**AGROINDUSTRIAL STRENGTHENING OF QUINOA PROJECT THROUGH THE SCALING OF PROTOTYPES IN RELEVANT ENVIRONMENTS FOR THE INDUSTRY IN THE DEPARTMENT OF CAUCA**

**Identification of Bacillus cereus NTC 4679**

**Guide Code: 004**

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1. **AIM**

Describe the methodology used to determine the presence of *Bacillus cereus* according to NTC 4679

1. **BASIS**

*Bacillus cereus:*it is a mobile, large (+), spore-forming aerobic rod, which also grows well anaerobically. Resistant to cooking or pasteurization in food, its presence may indicate poor manufacturing practices, which may foster suitable conditions for the proliferation of foodborne diseases (ETA) in men (Sánchez et al., 2016). It spreads easily to many types of food, especially of plant origin, the ability to produce different toxins that affect human health when food contaminated by the microorganism is consumed in doses of 105 to 108 CFU per gram of food. (Granum & Lund, 1997).

1. **MATERIALS**

|  |  |
| --- | --- |
| **MATERIAL** | **AMOUNT** |
| Petri boxes | 3 |
| Spatula | 1 |
| Glass clock | 3 |
| Rack tips 1000μL | 1 |
| rack tips 100μL | 1 |
| erlemeyer | 3 |
| Glass beads | 1 |

Note: Wash, dry and sterilize materials.

1. **REAGENTS**

|  |  |
| --- | --- |
| **agar** | **Quantity** |
| MY P | According to supplier specifications |
| Distilled water |  |
| Yolk | According to supplier specifications |

1. **TEAMS**

|  |  |
| --- | --- |
| **Team** | **Quantity** |
| Autoclave | 1 |
| Incubator | 1 |
| Kiln | 1 |
| shaker | 1 |
| Colony count equipment | 1 |
| micropipette 1000 μL | 1 |
| micropipette 100μL | 1 |
| Laminar flow cabinet | 1 |

1. **PROCESS**
   1. **Preparation of sample**

Mix 10 g of sample with 90 mL of distilled water in an Erlenmeyer flask, shake in a shaker (MaxQ 4450 orbital Thermo Ficher Scientific USA) for 10 min at 150 rpm and allow to settle. Repeat procedure with the number of samples. Depending on the initial count, dilutions are prepared according to the NTC 4491-1 standard.

* 1. **agar preparation**

MYP agar is prepared according to the specifications of the technical data sheet of the container. Weigh quantity (g) of MYP agar, then add to the volume of distilled water; then it is left to boil on a heating plate with constant stirring. Later, it is sterilized in the autoclave for 20 min at 259 °C and 20 psi, after the specific egg yolk supplement for Bacillus cereus is added.

* 1. **Process**
     1. **Inoculation and incubation**

Using a micropipette, transfer1000 μL of sample from the first decimal dilution (10-1) to the next (10-2); the described procedure is repeated with the additional dilutions, using a different tip for each decimal dilution.

Pour MYP agar into Petri dishes so that it gels, once gelling is complete, take a 100 μL aliquot of the dilutions and place them in the agreed Petri dishes for repetitions, shake vigorously in the box with the help of glass beads Petri dish, repeat the process with each of the repetitions.

The solidified Petri dishes are inverted and packed in vinyl and incubated at 37 °C for 24 h. Monitor during the incubation of the microorganism.

* + 1. **Colony count**

After the specified period for incubation, select the Petri dishes containing colonies ≤ 300 colonies and count them in the colony counting equipment, using dim light, taking care to mark the counted colonies, to avoid confusion.

1. **DISPOSAL OF CHEMICAL AND/OR BIOLOGICAL WASTE.**

Once the determination of Bacillus cereus is finished, the boxes must be deactivated in an autoclave. The medium is then thrown into a bag indicating biohazard.

**BIBLIOGRAPHY**

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