A review on characterization, applications and structure-activity relationships of *Bacillus* species-produced bacteriocins

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**SUMMARY** Antimicrobial peptides (AMPs) are inherently occurring proteins that are produced by microorganisms as secondary metabolites. Members of genus *Bacillus* produce many types of AMPs by ribosomal (bacteriocins) and non-ribosomal (polymyxins and iturins) mechanisms. Bacteriocins are ribosomally synthesized peptides that inhibit the growth of closely related bacterial strains. Moreover, bacteriocins produced by *Bacillus* species have been widely used in pharmaceutical, food industry, fishery, livestock as well as in agriculture sector. The objective of this review is to assess the characterization of the *Bacillus*-derived bacteriocins, their potential use in different sectors and structure-activity relationships.

**Keywords** Antimicrobial agents, lantibiotics, bacteriocin-like inhibitory substance (BLIS), probiotics

1. Introduction

Microorganisms are good source of antimicrobial agents. The production of antibiotics by microorganisms and use of antimicrobial peptides (AMPs) as therapeutics has been one of the major achievements in medicine (1). *Bacillus* is Gram-positive, rod shaped and spore-forming bacteria. They are aerobic and catalase producing bacteria. They are aerobic and catalase producing bacteria and found in different natural environments such as soil, rocks, dust, marine, agricultural produce, and the gastrointestinal tract of animals (2). Among the strain for producing antimicrobial compounds, *Bacillus subtilis* is the major producer followed by other Bacilli such as *Bacillus brevis* (brevistin, edeines, gramicidines, tyrocidin), or *Bacillus amyloliquefaciens* (3). The bacteriocin is one of a heterogenous subgroup of ribosomally synthesized antimicrobial peptides that have bacteriocinogenic plasmids which are lethal to closely related bacteria (4). Both Gram-positive and Gram-negative bacteria produce bacteriocins.

Members of the *Bacillus* group are known to be a major producer of antimicrobial substances (4). Some of its members, such as *B. subtilis*, devote more than 4% of its genome for the synthesis of polyketides (PKs), non-ribosomal peptides (NRPs), bacteriocins as well as other uncommon antibiotics (5). The antimicrobial agents produced by various strains of *Bacillus* are found to exhibit antibacterial as well as antifungal activity against many pathogenic microorganisms including phytopathogens (6).

Because of potency of bacteriocins in different sectors, their study is important notion. This review illustrates an overview of bacteriocins produced by *Bacillus* including its classification as well as their applications in different sectors such as human health, food industry, fishery, agriculture, and environment.

2. Bacteriocins produced by *Bacillus* species

*Bacillus* genus strains produce large number of antimicrobial peptides with different chemical structures. Specifically, they produce antimicrobial substances including peptides, lipopeptides and bacteriocins (4). Similarly, *Bacillus* species produce major antibiotics that are made by ribosomal (bacteriocins) or non-ribosomal (polymyxins and iturins) pathway according to their mechanism of action. Among them, high number was produced by *B. subtilis*, followed by *B. brevis* and few by other *Bacillus* species (3). Different strains of *B. subtilis* produce variety of bacteriocins. For example, *B. subtilis*, *B. subtilis* A1/3, *B. subtilis* 168, *B. subtilis* strain HILY-85 produces subtilin, ericin S and ericin A, sublancin 168, mersacidin, respectively. Other *Bacillus* species like *B. licheniformis*, *B. cereus*, *B. thuringiensis*, and *B. pseudomycoides*, etc. also produce bacteriocins like bacillocin 490, cerein 8A, thuricin 7, and pseudomycoicidin respectively (7-11). Recently, a new bacteriocin was reported namely amylocyclicin which was produced by *B. amyloliquefaciens* FZB42 (12). Sonorensin is a new peptide belonging to
heterocycloanthracin, subfamily of bacteriocin isolated from marine bacteria B. sonorensis MT 93. This peptide showed activity against broad spectrum bacteria including B. subtilis, E. coli, Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio vulnificus (13). Other class iii bacteriocins produced by B. subtilis group are baciamin and Bac 14 B which have antibacterial as well as antifungal activity (5) Abriouel et al. in 2011 reported a long list of bacteriocins/BLIS produced by Bacillus species (14). Some of the reported Bacillus produced bacteriocins are summarized in Table 1.

