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# On the industrial use of *Bacillus licheniformis:* a review

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**Abstract.** We document here that in those rare cases where disease has been related to *Bacillus licheniformis,* infection was associated with bypassing the normal biological protective barriers or severely debilitated patients. No case suggests any invasive properties of this bacterium. *B. licheniformis* can therefore be considered non-pathogenic to humans in general. Food-borne illness caused by possible *B. licheniformis* toxins have been reported, but only in a very few cases and only in connection with consumption of inappropriately prepared food. Considerable experience concerning the industrial use of recombinant *B. licheniformis* strains has now accumulated and authorities in the United States, Europe and Japan have approved production with and products from recombinant *B. licheniformis* strains. We conclude that *B. licheniformis* is a safe host for the production of harmless, industrial products.

## Introduction

For many years the fermentation industry has used micro-organisms isolated from nature to produce antibiotics, amino acids, enzymes and other useful compounds. These micro-organisms have proved safe in industrial settings, and are therefore ideal as hosts for recombinant DNA since it is generally accepted that a genetically engineered microorganism is as safe as the host, provided the products of the transferred genes are harmless. We therefore find it useful to review the safety of some industrial micro-organisms that are, or are likely to become, hosts for the construction of industrial production strains by genetic engineering. The series so far includes reviews of *Bacillus subtilis* and *B. amyloliquefaciens* (de Boer and Diderichsen 1991) and of *Aspergillus oryzae* (Barbesgaard et al. 1992). The purpose of this article is to review the safety of *B. licheniformis*.

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Endospore-forming bacteria of the genus *Bacillus* are used for the manufacture of several industrial products, and in particular extracellular enzymes (largely amylases and proteases) and bioinsecticides (Priest 1989a). Surfactants may become an important addition to this list (Fiechter 1992). With the exception of the bioinsecticides of *B. thuringiensis,* these products are mainly derived from *B. amyloliquefaciens, B. licheniformis, B. subtilis, B. lentus* and alkalophilic bacilli related to *B. lentus. B. licheniformis* is able to secrete proficient amounts of indigenous proteins and has simple growth requirements.

*B. licheniformis* strains are listed in the Third edition of the Food Chemicals Codex (1981) as a source of carbohydrase and protease enzyme preparations used in food processing. Since 1972, it has been safely used for large-scale industrial fermentation to produce amylase. *B. licheniformis* is therefore an attractive host for the expression of cloned gene products on an industrial scale. Several recent reports describe cloning and expression of industrially relevant proteins in *B. licheniformis* (Andreoli et al. 1988; Diderichsen et al. 1991; van Leen et al. 1991).

## Taxonomy and ecology

Within the genus *Bacillus,* the *B. subtilis* group is traditionally recognised as encompassing five physiologically similar species (Gordon et al. 1973): *B. amyloliquefaciens,* the recently described *B. atrophaeus* (previously *B. subtilis* var. *aterrimus,* Nakamura 1989), *B. licheniformis, B. pumilus* and *B. subtilis.* Although these bacteria are phenotypically very similar, they can easily be distinguished by DNA homology studies. In particular, *B. licheniformis* strains share an average of only 10% DNA homology with other members of the group; its closest relative is probably *B. amyloliquefaciens* with 9–15% homology, the most distant being *B. pumilus* with about 8% homology (Seki and Oshima 1989). Similarly, small subunit rRNA sequence comparisons have established that this is a group of distinct, but closely related species (Ash et al. 1981).

Some classical biochemical tests can be used to distinguish *B. licheniformis* from other species of the *B. subtilis* group. Thus, *B. licheniformis* strains react positively for anaerobic growth, arginine dehydrolase production, starch hydrolysis and utilization of propionate as a carbon source; no other species in the group displays this series of reactions (Priest et al. 1987). Thus, the distinction between *B. licheniformis* and *B. subtilis* is now easily made.

Strains of *B. licheniformis* are widely distributed in the environment; they are common in most soils and dominate in nutrient-poor soils such as moorland and deserts. *B. licheniformis* is also common in foods including natural agricultural products such as cereals, which it presumably colonizes from wind-blow dust and soil particles. Large numbers of spores of this bacterium may be found in processed or dried foods and herbs such as cocoa and spices (reviewed by Priest 1989b).

## Infections

A database search of the literature since 1966 was made for reports describing human infections with *B. licheniformis*. This search was supplemented with a review of our own collection of references on *Bacillus* pathogenicity. The search resulted in fewer than ten relevant articles describing approximately 20 cases of *B. licheniformis* infections.

Three cases on local infection with *B. licheniformis* have been published. Young et al. (1982) described a case of post-surgical infection with *B. licheniformis*. The infection developed after removal of a brain tumor. Tabbara et al. (1979) reported on an eye infection with *B. licheniformis* following a corneal injury, and Thurn and Goodman (1988) described a case of eye infection after injury of the eye by a metal splinter. The patient lost vision in the injured eye.

