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Wissensgrundlagen

# RISK PROFILES OF FOOD-BORNE PATHOGENS
# *BACILLUS CYTOTOXICUS*

*Bacillus cytotoxicus is a bacterium of emerging interest due to its ability to cause foodborne illness. It has been detected in a range of plant-based foods, particularly in products containing potato, as well as in edible insects. It can cause acute gastrointestinal symptoms and has been linked to severe, sometimes fatal, foodborne outbreaks. Standard food screening methods fail to detect it reliably, limiting the ability to routinely assess its presence in food products.*

## Hazard Identification

*Types and strains: Bacillus cytotoxicus* is a spore-forming bacterium belonging to the *Bacillus cereus* group. It was first identified as a distinct species in 2013, following a fatal foodborne outbreak in France associated with severe diarrheal illness [1], [2]. To date, *B. cytotoxicus* has been studied based on a limited number of isolates obtained from foodborne disease outbreaks and scientific investigations. However, the virulence potential of *B. cytotoxicus* seems to be highly variable [3]. Some strains are highly cytotoxic, but it is not yet clear whether the differences in cytotoxicity are solely attributed to Cytotoxin K-1 expression of the bacteria, or stem from a combination of different enterotoxins [3]. As a result, the role of *B. cytotoxicus* as a foodborne pathogen remains under investigation. Key properties of *B. cytotoxicus* are summarized in Table 1.

Table 1: Characteristics of *B. cytotoxicus*

| **General characteristics of the bacteria** | |
|---|---|
| **Family/Genus** | *Bacillaceae / Bacillus* |
| **Strains** | Strains that have been characterized to date range from non-toxic to extremely toxic [4]. |
| **Gram stain** | Gram-positive rods |
| **Cell diameter** | ≥1.0 µm [5] |
| **Spore formation** | Yes |
| **Motility** | Not defined |
| **Toxins** | The Cytotoxin K-1 (CytK-1) gene is present in all strains, but not all express it. CytK-1 displays hemolytic, necrotic, and cytotoxic activity towards intestinal cells [6].<br>Some strains also carry genetic markers for the non-hemolytic enterotoxin (Nhe), which may contribute to cytotoxicity, though their functional role remains unclear [6, 7] |

Figure 1:Illustration of the Bacillus genus of bacteria, commonly found in soil and food (Source: freepik.com).

*Growth and inactivation*: *B. cytotoxicus* is heat resistant and can survive the heating and drying processes used in the production of dehydrated foods like mashed potato powder [3], [8]. Like other *B. cereus* group strains, *B. cytotoxicus* is capable of sporulation, which may enable it to persist on industrial surfaces and potentially contaminate food [6]. Knowledge of the growth dynamics, toxin production, and inactivation of *B. cytotoxicus* is still incomplete. Further research is required to identify factors influencing its persistence and to develop effective control strategies during food production and preservation, as shown in Tables 2 and 3. As with the *B. cereus* group in general, strict temperature control during food preparation and storage helps to reduce bacterial growth and toxin formation.

Table 2: Growth and toxingenesis of *B. cytotoxicus*

| **Growth (vegetative cells)** | | | |
|---|---|---|---|
| **Parameter** | **Min** | **Opt.** | **Max** |
| **Temperature** [5, 7] | 18 °C | 37-47 °C | 52°C<br>Some non-food related isolates up to 60°C [6] |
| **pH** [5, 9] | 4.3 - 5 | - | 8.8 |

| | | | |
|---|---|---|---|
| **$a_w$** [1, 7] | 0.93 | - | - |
| **Salt** | Not defined | - | - |
| **Toxin Production** | | | |
| **Parameter** | Min | Opt. | Max |
| No defined parameters | - | - | - |
| **Toxin Stability** | | | |
| No defined parameters | - | - | - |

Table 3: Inactivation of *B. cytotoxicus*

| **Inactivation of vegetative cells and spores** | |
|---|---|
| **Parameter** | **Conditions** |
| **Heat** | The gold standard for inactivating spores is 121 °C for 3 min, which is used during the production of canned foods [8] |
| **Irradiation** | Not defined |
| **High pressure** | Not defined |
| **Disinfectants** | Not defined |

## Hazard Characterisation

*Disease characteristics: B. cytotoxicus* can produce the toxin cytK-1, which belongs to the cytK group associated with the diarrheal form of *B. cereus* food poisoning [8]. Typically, diarrheal illness occurs when members of the *B. cereus* group produce enterotoxins in the intestine. For this process, bacterial spores must survive gastric passage, germinate, exhibit motility, and produce enterotoxins under intestinal conditions [10]. These specific functionalities are still under investigation for *B. cytotoxicus* and are currently inferred from general knowledge of the *B. cereus* group. A summary of disease characteristics is presented in Table 4.