2.1. Classification of Bacillus bacteriocins

The classification of bacteriocins was initially done by Klaenhammer in 1993, Nes and colleagues in 2007, and by Abriouel and his coworkers in 2011 (14,20). They classified bacteriocins into three classes, the first class (I) include antimicrobial peptides that undergo different forms of post-translational modifications; the second class (II) presents nonmodified and linear peptides, and the last class (III), which includes large proteins (> 30 kDa). Recently, Soltani et al. (2021) reclassified bacteriocins into two large classes. Class I resembles peptides group with molecular masses < 5 kDa and that contain post translationally modified bacteriocin. Class II bacteriocins contain unmodified peptides with molecular masses of 6-10 kDa including peptides with unstable disulfide bridges (21).

Class I. Post-translationally modified peptides

The class I bacteriocins include small (< 5 kDa) heat stable peptides. This class can be further divided into 4 subclasses (subclasses I.1, I.2, I.3, and I.4). Subclasses I.1-1.3 includes lantibiotic peptides, containing lanthionine and methyllanthionine residues. While the subclass I.4 includes peptide with unique modifications (14).

Subclass I.1. Single peptide, elongated lantibiotics

This group is represented by lantibiotics, are small peptides (22,23) (19-38 aminoacids) and contains dehydrated amino acids (lanthionine and methyllanthionine) introduced by posttranslational modifications (24,25). Unusual amino acids lanthionine and 3-methyllanthionine are in the form of ring structures that make lantibiotics more stable against heat, wide range of pH and proteolytic enzymes, thus these properties differentiate them from other antimicrobial peptides (25). Subtilin is a lantibiotic produced by B. subtilis, one of the extensively studied peptide that belongs to type A lantibiotics. It is active against most of the Gram-positive and some Gram-negative bacteria (26). Ericin S (3,442 Da) and ericin A (2,986 Da) are two related bacteriocins produced by B. subtilis A1/3 with strong resemblances to subtilin (27).

Subclass I.2. Other single peptide lantibiotics

Subclass I.2 includes globular lantibiotics mersacidin and other lantibiotics namely sublancin 168 and paenibacillin. Mersacidin which is produced by Bacillus sp. strain HIL Y-85.54728 shows more globular structure due to the presence of four intermolecular thioether bridges. It inhibits the growth of Gram-positive bacteria including methicillin resistant Staphylococcus aureus (MRSA) (22). Sublancin 168 is produced by B. subtilis 168, consists of one lanthionine linkage and two unusual disulfide bonds. It shows activity against Gram-positive bacteria, including B. cereus, Streptococcus pyogenes and S. aureus. Since a lot is known about sublancin, it has potential for novel biomaterial engineering (28). In 2013, Arias and colleagues identified amylolysin from B. amyloliquefaciens GA 1, a type B lantibiotic which exhibits antibacterial activity against Gram-positive bacteria including MRSA and Listeria monocytogenes (19).

Subclass I.3. Two-peptide lantibiotics

This subclass includes lantibiotics containing two components. The two peptide bacteriocins produced by Bacillus species are haloduracin and lichenicidin produced by B. halodurans C-125, B. licheniformis DSM 13 respectively (29,30). These peptides are closely related to two peptide bacteriocins produced by other bacteria such as, cytolysin from Enterococci, lactacin 3147 from Lactococcus lactis DPC3147, staphylococcin C55 produced by S. aureus C55, plantaricin W from

<table>
<thead>
<tr>
<th>Bacillus species</th>
<th>Bacteriocin/BLIS</th>
<th>Study reports</th>
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<tbody>
<tr>
<td>Bacillus subtilis GAS101</td>
<td>GAS101</td>
<td>Sharma et al. (2018)(15)</td>
</tr>
<tr>
<td>Bacillus subtilis KIBGE-17</td>
<td>Bac-tB17</td>
<td>Ansari et al. (2012)(16)</td>
</tr>
<tr>
<td>Bacillus subtilis SN7</td>
<td>Mejacin</td>
<td>Lee et al. (2018)(73)</td>
</tr>
<tr>
<td>Bacillus subtilis L-Q11</td>
<td>Subtilin L-Q11</td>
<td>Qin et al. (2019)(17)</td>
</tr>
<tr>
<td>Bacillus subtilis EMD4</td>
<td>Subtilosin A, BacEMD4</td>
<td>Liu et al. (2015)(18)</td>
</tr>
<tr>
<td>Bacillus amyloliquefaciens FZB42</td>
<td>Amylocyclin</td>
<td>Scholz et al. (2014)(12)</td>
</tr>
<tr>
<td>Bacillus amyloliquefaciens GA1</td>
<td>Amyloyclin</td>
<td>Arias et al. (2013)(19)</td>
</tr>
<tr>
<td>Bacillus sonorensis MT 93</td>
<td>Sonorensin</td>
<td>Chopra et al. (2014)(13)</td>
</tr>
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</table>
Klebsiella pneumoniae, helveticin I (from Lactobacillus helveticus), and enterolysin (from Enterococcus faecalis) are the members of this group (41).