Septicaemis caused by *B. licheniformis* have been reported in four instances. Sugar et al. (1977) isolated *B. licheniformis* from the blood of a man suffering from a torsion of the small bowel and jejunal perforation. The septic condition was related to the stasis in the intestine, which made it possible for bacteria to pass into the bloodstream. Peloux et al. (1976) described a case of septicaemia in a pregnant woman, given several blood transfusions within a short space of time. The route of infection was in this case either contaminated blood cultures or contaminated injection remedies. Fauchere et al. (1977) reported a fatal septicaemic condition. The person had previously suffered from a perforated gastric ulcer, and the following surgery had revealed a hepatic abscess. *B. licheniformis* was isolated several times in the patient's blood. After death, *B. licheniformis* was isolated from the hepatic abscess. The route of infection might have been the abscess, which again was probably established in connection with the gastric ulcer. Hardy et al. (1986) reported a case of septicemia following an aortographic investigation. The infection was linked to the invasive investigation procedure. With the exception of the case described by Fauchere et al. (1977), all patients were successfully treated.

Cotton et al. (1987) retrospectively examined episodes of *Bacillus* bacteraemias at a hospital with a large proportion of immunocompromised patients. Seventeen cases were found and, of these, 12 were identified to the species level. Three of the infections were caused by *B. licheniformis*. Fifteen of the patients had advanced malignancies and fourteen had intravenous catheters through which the bacteria had possibly been introduced. A similar situation was described by Banerjee et al. (1988). During an 8-year period they isolated *Bacillus* species in the blood from 24 patients all suffering from cancer. *B. licheniformis* was isolated in one case.

Other reviews on *Bacillus* infections recorded in hospitals reveal no infections caused by *B. licheniformis*. Sliman et al. (1987) reviewed the records of five Cleveland hospitals, and found 38 patients with infections caused by *Bacillus* species. Fifteen of the isolates were identified to the species level, but none were identified as *B. licheniformis*.

The microbial contamination of 49 drug samples as well as injection remedies was investigated by Shamsuddin et al. (1982). *Bacillus* species, i.e. *B. cereus, B. subtilis, B. pumilus* and *B. licheniformis*, were found most frequently, but *B. licheniformis* was not identified as the infective organism in any of four cases of infection mentioned by Shamsuddin et al. (1982).

Finally, a review by Tuazon et al. (1979) dealt with disseminated infections caused by *Bacillus* spp. but *B. licheniformis* was not isolated from a total of 32 cases. Pearson (1970) did not identify *B. licheniformis* as the cause of infection in 48 patients, all suffering from traumatic wounds.

In summary, very few cases of infections associated with the presence of *B. licheniformis* have been described. The case stories summarized above all involve preceding tissue injury, intravenous injection or catheter implantation. *B. licheniformis* strains have in no publications been described as the cause of disease without preceding traumatic bypass of the normal barriers of the body, i.e. skin, gastrointestinal tract or lung tissue. Neither is *B. licheniformis* commonly reported as the infectious agent in wounds. Therefore, it is concluded that *B. licheniformis* is non-pathogenic to humans.

## Food poisoning

*B. licheniformis* as the cause of food-borne illness has been described by Kramer and Gilbert (1989). During the 10 years from 1975 to 1986, only 24 episodes of food-borne illness caused by *B. licheniformis* were reported in the UK. In comparison, during the period from 1971 to 1984, 192 episodes of the emetic type of food-borne illness caused by *B. cereus* were reported in the UK. The best proof that possible *B. licheniformis* toxins are not any reason for concern is the long history of safe industrial use of *B. licheniformis* for the production of food enzymes.

## Industrial use of recombinant strains

In 1989, the Danish Ministry of Health gave the first production permissions and environmental certifications concerning the industrial use of genetically engineered strains of *B. licheniformis*. Subsequently, the Ministry stated that the recombinant organisms complied with the criteria of Good Industrial Large Scale Production organisms (GILSP organisms), recommended by the OECD (Recombinant Safety Considerations, Paris 1986).

The National Institutes of Health of the United States (NIH) has in part exempted *B. licheniformis* host/vector systems from its Guidelines for Research Involving Recombinant DNA Molecules (1988). In this respect, *B. licheniformis* has obtained a status similar to that of *B. subtilis*. In 1991, the NIH amended its Guidelines to introduce a GLSP (Good Large-Scale Practice) level of containment for large-scale culture for research and production, using recombinant microorganisms fulfilling GLSP criteria equivalent to the GILSP criteria recommended by the OECD (1991).

Production permissions have been given for several recombinant *B. licheniformis* strains by the Environmental Protection Agency of the United States (EPA). See for example approval of production using recombinant *B. licheniformis* strains given by the EPA to the companies Gist-Brocades and Novo Laboratories, based on Pre-manufacturing Notices PMN 87-1511 and PMN 89-1071, respectively. Moreover, production with recombinant *B. licheniformis* has been approved by the Ministry of International Trade and Industry in Japan (1988).

Carbohydrase and protease enzyme preparations produced by *B. licheniformis* have been affirmed as GRAS (Generally Recognized As Safe) by the Food and Drug Administration of the United States (FDA; 1983). In the supplementary information to the final rule in the Federal Register, FDA emphasized that "Published scientific literature as well as standard books on food microbiology demonstrate that *B. licheniformis* is widely recognized as a common contaminant found in many foods. None of these references report any toxicity or pathogenicity associated with the presence of this organism in food."

A GRAS affirmation petition requesting formal affirmation of the GRAS status of an amylase from *B. stearothermophilius* expressed in *B. licheniformis* has been accepted for filing by the FDA (1991). The petition includes information that demonstrates that the *B. licheniformis* production strain is neither pathogenic nor toxinogenic.

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