Table 3: Disease characteristics of *B. cytotoxicus*

| **Disease Characteristics** | |
|---|---|
| **Incubation period** | Typically, 8-16h for *B. cereus* group. |
| **Infectious dose** | There is no literature data on the infectious dose of cytotoxic strains. |
| **Mode of actions** | The diarrheal illness is generally considered a toxico-infection resulting from the ingestion bacterial spores, which germinate in the intestine and produce toxins [8]. |
| **Symptoms** | Watery diarrhoea, abdominal pain. Rare severe cases with bloody diarrhoea [1]. |
| **Progression** | Typically, self-limiting within 12-24 hours for the *B. cereus* group. May progress to necrotic enteritis in rare severe cases [1]. |
| **Infectious period (shedding)** | Not relevant for foodborne intoxication. |
| **Shedders** | Not applicable |
| **Vulnerable populations** | Individuals with underlying health conditions may be more susceptible to severe outcome of the illness. |
| **Dose-response relationship** | Standardized dose-response relationships for *B. cytotoxicus* have not been established. The concentrations of *B. cytotoxicus* found in food associated with outbreaks have been ranging from 4.0 × $10^2$ to 9.20 × $10^5$ CFU/g [11]. |

*Occurrence*: *B. cytotoxicus* is considered an emerging food safety concern due to its ability to survive food processing and its association with severe illness. Although documented outbreaks caused by *B. cytotoxicus* are rare.

Table 5: Epidemiological situation in Switzerland

| **Epidemiological Situation in Switzerland** | |
|---|---|
| **Annual reported cases** | No documented cases of illness in Switzerland |
| **Mandatory reportable disease** | Not listed as a notifiable disease |

## Exposure Assessment

*Sources and reservoirs:* Most reports of *Bacillus cytotoxicus* strains have originated from France, Germany, Norway, Switzerland, and the United Kingdom. [6]. It has been found in a range of plant-based foods with potato-containing products being the most commonly affected, but also in edible insects. In Switzerland, a study of selected retail foods found *B. cytotoxicus* in mashed potato products, instant soups, potato flakes and gnocchi, with the highest prevalence in mashed potato products (82%) and potato flakes (67%) [3]. Prevalence in mashed potato powders has also been reported in other studies from Switzerland (45%) [12] and Germany (71%) [13].

In the UK, there have been two incidents of food contamination involving *B. cytotoxicus*: one involving protein bars made with insect flour (9.6 x $10^5$ CFU/g) and the other involving food products made with insect flour (1.9–5.4 x $10^6$ CFU/g) [6]. *B. cytotoxicus* has been also detected in dried edible insects supplied by online retailers [14].

The typical level of *B. cytotoxicus* found in foods is ≤ $10^2$ CFU/g [4]. However, when for example, potato purée is stored at room temperature, bacterial counts can rise, reaching up to $10^5$ CFU/g within two days [4].

*Outbreaks:* Although rare, some foodborne outbreaks have been confirmed. Notable cases include a fatal outbreak in France in 1998 linked to vegetable purée, which affected 44 people and resulted in three deaths [1]; a 2008 outbreak in a French school canteen associated with semolina, involving 61 cases [5]; and two additional cases connected to potato purée, reported in France (2003) and Germany (2007) [6].

Table 4: Main sources and transmission routes in Switzerland.

| **Main Sources and Transmission Routes in Switzerland** | |
|---|---|
| **Main reservoir** | Possible reservoirs are being considered soil or insects, with genetic evidence of the bacteria suggesting soil adaptation [2, 12].<br><br>A Swiss study also indicates that the bacterium can persist in food production facilities, potentially playing a significant role in the re-contamination of food products [5]. |
| **Transmission** | Foodborne, through consumption of products containing spores or vegetative cells of the bacterium. |
| **Main source of infection** | Swiss studies on selected retail products have detected highest prevalence in **mashed potato powder** and **potato flakes** [3], [12].<br><br>In other European countries *B. cytotoxicus* has been linked to foodborne outbreaks involving **vegetable purée** [1], **potato purée** [6], [7], and **semolina** [5]. Additionally, incidents of food contamination have been reported in products containing **insect flour** [6]. |
| **High risk food items** | Plant-based foods, especially those containing potato. |
| **Populations at elevated risk for infection** | Unknown |
| **Major outbreaks** | No outbreaks reported in Switzerland. |

## Regulatory Aspects

*Regulation*: While general food hygiene regulations apply to all food production and handling processes to minimize contamination risk, there are currently no specific Food Safety Criteria established for *B. cytotoxicus,* in the EU and in Switzerland.

*Detection*: Classical screening protocols performed at mesophilic temperatures (30–42 °C) fail to detect the majority of *B. cytotoxicus* present in foods. For research purposes, screening for *B. cytotoxicus* in food has been successfully performed by initially incubating samples in CGY medium at 50 °C overnight [3]. The broth is subsequently inoculated onto selective Mossel/MYP agar and further incubated at 37 °C. Suspect colonies are then confirmed as *B. cytotoxicus* by PCR targeting the cytK-1 gene [15].

## RESOURCES

Website of the Federal Food Safety and Veterinary Office: https://www.blv.admin.ch/blv/en/home/lebensmittel-und-ernaehrung.html

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