2.2. Application of Bacillus bacteriocin

The Bacillus species are industrially important because of their excellent safety record, rapid growth rates, short fermentation cycles and their high capacity for protein secretion into the extracellular medium (42). Peptides derived from Bacillus species have shown antibacterial, antifungal, antiviral, antitumor, antiamebocytic, and antimycoplasmic activities (4, 42). Similarly, many Bacillus species, such as B. subtilis, B. clausii, B. cereus, B. coagulans, and B. licheniformis, have been used as probiotic supplements in both animals and humans (23, 43). Some of the applications of bacillus bacteriocin are discussed below (Figure 1).

2.2.1. Application in human health

Bacteriocins are considered as alternative antimicrobials for treatment of human infections, as there is increasing bacterial resistance to conventional antibiotics (14). Bacteriocins or bacteriocin like substances (BLIS) produced by bacillus have shown antimicrobial activity against multi drug resistant bacteria such as MRSA, vancomycin resistant enterococci (VRE), etc. In addition to bacteriocins, lantibiotics has recently been the peptide of interest (14). Bacitracin is one of the important polypeptides, which is effective against Streptococcus pyogenes and Staphylococcus aureus. Bacitracin has been used clinically in combination with other antimicrobial agents (44). Others have reported that oral administration of bacitracin daily for 7-10 days was successful in the treatment of antibiotic-associated colitis and diarrhea caused by Clostridium difficile (45). Amylolysin, a novel bacteriocin produced by the B. amyloliquefaciens GA1 strain, has been reported to exhibit activity against L. monocytogenes strains, which are responsible for food-borne listeriosis. Mersacidin, a lantibiotic shows strong antimicrobial activity against S. aureus both in vitro and in vivo studies (19, 46). The lantibiotic subtilosin A shows antimicrobial activity against pathogens such as L. plantarum, and Smb produced by Streptococcus mutans GS5 (31-35). In two peptide lantibiotics group, the antimicrobial activity is exhibited due to the synergistic activities of two lanthionine containing peptides (A1 and A2) (14). Haloduracin consist of two post translationally modified peptides Halα/A1 and Halβ/A2, both of which act synergistically to produce bactericidal activity. Similarly, lichenicidin also consist of two prepeptides Bliα/A1 and Bliβ/A2 that has 38% and 52% similarity to HalA1 and Hal A2 respectively (29).

Class II. Non-modified peptides

The class II bacteriocins are heterogenous group of small peptides having size of less than 10 kDa (25). These are heat stable, non-modified cationic peptides that are hydrophobic in nature. Klænhammer et al. (1993) had sub divided these peptides under three subgroups: class IIa pediocin like, class IIb two-component peptide and class IIc thiol activated peptides. In 1996, Nes and colleagues have suggested that, based on some common characters, class II bacteriocins can be divided as pediocin like and anti- listeria bacteriocins, two peptide bacteriocins and bacteriocins with sec-dependent signal sequence (20, 36). Later, Cotter et al. (2005) have suggested 4 subdivisions, retaining class IIa and IIb with two new subdivisions (class IIc and IId). The class IIc included cyclic bacteriocins while class IId has non-pediocin single linear peptides (37). Nissen-Meyer et al. (2009) have maintained this classification scheme in their review about the structure and function relationship of class II bacteriocins (38). In 2011, Belkam and coworkers suggested that circular bacteriocins as a separate class of bacteriocin (39).

Class III. Large proteins

This class includes large proteins (30 kDa), which have phospholipase activity such as megacins A-216 and A-19213 produced by Bacillus megaterium ATCC 19213. Megacin A-216 contains 293 amino acid residues and shows a native molecular weight of c. 66 kDa (40). Other proteins like colicins, klebicin (from Klebsiella pneumoniae), helveticin I (from Lactobacillus helveticus), and enterolysin (from Enterococcus faecalis) are the members of this group (41).

