of the brains of cattle infected with bovine spongiform encephalopathy, it can be seen that a unique pattern of spongiform vacuoles was formed in the brain due to the destruction of neurons and the deposition of abnormal protein fibers.

[14] The pathogen believed to be the cause of transmissible spongiform encephalopathy is an infectious protein called an abnormal prion. Unlike regular viruses that require nucleic acids, the abnormal prion is an infectious particle composed only of protein and containing no nucleic acid. Regarding TSE, it is known that, when an abnormal prion (PrPsc) that is an infectious particle binds to a normal prion (PrPc), it is converted to a pathogenic prion which then accumulates in the brain (Prusiner SB, *Alzheimer Dis Assoc Disord.*, 3:52-78, 1989).

[15] Creutzfeldt-Jakob disease is a rare neurodegenerative disorder of human transmissible spongiform encephalopathy (TSE) where the transmissible agent is apparently an abnormal isoform of a prion protein. An individual with Creutzfeldt-Jakob disease can deteriorate from apparent perfect health to akinetic mutism within six months. Thus, when a pharmaceutical composition containing a biologic, such as botulinum toxin, obtained using animal-derived products, there is a risk of developing a prion-mediated disease such as Creutzfeldt-Jakob disease. Thus, if

[55] In another example of the present invention, an experiment was conducted to compare the growth pattern and toxin concentration of the *Clostridium botulinum* strain between when grown in an APF medium containing the final selected plant peptone of the present invention and when grown in a medium (Comparative Example 7) described in Korean Patent Application Publication 10-2022-0140696, which was published prior to the present invention. As a result, as shown in Tables 13 to 15 below and FIGS. 20 and 21, in the case of the medium of the present invention, the OD_{540nm} value was 7.1021 after 28 hours of culture of the *Clostridium botulinum* strain, and then the OD value gradually decreased, the OD_{540nm} value was 4.5636 after 48 hours, and the toxin concentration in the supernatant of *Clostridium botulinum* toxin was 588.8 μg (10 mL of culture medium) after 48 hours of culture. Meanwhile, as shown in Tables 13 to 15 below and FIGS. 20 and 21, in the case of the medium of Comparative Example 7, the OD_{540nm} value was 6.7739 after 28 hours of culture of the *Clostridium botulinum* strain, and the toxin concentration in the supernatant of *Clostridium botulinum* toxin was 263.7 μg (10 mL of culture medium) after 48 hours of culture, suggesting the toxin expression level decreased by 55.2% compared to that in the case of the medium of the present invention.

only to illustrate the present invention, and it will be obvious to those skilled in the art that the scope of the present invention is not to be construed as being limited by these examples.

[77]

[78] Example 1: Selection of plant peptones

[79] 1-1: Composition of CYG medium used for culture (control)

[80] CYG medium (casein, yeast extract and glucose) for culturing *Clostridium botulinum* was prepared by dissolving 2% casein hydrolysate [20 g/L], 1% yeast extract [10 g/L], and 1% glucose [10 g/L] in 90 mL of distilled water, adjusting the final volume to 100 mL, and dispensing 10-mL aliquots of the prepared medium into 15-mL culture tubes. Thereafter, the medium was autoclaved at 121°C for 30 minutes and then kept under anaerobic conditions in an anaerobic incubator.

[81] 1-2: Compositions of APF media used for culture

[82] As shown in Tables 1 and 2 below, 9 types of 2% plant peptone candidates (Sigma™ C8160-500G, Millipore™ 96174, Millipore™ 93492-500G-F, Sigma-Aldrich™ G5004-500G, Millipore™ 38143-500G, Organotechnie™ 19425, Sigma-Aldrich™ B4032-500G, Sigma-Aldrich™ 17218-500G, and Merck™ 1.05391.0500) [20 g/L] were each added to 1% yeast extract [10 g/L] and 1% dextrin or glucose [10 g/L], and then dissolved in 90 mL of water for injection. Then, the final

Peptone 1: peptone from pea; Peptone 2: wheat peptone; Peptone 3: potato peptone).

[105] The experimental design for OD_{540nm} values according to the proportion of plant peptones obtained through custom design is shown in Table 6 below, and the toxin concentration after the completion of culture under each condition was measured and added to the analysis items (Peptone 1: peptone from pea; Peptone 2: wheat peptone; Peptone 3: potato peptone).

[106] [Table 6]

	Peptone 1	Peptone 2	Peptone 3	OD540nm
1	10	20	0	
2	10	0	10	
3	20	10	0	
4	10	0	0	
5	10	10	20	
6	0	10	0	
7	0	0	0	
8	20	20	0	
9	0	20	20	
10	0	20	10	
11	20	20	20	
12	20	0	0	
13	20	0	20	
14	0	20	0	
15	20	10	10	
16	0	0	20	

[107] 2-2: Compositions of APF media used for culture

[108] As shown in Table 7 below, to 1% glucose [10 g/L] as a carbon source and 1% yeast extract [10 g/L], 2% three plant peptones [20 g/L] were added. Each of the mixtures was dissolved in 90 mL of water for injection, and the final volume was adjusted to 100 mL. 10-mL aliquots of the prepared