source (Loessner, 2005)

SOME POTENTIAL APPLICATIONS OF ENZYBIOTICS

In Food industry: Enzybiotics have been widely used in food industry as food additives and preservatives like in the production of cheese and wine. The Food and Drug

Administration (FDA) has given a nod to the use of enzybiotics to control *Listeria monocytogenes* in cheese, classifying them as GRAS (generally recognised as safe) in the year 2006 which was later extended to enzybiotic use on all food products in 2007. Bacteriophage encoded endolysins have been recently deemed as new emerging biocontrol tools to inhibit and check the food contaminations by pathogens in food industry. The ionic concentration plays an important role for optimal lytic activity of lysins such as LYSH5, the endolysins encoded by the staphylococci bacteriophage phi-SauS-ipa88. Mg and NaCl enhances the activity of LysH5. The activity was inhibited by the presence of Mn and Zn. Along with another food biopreservative, bacteriocin nisin LysHsa potray strong synergistic effect specifically against S.aureus. such study paves the way to exploit the possibilities of hurdle technology, part of Harzard Analysis and Critical Control Point (HACCP) combining a phage-encoded endolysins and the bacteriocin for efficient *S.aureus* inhibition in milk and other dairy products (brussow, 2001; Garcia *et al*., 2010) . currently

In Medical industry: Their novel approach for antibacterial action, ability to kill antibiotic-resistant bacteria and very low possibility to develop bacterial resistance make them potent antibacterial and antifungal agents. They are used in eye drops, toothpastes etc. Their other uses are well documented above. Emerging resistance to antibiotics along the threat of antibiotic residue have got negative influence on human health. Both European Union and United States have prohibited the usage of antibiotics in this context. For developing bacteriocins and antimicrobial peptides; bacteriophages is in further progress (Pritchard *et al*., 2007). Putative lysine is a new antimicrobial growth promoting agent which is suggested by the European Union. It is found to be a better alternatives than prebiotics or probiotics; as well as phytonutrients and hyperimmune antibodies (Seal, 2013). In the intrapartun prophylaxis in case of early onset of neonatal infections due to *Staphylococcus agalacticiae* that colonizes the genital tract, the major potential application for the lytic enzymes have been proposed (Pritchard *et al*., 2004; Cheng *et al.*, 2005). PlyGBS is the only lysine specific to *S. galacticae* whose efficacy has been evaluated in vivo (Cheng *et al.*, 2005). In case of colonization in the vagina in murine model one topical dose of lysin when administered has resulted in 3-log decrease in the level of bacteria in comparison to mice in control group. One such dose of PlyGBS topically is sufficient to cause reduction in colonization of bacteria of the mucosa of oropharnynx sufficiently, it thus

appears that lytic enzymes specific to *S.agalaticae* may be used not only for the elimination of colonization in the vagina before delivery in case of pregnant animals but also for decontamination of new borns; thereby causing decrease in the incidence of infections in neonates.

In the prophylaxis as well as treatment of several bacterial infections that include pharyngitis and tonsillitis, dysentery as well as infections caused by wound, there has been use of lysozymes in combination with other antibiotics for the last several decades (Cheng *et al*., 2005).

There has been the use of formulation of lysozymes either as gel for treatment topically in case of wounds ; acne's treatment by using several formulation of enzymes; and infection prophylaxis due to piercing of skin.

The use of mutants of lysozyme for neutralizing the activity of a lysozyme inhibitor produced by *Treponema pallidum* is another interesting application of lysozyme. As a component of oral health products including mouthwash s, lysozymes has also been used for the purpose of killing several bacteria in the oral cavity (Tenovuo, 2002; Gil-Montoya *et al*., 2008).

Among the endopeptidase, lysostaphin is a major one having potential theraupetic application. The foremost medical application of Lystotaphin is Staphylococcal elimination that colonizes the membrane of the nasal mucosa. (Veiga-Crespo *et al*., 2007). The enzyme can exert antibacterial activity even after injection repeatedly.

In Farm industry: In the recent years the misuse and overuse of antibiotics in food producing livestock have raised a serious alarm on increasing number of antibiotic resistant bacteria, thus, escalating the risk of antibiotic resistant infections in humans. Enzybiotics present a novel way to combat the problem. They have been successfully used in treating septicaemia and meningitis in chickens and calves and also used as biocontrol agenets to control the number of *Salmonellla* in poultry products.

Enzybiotics present a redoubtable cover over antibiotics. Their objectionable side effects have been rarely reported. Therefore, they are very effective to be used as antibacterial and antifungal agents. Researches are being carried out on further potentials of enzybiotics and it is hoped that they present a promising future to medical community by help combating the antibiotic resistant strains of pathogens.

COMPARISON WITH OTHER EMERGING ALTERNATIVE BIOLOGICAL THERAPIES.