![Figure 1. Illustration showing applications of bacteriocins in different sectors.](www.ddtjournal.com)
monocytogenes, Gardnerella vaginalis and Streptococcus agalactiae. Similarly, Pep5 and epidermin prevent the adhesion of coagulase-negative staphylococci, specifically Staphylococcus epidermidis, to siliconised catheters (47). Besides antibacterial activity of bacteriocin, it has been found to have antifungal activity. For example, baciamin, an antifungal protein produced by B. amyloliquefaciens was reported to be active against various fungi like Botrytis cinerea, Helminthosporium turcicum, Harpophora maydis, Valsa mali, Mycosphaera arachidicola, Pythium aphanidermatum, Rhizoctonia solani, and Fusarium oxysporum (14,48).

Bacteriocin producing bacterial strains can be used as probiotic supplement for human and animals as they can inhibit the intestinal pathogens such as Clostridium perfringens, Clostridium difficile and others (49,50). For example, B. clausii produces inhibitory substances against S. aureus, Enterococcus faecium and C. difficile (50). Bacillus polyfermenticus SCD (polyfermenticin SCD producer) is a probiotic, commercially used for the treatment of long-term intestinal disorders as it inhibits the growth of C. perfringens (51).

2.2.2. Application in food industry

LAB derived bacteriocins are promising food preservatives and they are safe for human use because they are non-toxic compound. Nisin (as nisinpal) and pediocin PA-1 (as ALTA 2341) are commercially available food additives (38). There are two bacteriocins from Bacillus that have potential preservative application in dairy products (14). For example, bacillolcin 490 showed activity against closely related Bacillus spp. The bactericidal activity was found to be stable at 4°C, wide pH range and high temperature (8). Another is cerein 8A produced by B. cereus 8A, was used to control cheese surface contamination by L. monocytogenes. In 2008, Bizani and colleagues showed that cerein 8A only caused a delay in the start of exponential growth phase in soft cheese (10). Furthermore, BLIS produced by B. amyoliquefaciens GA1 was used as biopreservatives in poultry meat (14). These days there is a trend of discouraging the use of chemical preservatives which has increased the interest in the application of natural preservatives. Recently, the antimicrobial lipopeptide microcapsules made from B. amyoliquefaciens ES2 was tested as food additives (52).

2.2.3. Application in livestock

Bacteriocin producing Bacillus strain could be used as probiotics in livestock to improve the health of animals (53). For example, a lichenin derived from B. licheniformis was found to exhibit antibacterial effect against Eubacterium ruminantium and Streptococcus bovis and it also possessed the hydrolytic activity against polysaccharides (54). Therefore, Pattnaik and colleagues (2001) postulated that lichenin have potential applications to improve rumen fermentation due to its role as a digestive aid and due to its antimicrobial properties (54). Amylolsyn at concentration of 5-10 µg/g has shown to inhibit the growth of L. monocytogenes in poultry meat (19). The bacteriocin-producing strains Paenibacillus polymyxa NRRL B-30507, NRRL B-30508, NRRL B-30509 and Bacillus circulans NRRL B-30644 were used to control Campylobacter jejuni for treating animals carrying zoonoses. Spores of the B. amyoliquefaciens CECT 5940 are used as a probiotic in poultry feeds (Ecobiols, Norel & Nature Nutrition) to reduce the effect of pathogenic bacteria such as C. perfringens, E. coli and Yersinia (14).

2.2.4. Application in fishery

Probiotics produced by Bacillus strain could be used in two ways: as preservative to improve the storage in fish procession industry and as an antimicrobial to improve fish health in aquaculture. Bacteriocin, an antimicrobial peptide, possess antagonistic activity against other bacteria showing immunoprotective effects against fish bacterial infections (55). Probiotics or bioactive molecules isolated from fish gut-derived Bacillus spp., are found promising source of natural antimicrobial compounds against fish bacterial diseases. Similarly, bacteriocin TSU4 isolated from fish inhabited Lactobacillus showed a wide range of antimicrobial activity against Aeromonas hydrophila (MTCC 646) and Pseudomonas aeruginosa (MTCC 1688) and showed pH and thermal stability as well. Thus, bacteriocin "TSU4" has potentiality for using as preservative in fish processing industry (56). Likewise, nisin was found as effective bio-preservative agent to increase shelf life of rainbow trout (Oncorhynchus mykiss) storage. Analogously, nisin, which is produced by Lactobacillus lactis subsp. lactis, is allowed to use as food additive. Nisin-treated vacuum packaged rainbow trout increased the self-life from 12 days to 16 days at 4°C (57). These evidences show the usefulness of bacteriocins in fishery industry.

2.2.5. Application in environment

Bacillus species is naturally found in soil and plants. Thus, the bacteriocins or BLIS produced by Bacillus could be acquiescent to be used as biocontrol agent (14). For example, ericin S is active against Clavibacter michiganensis, the causative agent of tomato bacterial canker. Therefore, purified ericin or its producer strain could be developed as a bioprotectant on tomato cultivation against bacterial canker disease. As, the bioactivity of BLIS produced by B. subtilis 14B, Bac 14B, is active against Agrobacterium tumefaciens, thus it could be used as a biocontrol agent against A. tumefaciens associated infections. Moreover, some...
of the BLIS are effective against fungal strains, thus there is potential of using those BLIS as biocontrol agent to preserve plants decay and postharvest control of fruits and vegetables (14,18,27). Likewise, a BLIS produced by B. amyloliquefaciens AC 2 is bioactive against Colletotrichum dematium, mulberry anthracnose fungus and several other phytopathogenic fungi as well as bacteria, such as Rosellinia necatrix, Pyricularia oryzae, A. tumefaciens and Xanthomonas campestris pv. campestris (14). Furthermore, lipopeptides such as fengycin and iturins have antifungal activity (58). Moreover, surfactin has surfactant activity and emulsification properties, indicating that these peptides might be applied in bioremediation. The surfactin lipopeptide has also demonstrated activity as antitumor, antiviral, antibacterial activities and hypcholesterolemic agent (59). Many Bacillus-derived antimicrobial peptides can be used to inhibit plant pathogens and preserve grain. The B. subtilis species is widely used in the biocontrol of plant diseases. Recently, Guo and colleagues (2014) discovered that the B. subtilis NCD-2 strain secretes fengycin-type lipopeptides that exhibited antifungal activity against Rhizoctonia solani, the causative agent of cotton damping-off disease (58).

BLIS producing bacilli also have other environmental applications. The antimicrobial substances (AMS) produced by strains B. licheniformis T6-5 and Bacillus firmus H(2)O-1 prevented the formation of Bacillus pumilus LF4 biofilm and eliminated pre-established LF4 biofilm (60). In addition, Korenblum and coworkers reported that the presence of AMS produced by B. firmus H(2)O-1 reduced the viability and attachment of the SRB consortium biofilm thus, suggested that the AMS produced by Bacillus strains T6-5 and H(2)O-1 may have a potential for pipeline-cleaning technologies to inhibit biofilm formation and consequently reduce biocorrosion (60).

3. Structure-activity relationships (SARs) of bacteriocins

The molecular structure of antimicrobial peptides (AMPs) affects their mechanism of action and therapeutic effects. Etayash and his colleagues reported the structure-activity relationships (SARs) of seven bacteriocins (nisin, microcin J25, microcin B17, microcin C, leucocin A, sakacin P and pediocin PA-1 (61). It is relevant to discuss some examples of structure-activity relationships of nisin, a type of bacteriocins. Nisin is one of the most well studied peptides. This highly potent peptide inhibits food-spoilage bacteria and used to treat drug-resistant bacterial infections. Nisin has two most common forms; they are nisin A and nisin Z. Many analogues of nisin were synthesized using site directed mutagenesis and chemical synthesis. After the mutations at rings A and B of nisin, the mutants retained the biological activity of the peptide (62). The hinge region of nisin was mutated in many ways and the mutants N20P, M21V and K22P showed activity greater than the native type nisin A against S. aureus, L. monocytogenes and S. agalactiae respectively (63). Field et al. isolated novel nisin variant with increased activity against clinical and foodborne pathogens by bioengineering process (64). They identified a variant with a serine to glycine change at position 29 (S29G). Moreover, they made three nisin A derivatives (S29A, S29D and S29E) which are active against Gram-positive drug resistant bacteria, by site-directed mutagenesis.