Several other contemporary alternative are also evolving in parallel to enzybiotics therapy. These are:

Bacteriophage therapy: bacteriophages are viruses of a bacterium which invades their host cell bacterium by using specific receptors but does not affect eukaryotic cells being host specific. In their lytic mode of life cycle, by secreting endolysins and holins enzyme phages can kill Gram-positive, gram negative, acid fast and many other bacteria as well. Bacteriophage therapy has been tried for a wide range of bacterial infections for animals and humans (Pritchard *et al*., 2004).

Cytokine therapy: Cytokines are intercellular regulatory proteins , which play a pivotal role in initiation, maintainance and regulation of immunological homeostatic and inflammatory processes. Due to their multiple functions, they are promising candidates for therapeutic interference in infectious and autoimmune diseases, especially immunosupressed patience receiving long term treatment for cancer or AIDS. The immunoglobulin Fe fragment based cytokines provides superior therapeutic approach. Nevertheless, the development of new vaccines necessitates the development of new types of cytokines adjuvants to ensure an appropriate immune response. (Rahimi *et al*., 2007).

Avian egg antibody therapy: chicken are capable of producing antigen specific antibodies (IgY) which have function similar to IgG in response to antigen. It can be used to treat microbes which do not respond to antibiotics treatment with these antibiotics produced in eggs of hyper immune birds is safer, more efficient and less expensive in comparison to antibiotics. Specific IgY antibiotic have been developed against different viral or bacterial viz, rotavirus, bovine respiratory syncital virus, coronavirus, infectious bursal disease virus. *E.coli, Salmonella, Edwardsiella, Yersinia, Staphylococcus* and *Pseudomonas* (Rahimi et al., 2007; Michael *et al.*, 2010).

Herbal therapy: various herbs and their extracts have been proved to have potent antimicrobial, antiviral or antifungal activities (Lai *et al*; 2002). For example, neen, ashwagandha, gloy, onion, garlic, mustard, red chilli, turmeric, clove, rosemary, cinnamon, ginger etc have been found to be highly usefell in this aspect. Also, herbs do not possess developmental resistance like that of

antibiotics and are comparatively safer and cost-effective. Herbal therapy is also gaining much attention these days in the treatment of subclinical mastitis and uses of *Terminalia chebula* and *Terminalia belerica* in this regard are found to be significant. (Akesson *et al*; 2007).

Panchgavya therapy: nowadays, panchavya therapy (cowpathy) is also gaining much importance because cow urine (an important component of panchavya) is able to kill a lot of bacteria that shows antibiotic resistance. The antibiotic resistant germs of tuberculosis can be killed by cow dung and urine, particularly cow urine which acts as a bioenhancer for anti-tuberculosis drugs , for which it is gaining much importance in the international market as an ant tubercular agent. (Gründling *et al;* 2006).

IMPORTANT ASPECTS OF ENZYBIOTIC THERAPY

Moreover, side effects associated with enzybiotic therapy might occur following the massive release of preformed bacterial toxins from the cytoplasm of bacteria during bacteriolysis. In fact, autolysins of some bacterial species may be involved in the pathogenesis of infections in the mechanism based on the release of different toxins. For instance, autolysins of *Clostridium difficile* may be involved in the release of toxin A and toxin B (Dhalluin *et al.,* 2005). This potential effect should also be taken into account in the discussion about the safety of enzybiotic therapy.

CONCLUSION AND FUTURE PERSPECTIVES

This review discusses the prophylactic and therapeutic applications of enzybiotics, especially with respect to their potential use in human and animal medicine. Due to increase in the prevalence of multidrug resistant bacteria dramatically and continuously, the most crucial characteristic of enzybiotics is their mode of action which is novel along with the ability to combat bacteria that are resistant to antibiotics. In relation to traditional antibiotics the risk of development of resistance is relatively lower for certain lytic enzymes. Enzybiotics that are unmodified importantly lyse solely gram positive bacteria but certain modifications that are developed enable them to kill gram negative bacteria as well. Various forms of prophylaxis as well as treatment of bacterial infections are included in the potential medical applications of enzybiotics. In animal models for instance certain lytic enzymes have been shown to be very

effective in killing bacteria that colonize mucous membranes upon administration topically. Employment of such enzymes can be done as unique means of prophylaxis on the basis of clearance of bacteria that represents a starting point for infections potentially. In the treatment of several systemic infections (including bacteremia) in animals that are immunized too it has been shown by various experimental studies that lytic enzymes are efficacious. Being most abundant biological entities on earth phages are a rich natural source of endolysin emzymes, hence with enormous potentials Lysins can be explored against infectious disease even in the dilemma of multi-drug-resistance conditions also. Further digging will definitely lead to produce new opportunities for the production of specifically engineered designer lysins with diverse applications in biology and life sciences for the wellbeing of humanity against deadly pathogens and infections. On the basis of the unique therapeutic capabilities, enzybiotics certainly deserve attention in the wider sense of the medical community.

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