Similarly, Cotter and coworkers changed the three amino acids at the hinge region (N20, M21 and K22) of nisin to increase its bioactivity against many target strains (65). Likewise, Evelyn et al. created a bank of nisin A derivatives in which K 12 was substituted with all other standard amino acid residues to make more antibacterial peptide analogue, using bioengineering technology (Table 2) and the site-directed mutagenesis (66). Furthermore, Arnusch et al. (2008) conjugated nisin to vancomycin and the conjugate has 40-fold increase in antibacterial activity. The nisin fragment (1-12) was made by enzymatic cleavage and then it was conjugated to vancomycin by the click chemistry and the activity increased to wide strains of bacteria (67) (66). Pediocin PA-1 is a class II a bacteriocin that shows activity against Listeria monocytogenes (69). There are several studies regarding structure functional relationship of pediocin. Several mutants of pediocin were generated by chemical synthesis or by site directed mutagenesis (38). In the study done by Tomigana et al. (2007) showed some of the residues were essential for retaining activity of pediocin (70). They replaced each residue of the native codon with the NNK triplet Oligonucleotide by using NNK scanning method and generated 35 peptide mutants (Table 3). They found that, the bioactivity of pediocin was retained by almost all mutants having mutations at K1, T8, G10, S13, G19, N28 and N41, whereas the activity was completely lost in analogues with mutations at residues Y2, G6, C9, C14, C24, W33, G37, and C44, implying the importance of these residues for the bioactivity of pediocin (70).

Analogously, Song et al. made nine mutants of

### Table 2. Nisin A mutants obtained by bioengineering technology. Mutation at Lysine (K)12 with various amino acid substitutions exhibited increased, decreased and no activity as shown by Etayash et al. (2015) (61).

<table>
<thead>
<tr>
<th>Original residue</th>
<th>Mutant residue(s)</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K12</td>
<td>P,A,T,S</td>
<td>&gt; 125</td>
</tr>
<tr>
<td>K12</td>
<td>Q,M,C,N,V</td>
<td>100</td>
</tr>
<tr>
<td>K12</td>
<td>R,H,W,F,Y,I,G</td>
<td>50-70</td>
</tr>
<tr>
<td>K12</td>
<td>D,E</td>
<td>no activity</td>
</tr>
</tbody>
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pediocin with different substitution and increasing positively charged residues (71). They found two-fold increase in the activity of some of the mutants. The mutant S13K was found to be more potent than the native pediocin PA-1, which also indicated that charged mutant S13K was found to be more potent than the increase in the activity of some of the mutants. The positively charged residues (bacteriocins structure-activity relationships. This review characterized and classified them and discussed this review, we have explored the sources of bacteriocins, characterized and classified them and discussed bacteriocins structure-activity relationships. This review provides valuable information about multifarious use of bacteriocins.

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### References


### Table 3. Pediocin mutants obtained by NNK scanning where, N = A/C/G/T, K = G/T. Mutations at Y2, G6, C14, W33, G37 and C44 showed > 90% activity; while substitution at K1, T8, G10, S13, G19, N28, and N41 had < 10% relative activity described by Tomigana and Hatakeyama, et al. (2006) (68).

<table>
<thead>
<tr>
<th>Original residue</th>
<th>Mutant residue(s)</th>
<th>Activity (%)</th>
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<tr>
<td>K1</td>
<td>ND’</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Y2</td>
<td>Y</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>Y2</td>
<td>H</td>
<td>30-50</td>
</tr>
<tr>
<td>G6</td>
<td>G</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>T8</td>
<td>D,P</td>
<td>&lt;10</td>
</tr>
<tr>
<td>C9</td>
<td>C</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>G10</td>
<td>C,PQ</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>S13</td>
<td>C,P</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>C14</td>
<td>C</td>
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<tr>
<td>G19</td>
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<td>N28</td>
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<tr>
<td>C44</td>
<td>C</td>
<td>&gt; 90</td>
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activity = (diameter of the circle formed by the wild type/diameter of the circle formed by the wild mutant) 100%. P- Proline, A- Alanine, T- Threonine, S- Serine, Q- Glutamine, M- Methionine, C- Cysteine, N- Asparagine, V- Valine, R- Arginine, H- Histidine, W- Tryptophan, F- Phenylalanine, Y- Tyrosine, I- Isoleucine, G- Glycine, D-Aspartic acid. ND’, residues where the stop codon was introduced, or peptide was damaged by PCR error.


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